NORGEN BIOTEK CORP.

The Sample Preparation Experts

BLOOD, PLASMA & SERUM SAMPLE PREPARATION

The New Standard in RNA Purification, Best-in-Class, Pure & Simple

Collection, preservation & isolation of inhibitor-free RNA, microRNA and genomic DNA for any application

www.norgenbiotek.com

An ISO 13485:2003, ISO 9001:2008 & ISO 15189:2007 Certified Company

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Norgen Biotek is dedicated to providing our customers with first class sample preparation kits for RNA, microRNA, DNA and protein purification, clean-up and concentration for research and diagnostic applications; and to providing dedicated and expert support services to our customers and commercial partners worldwide.

Norgen is an **ISO 9001:2008, ISO 13485:2003 and ISO 15189:2007** certified company, indicating our commitment to quality.

ISO 9001:2008 ISO 13485:2003 ISO 15189:2007 CE SCC Accredited LAB™

Ordering Information

To Order by Phone:
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Telephone Orders
Customer service representatives are available to receive orders Monday through Friday from 9:00 A.M. to 5:30 P.M. EST (Eastern Standard Time).

When placing an order, please be prepared to provide us with the following information:

1. Purchase order number
2. Customer number (if known)
3. Billing Address
4. Shipping Address
5. Name of person to whose attention the order should be shipped
6. Name, telephone number and email of contact person
7. Product catalogue number, description, size and quantity

Email and Fax Orders
We also accept orders by email, Fax and mail. When placing an order by email, Fax or mail, please ensure that the information listed above is included in order to expedite ordering.

Technical Support

Contact our Technical Support Team between the hours of 9:00 and 5:30 EST (Eastern Standard Time) at (905) 227 - 8848 or Toll Free in North America at 1-866-667-4362.

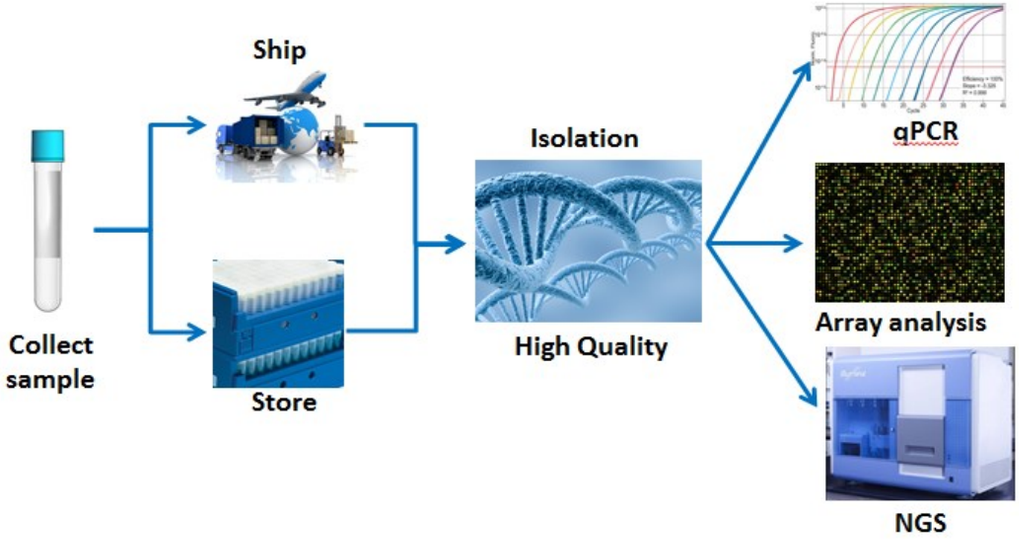
Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Table of Contents

A. Blood RNA Preservation & Purification Kits

Collection, Preservation and Isolation of high quality total RNA from blood using Norgen's RNA preservation and purification technologies. RNA can be preserved using Norgen's RNA Preservation tubes for up to 12 days at room temperature. Next, RNA can be isolated using Norgen's RNA purification technology that isolates all sizes of RNA including microRNA. In addition, Norgen offers different RNA purification kits to isolate RNA preserved in different Blood RNA Preservation Tubes including Norgen's Blood RNA Preservation Tubes, Tempus™ Blood RNA Tubes and PAXgene™ Blood RNA Tubes. All RNA isolated by Norgen's technology is of the highest quality and is suitable for sensitive downstream applications.

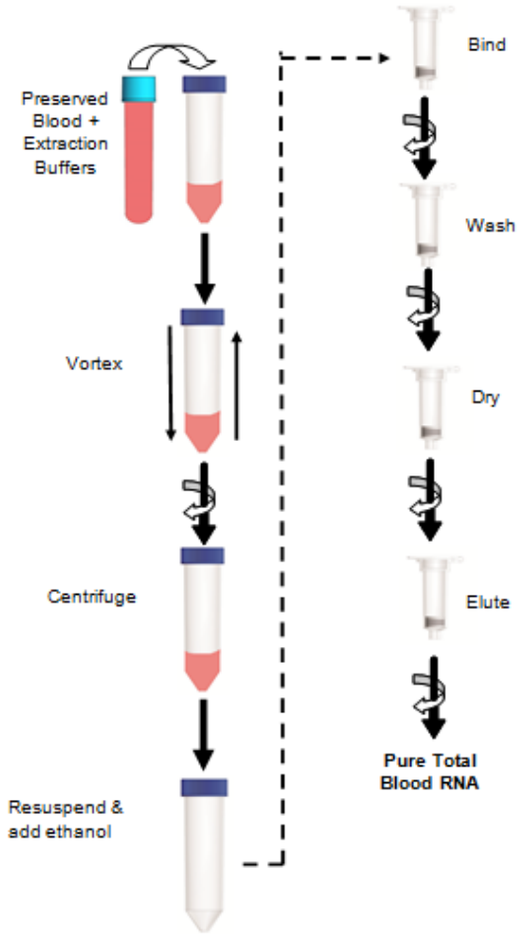
Kit	Cat. #	Kit size
A Complete System From Collection and Preservation to Purification of Total RNA From Blood		
Blood RNA Preservation and Purification System	52720, 52620	96, 48 preps
Blood RNA Preservation Tubes		
Blood RNA Preservative Tubes	52710, 52610	96, 48 preps
RNA Isolation Kits - For Preserved Blood RNA from Various Sources (Norgen, Tempus™ and PAXgene™)		
Preserved Blood RNA Purification Kit (for use with Norgen Blood RNA Tubes)	52700, 52600	96, 48 preps
Preserved Blood RNA Purification Kit I (for use with Tempus™ Blood RNA Tubes)	43400	50 preps
Preserved Blood RNA Purification Kit II (for use with PAXgene™ Blood RNA Tubes)	43500	50 preps



A Complete System From Collection and Preservation to Purification of Total RNA From Blood

Blood RNA Preservation and Purification System Cat. # 52720 & 52620

Rapid Flow Chart Procedure



A Complete System for the Preservation and Isolation of Whole Blood RNA

Product Description

Norgen has developed a system that enables the collection, stabilization, storage, and transportation of whole blood specimens, together with a rapid and efficient protocol for purification of intracellular RNA. The system requires the use of: **(A)** Norgen's Blood RNA Preservative Tubes for blood collection and RNA stabilization and **(B)** RNA purification using spin column chromatography based on Norgen's proprietary resin.

Collection of whole blood is the first step in many molecular assays used to study cellular RNA. However, a major problem in such experiments is the instability of the cellular RNA profile in vitro. Studies have shown that the copy numbers of individual mRNA species in whole blood can change more than 1000-fold during storage or transport at room temperature, due to rapid RNA degradation and induced expression of certain genes after the blood is drawn. Such changes in the RNA expression profile make reliable studies of gene expression impossible. A method that preserves the RNA expression profile is therefore essential for accurate analysis of gene expression in human whole blood.

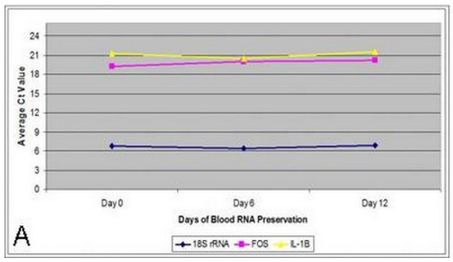
(A) Sample Collection and Stabilization

Norgen's Blood RNA Preservative Tubes (included in kit) feature an aqueous preservative that protects RNA molecules from degradation by RNases and minimizes induction of gene expression. Norgen's Blood RNA Preservative Tubes are intended for the collection of whole blood and stabilization of cellular RNA for up to 12 days at 18–25°C or up to 14 days at 2–8°C. Studies evaluating stability for longer time periods at other temperatures are ongoing, and further information regarding these findings can be obtained by contacting Norgen Biotek. The actual duration of RNA stabilization may vary depending upon the species of cellular RNA and the downstream application used. Five different transcripts were validated for stabilization specifications (IL-16, NFKβIA, RAS1, FOS and IL1B gene transcripts).

(B) RNA Concentration and Purification

Norgen's Blood RNA Preservation and Purification Kit provides a rapid method for the isolation and purification of total RNA from 3 mL of human whole blood collected in Norgen's Blood RNA Preservative Tubes. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA). The RNA is preferentially purified from other cellular components such as proteins, without the use of phenol or chloroform. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

Blood RNA Preservation and Purification System Cat. # 52720 & 52620



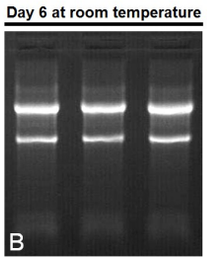


Figure 1. Stability of Blood RNA in Norgen's Blood RNA Preservative Tubes. A main component of Norgen's Blood RNA Preservation and Purification Kits are the Blood RNA Preservative Tubes. Blood samples were collected from the same donor in triplicate on Norgen's Blood RNA Preservative Tubes. The stability of the blood RNA in Norgen's Blood RNA Preservative Tubes was evaluated over a period of 12 days at room temperature. At each time point, the preserved blood RNA was isolated using Norgen's Blood RNA Preservation and Purification Kit. Three different genes, FOS, IL1B and 18S rRNA, were used to evaluate the stability of blood RNA over a period of 12 days at room temperature.
Figure 1A shows the efficiency of Norgen's Blood RNA Preservative for minimizing the induction of gene expression over a period of 12 days at room temperature. Each of the 3 genes tested showed no changes in gene expression over the 12 day preservation period.
Figure 1B shows the quality of the Blood RNA preserved for 6 days at room temperature.
Most importantly that Norgen's Preserved Blood RNA Purification method allows for the isolation of all sizes of RNA, from the very large RNA down to the microRNA without the use of phenol.

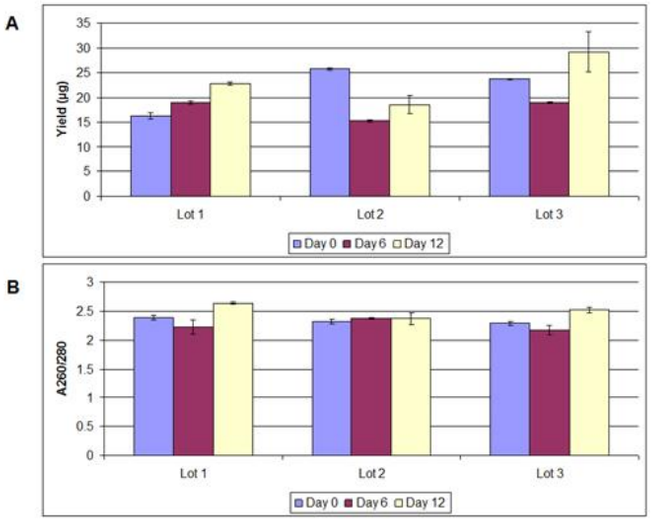


Figure 2. Qualitative and Quantitative Analysis of Blood RNA Preserved and Isolated using Norgen's Blood RNA Preservation and Purification Kit . Preserved blood RNA processed using three different lots of Norgen's Blood RNA Preservation and Purification Kit were analyzed qualitatively and quantitatively. Blood was collected from the same donor on Norgen's Blood RNA Preservative Tubes. Blood RNA preservation was evaluated for the three different lots over a period of 12 days at room temperature. Preserved blood RNA was isolated from each sample at each time point using Norgen's Blood RNA Purification Kit.
Figure 1A is showing the RNA yield from blood isolated and preserved for 12 days at room temperature.
Figure 1B is showing the high quality of the RNA isolated from blood preserved for 12 days at room temperature.

Features and Benefits

- **Isolate a diversity of RNA species -** All RNA species can be isolated including microRNA (miRNA)
- **Full gene expression profiling** - High stability of individual mRNA species.
- **Efficient Whole Blood RNA Preservation** - Preserve whole blood RNA for up to 12 days at 18–25°C or up to 14 days at 2–8°C
- **Fast and easy processing -** Rapid spin-column format allows for the processing of multiple samples in 45 minutes. No organics or phenol.
- **High Quantity and Quality -** No contamination or degradation of preserved blood RNA is observed.

Applications

- Quantitative, real-time RT-PCR for long non-coding RNA, mRNA and miRNA
- RT-PCR for both large mRNA and small RNA including miRNA
- Full expression profiling for mRNA and small regulatory RNA
- Next Generation Sequencing for RNA and miRNA
- microRNA cloning and amplification
- PCR-based virus detection

Feature	Specifications
Volume of Blood Collected	3 mL
Volume of Blood-Preservative Mix	9 mL
Preservation Time/ Temperatures	12 days at 18–25°C 14 days at 2–8°C
Maximum Spin Column Binding Capacity	50 µg
Maximum Spin Column Loading Capacity	650 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Time to Complete 10 Purifications	45 minutes
Average Yield	6 – 25 ug per 3 mL preserved human blood

Ordering information

Cat #	Quantity
52720	96 preps
52620	48 preps

Blood RNA Preservation Tubes

Blood RNA Preservative Tubes

Cat. # 52710 & 52610

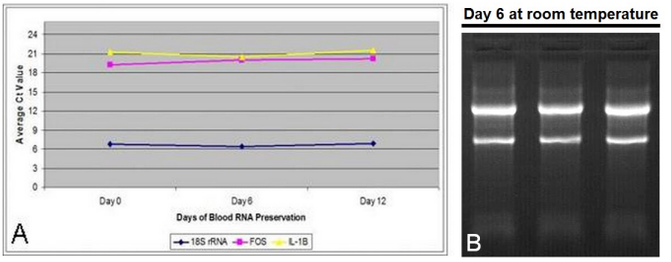


Figure 1. Stability of Blood RNA in Norgen's Blood RNA Preservative Tubes. A main component of Norgen's Blood RNA Preservation and Purification Kits are the Blood RNA Preservative Tubes. Blood samples were collected from the same donor in triplicate on Norgen's Blood RNA Preservative Tubes. The stability of the blood RNA in Norgen's Blood RNA Preservative Tubes was evaluated over a period of 12 days at room temperature. At each time point, the preserved blood RNA was isolated using Norgen's Blood RNA Preservation and Purification Kit. Three different genes, FOS, IL1B and 18S rRNA, were used to evaluate the stability of blood RNA over a period of 12 days at room temperature.
Figure 1A shows the efficiency of Norgen's Blood RNA Preservative for minimizing the induction of gene expression over a period of 12 days at Room temperature. Each of the 3 genes tested showed no changes in gene expression over the 12 day preservation period.
Figure 1B shows the quality of the Blood RNA preserved for 6 days at room temperature.
Most importantly that Norgen's Preserved Blood RNA Purification method allows for the isolation of all sizes of RNA, from the very large RNA down to the microRNA without the use of phenol.

Collect and preserve blood RNA at room temperature

Product Description

Norgen's Blood RNA Preservative Tubes are plastic evacuated tubes for whole blood collection and stabilization of blood RNA for up to 12 days at 18–25°C or up to 14 days at 2–8°C.. The tubes contain aqueous preservative that protects RNA molecules from degradation by RNases and minimizes induction of gene expression. The actual duration of RNA stabilization may vary depending upon the species of cellular RNA and the downstream application used.

Features and Benefits

- **Long-lasting evacuated tubes** - Collection under vacuum of 3 mL whole blood
- **Efficient Whole Blood RNA Preservation** - Preserve whole blood RNA for up to 12 days at 18–25°C or up to 14 days at 2 - 8°C
- Fully compatible with Norgen's Blood RNA Purification Kit (For Use with Norgen's Blood RNA Preservative Tubes)

Storage Conditions and Product Stability

The Blood RNA Preservative Tubes should be kept tightly sealed and stored upon arrival at 4°C - 8°C. The Blood RNA Preservative Tubes can be used until the end of the expiration month indicated on the tube label.

Applications

- Quantitative, real-time RT-PCR for long non-coding RNA, mRNA and miRNA
- RT-PCR for both large mRNA and small RNA including miRNA
- Full expression profiling for mRNA and small regulatory RNA
- Next Generation Sequencing for RNA and miRNA
- microRNA cloning and amplification
- PCR-based virus detection

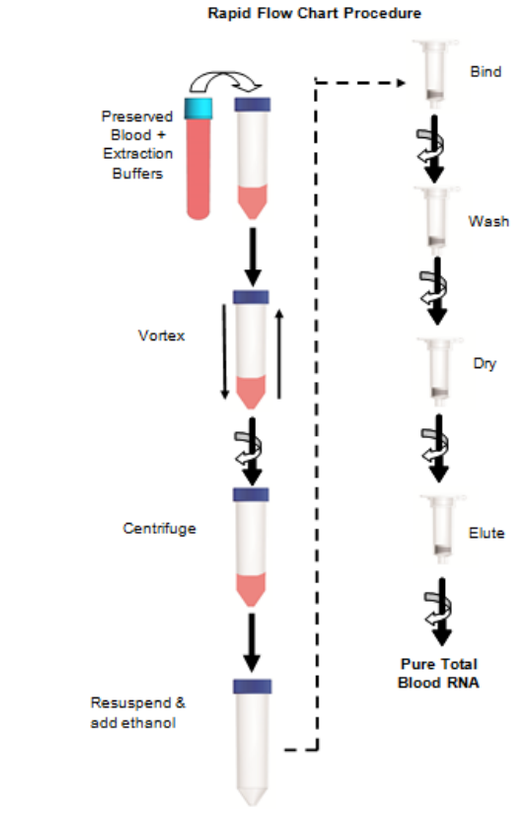
Feature	Specifications
Volume of Blood Collected	3 mL
Volume of Blood-Preservative Mix	9 mL
Preservation Time/ Temperatures	12 days at 18–25°C 14 days at 2–8°C

Ordering information

Cat #	Quantity
52710	96 preps
52610	48 preps

Preserved Blood RNA Purification Kit
(for use with Norgen Blood RNA Tubes)

Cat. # 52700 & 52600



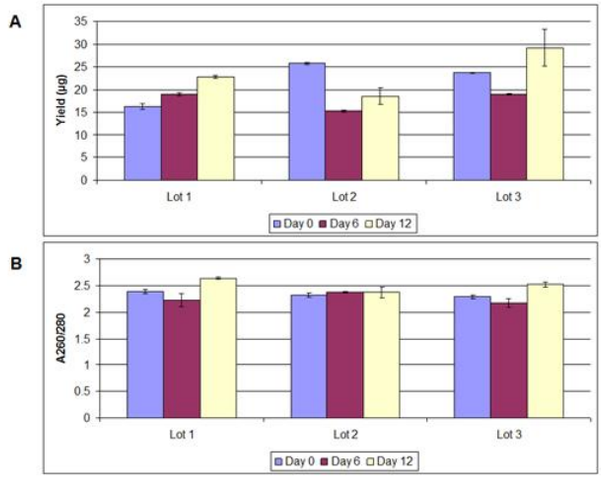


Figure 1. Qualitative and Quantitative Analysis of Blood RNA Preserved and Isolated using Norgen's Blood RNA Preservation and Purification Kit. Preserved blood RNA processed using three different lots of Norgen's Blood RNA Preservation and Purification Kit were analyzed qualitatively and quantitatively. Blood was collected from the same donor on Norgen's Blood RNA Preservative Tubes. Blood RNA preservation was evaluated for the three different lots over a period of 12 days at room temperature. Preserved blood RNA was isolated from each sample at each time point using Norgen's Blood RNA Purification Kit.
Figure 1A is showing the RNA yield from blood isolated and preserved for 12 days at room temperature.
Figure 1B is showing the high quality of the RNA isolated from blood preserved for 12 days at room temperature.

Rapid isolation and purification of total RNA from blood that has been preserved using Norgen Blood RNA Preservative Tubes

Product Description

Our Preserved Blood RNA Purification Kit (for use with Norgen Blood RNA Tubes) provides a rapid method for the isolation and purification of total RNA from 3 mL of human whole blood collected in Norgen's Blood RNA Preservative Tubes. The kit purifies all sizes of RNA including microRNA (miRNA).

Features and Benefits

- **Isolate a diversity of RNA species -** All RNA species can be isolated including microRNA (miRNA)
- **Full gene expression profiling** - High stability of individual mRNA species.
- **Efficient Whole Blood RNA Preservation** - Preserve whole blood RNA for up to 12 days at 18–25°C or up to 14 days at 2–8°C
- **Fast and easy processing -** Rapid spin-column format allows for the processing of multiple samples in 45 minutes. No organics or phenol.
- **High Quantity and Quality -** No contamination or degradation of preserved blood RNA is observed.

Applications

- Quantitative, real-time RT-PCR for long non-coding RNA, mRNA and miRNA
- RT-PCR for both large mRNA and small RNA including miRNA
- Full expression profiling for mRNA and small regulatory RNA
- Next Generation Sequencing for RNA and miRNA
- microRNA cloning and amplification
- PCR-based virus detection

Feature	Specifications
Volume of Blood Collected	3 mL
Volume of Blood-Preservative Mix	9 mL
Preservation Time/ Temperatures	12 days at 18–25°C 14 days at 2–8°C
Maximum Spin Column Binding Capacity	50 µg
Maximum Spin Column Loading Capacity	650 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Time to Complete 10 Purifications	45 minutes
Average Yield	6 – 25 ug per 3 mL preserved human blood

Ordering information

Cat #	Quantity
52700	96 preps
52600	48 preps

RNA Isolation Kits - For Preserved Blood RNA (Norgen, Tempus™ and PAXgene™)

Preserved Blood RNA Purification Kit I

(for use with Tempus™ Blood RNA Tubes)

Cat. # 43400

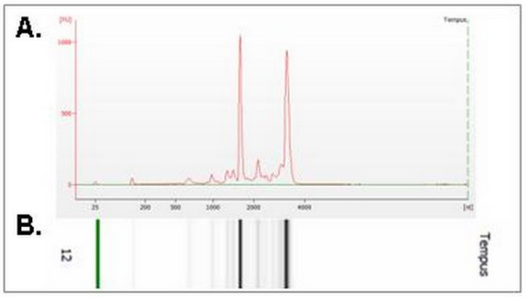


Figure 1. Isolation of High Quality Total RNA. Total RNA was isolated in duplicate from 3 mL hamster blood samples collected into Tempus™ RNA Blood Tubes using Norgen's Preserved Blood RNA Purification Kit I and was subsequently analyzed using an Agilent BioAnalyzer. Panel A shows the electropherogram from the Agilent Bioanalyzer, where the y axis represents fluorescence units and the x axis represents the runtime (s). The bands of the 18S and 28S rRNA fragments are clearly visible and the RNA integrity number score is 9.0. Panel B demonstrates the gel banding pattern of the RNA species purified from hamster blood. Abundant blood transcripts such as the globin mRNAs constitute the additional bands to the two major rRNA bands.

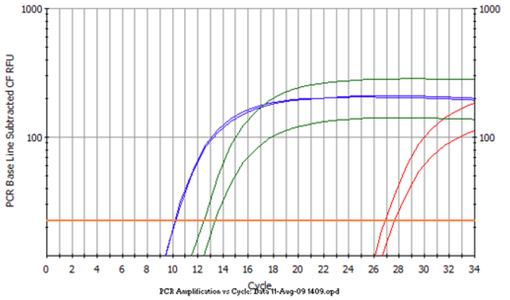


Figure 2. Isolate a Diversity of RNA Species, from large mRNA to microRNA. Total RNA was isolated in duplicate from 3 mL hamster blood samples collected into Tempus™ RNA Blood Tubes using Norgen's Preserved Blood RNA Purification Kit I and was subsequently used as the template in a RT-qPCR reaction for the detection of various sizes RNA. All sizes of RNA were successfully amplified including large RNA (Beta-Actin = green), small RNA, such as the 5S rRNA (blue) and microRNA, miR-21 (red), indicating both the size diversity of the purified RNA, as well as the high quality of the RNA. No template control is shown in yellow.

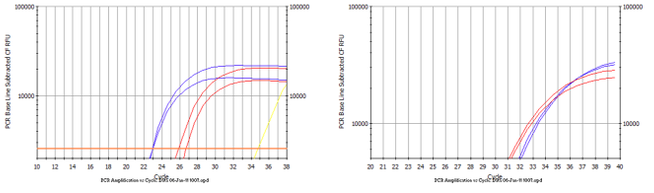


Figure 3. High Yield of a Diversity of RNA Species. Norgen's Preserved Blood RNA Purification Kit I (for use with Tempus™ Blood RNA Tubes) effectively recovers all sizes of RNA from large mRNA to small RNA, including microRNAs. Total RNA was isolated from equal amounts of goat blood collected in Tempus™ Blood RNA Tubes followed by RNA isolation using Norgen's Preserved Blood RNA Purification Kit I and a competitor's kit. The purified RNA was then used as the template in a RT-qPCR for detecting the beta-Actin gene (Left Panel) and for detecting miR-21 (Right Panel). In both graphs the blue lines correspond to Norgen isolated-RNA and the red lines correspond to competitor-isolated RNA. As it can be seen, Norgen's kit isolated higher yields of microRNA, as indicated by the lower Ct values of the blue lines (Right Panel). Also, Norgen's kit successfully isolated similar amount of large RNA to that of the competitor's kit (Lower Panel) indicating the full diversity of RNA species isolated.

Rapid isolation and purification of total RNA from blood that has been preserved using Tempus Blood RNA Tubes

Product Description

Norgen's Preserved Blood RNA Purification Kit I provides a rapid method for the isolation and purification of total RNA from blood that has been preserved using Tempus™ Blood RNA Tubes. The kit purifies all sizes of RNA, including microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other cellular components such as proteins, without the use of phenol or chloroform.

Norgen's proprietary resin provides superior affinity to the full size range of RNA molecules, resulting in large and small RNA (miRNA) purification with better linearity and sensitivity. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

Features and Benefits

- **No phenol:chloroform extractions** - Total RNA is isolated without the use of harmful chemicals such as phenol or chloroform
- **Isolate a diversity of RNA species** - All RNA species can be isolated, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA)
- **High quality total RNA -** Isolate inhibitor-free RNA that can be used direclty in sensitive downstream applications including RT-qPCR
- **Fast and easy processing** - Rapid spin-column format allows for the processing of 10 samples in 30 minutes

Applications

- Quantitative, real-time RT-PCR for long non-coding RNA, mRNA and miRNA
- RT-PCR for both large mRNA and small RNA including miRNA
- Full expression profiling for mRNA and small regulatory RNA
- Next Generation Sequencing for RNA and miRNA
- microRNA cloning and amplification
- PCR-based virus detection

Feature	Specifications
Maximum Column Binding Capacity	50 µg
Maximum Column Loading Volume	650 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Time to Complete 10 Purifications	30 minutes
Average Yield	5 – 25 µg per 3 mL preserved human blood

Ordering information

Cat #	Quantity
43400	50 preps

Preserved Blood RNA Purification Kit II

(for use with PAXgene™ Blood RNA Tubes) **Cat. # 43500**

Figure 1. High Quality of Isolated RNA with Complete Size Range. Unlike most competitor's kits, Norgen's Preserved Blood RNA Purification Kit II (for use with Paxgene™ Blood RNA Tubes) allows for the isolation of all sizes of RNA, from the very large RNA down to the microRNA without the use of phenol. Total RNA was isolated from preserved blood using Norgen's Preserved Blood RNA Purification Kit II (for use with PAXgene™ Blood RNA Tubes) and a leading competitors kit. The purified RNA was then resolved on a 1.2% formaldehyde-agarose gel. As it can be seen, only Norgen's kit was able to isolate the full diversity of RNA species, including all the small RNA species.

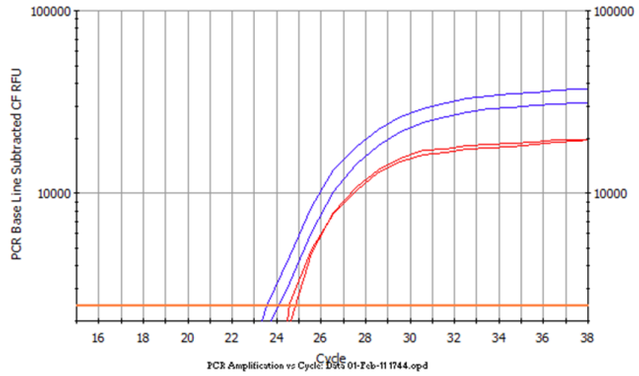


Figure 2. High Yield of a Diversity of RNA Species. Norgen's Preserved Blood RNA Purification Kit II (for use with PAXgene™ Blood RNA Tubes) effectively recovers all sizes of RNA from large mRNA to small RNA, including microRNAs. Total RNA was isolated from human blood collected in a PAXgene™ Blood RNA Tube followed by RNA isolation using Norgen's Preserved Blood RNA Purification Kit. II The purified RNA was then used as the template in a RT-qPCR for detecting the S15 gene (blue lines, in duplicate) and for detecting miR-21 (red lines, in duplicate). As it can be seen, Norgen's kit isolated complete range of RNA including high yields of mRNA and microRNA.

Rapid isolation and purification of total RNA from blood that has been preserved using PAXgene Blood RNA Tubes

Product Description

Norgen's Preserved Blood RNA Purification Kit II provides a rapid method for the isolation and purification of total RNA from blood that has been preserved using PAXgene™ Blood RNA Tubes. The kit purifies all sizes of RNA, including microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other cellular components such as proteins, without the use of phenol or chloroform.

Norgen's proprietary resin provides superior affinity to the full size range of RNA molecules, resulting in large and small RNA (miRNA) purification with better linearity and sensitivity. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

Features and Benefits

- **No phenol:chloroform extractions** - Total RNA is isolated without the use of harmful chemicals such as phenol or chloroform
- **Isolate a diversity of RNA species** - All RNA species can be isolated, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA)
- **High quality total RNA -** Isolate inhibitor-free RNA that can be used directly in sensitive downstream applications including RT-qPCR
- **Fast and easy processing** - Rapid spin-column format allows for the processing of 10 samples in 30 minutes

Applications

- Quantitative, real-time RT-PCR for long non-coding RNA, mRNA and miRNA
- RT-PCR for both large mRNA and small RNA including miRNA
- Full expression profiling for mRNA and small regulatory RNA
- Next Generation Sequencing for RNA and miRNA
- microRNA cloning and amplification
- PCR-based virus detection

Feature	Specifications
Maximum Column Binding Capacity	50 µg
Maximum Column Loading Volume	650 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Time to Complete 10 Purifications	30 minutes
Average Yield	5 – 25 µg per 3 mL preserved human blood

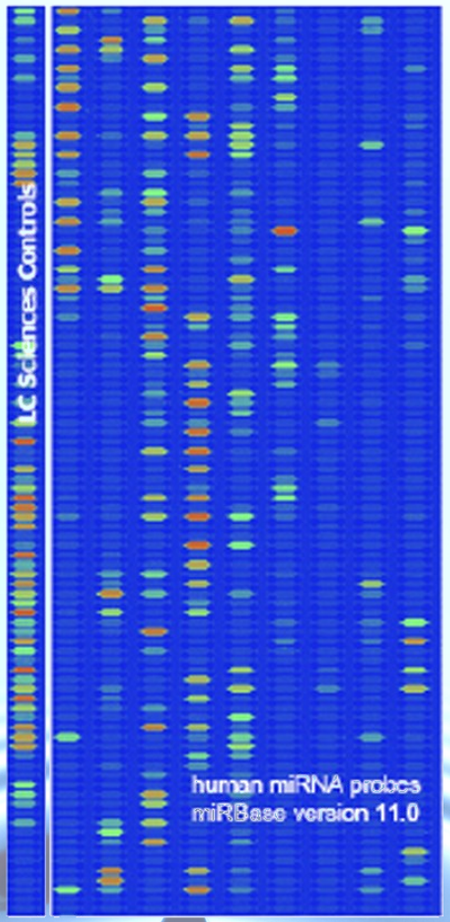
Ordering information

Cat #	Quantity
43500	50 preps

A proportion of samples isolated by phenol-based methods are inhibited during enzymatic downstream analysis such as PCR, arrays and NGS

TRY THE NON-PHENOL BASED METHOD

Non-phenol based method
Norgen



Phenol based method
Competitor

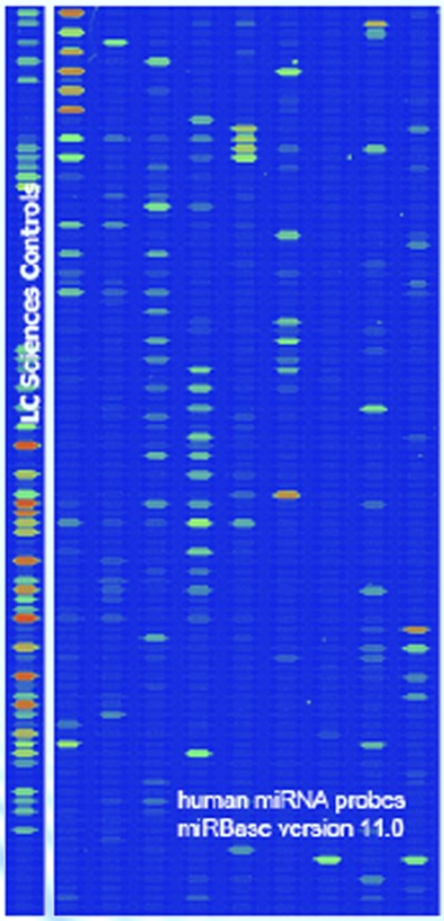


Image courtesy of LC Sciences

Better Diversity of miRNA Detected from Plasma. Norgen's Total RNA Purification Kit isolates miRNA from plasma with better diversity than a leading competitor. Total RNA including miRNA was isolated from 100 µL of plasma using Norgen's Total RNA Purification Kit or 625 µL of plasma using Competitor A's leading miRNA Kit, and was applied to an NCode expression profiling kit. Microarray images suggested that Norgen's Total RNA Purification Kit (left) isolates a better diversity of miRNA from a smaller input amount of plasma than the competitor's miRNA kit (right). Image courtesy of LC Sciences, Houston. (www.lcsciences.com).

BEST-IN-CLASS
PURE & SIMPLE

NORGEN BIOTEK CORP.

www.norgenbiotek.com

B. Blood Sample Preparation Kits

Isolation of high quality DNA or RNA from blood using Norgen's nucleic acid isolation technologies. DNA can be isolated from fresh or frozen blood using a single column-based preparation (different sizes: Micro, Mini, Midi, Maxi or dried blood spots), alcohol precipitation (0.3 mL to 10 mL) or 96-well plate for high throughput isolations. Blood RNA can be isolated from whole blood or leukocytes using a single column-based preparation. All nucleic acids isolated by Norgen's technology are of the highest quality and are suitable for sensitive downstream applications.

Sample Preparation Selection Table:

Kit	Cat. #	Kit size	Sample size	Yield
Single Preparation - DNA Isolation Kits				
Blood Genomic DNA Isolation Micro Kit	52100	50 preps	1-100 µL	2-6 µg
Blood Genomic DNA Isolation Mini Kit	46300, Dx46300*	50 preps	20-200 µL	4-12 µg
Blood Genomic DNA Isolation Midi Kit	51400	20 preps	300 µL – 2 mL	20-60 µg
Blood Genomic DNA Isolation Maxi Kit	31200	12 preps	3-10 mL	200-600 µg
Dried Blood Spot (DBS) Genomic DNA Isolation Kit	36000	50 preps	3 x 3mm punches	50-150 ng
Alcohol Precipitation - DNA Purification Kits				
Blood DNA Purification Kit - 30 mL	52500	100 preps (0.3 mL)	0.3-10 mL	~24 µg
Single Preparation - RNA Isolation Kits				
Total RNA Purification Kit Specific protocol for blood samples	17200, 37500, Dx17200*	50, 100 preps	Up to 200 µL	1-5 µg
Leukocyte RNA Purification Kit	21200	50 preps	10 µL – 2 mL	Up to 50 µg
High Throughput DNA Preparation				
Blood Genomic DNA Isolation 96-Well Kit	50500	2 x 96-well plates	20-200 µL	4-12 µg
Blood DNA Preservation				
Blood DNA Preservation Buffer (3X)	29111, 29112	25, 100 mL	Variable	Variable

*CE-certified kit for in-vitro diagnostic purposes - not available in all regions - very similar protocol to non-Dx kit.

Single Preparation - DNA Isolation Kits

Blood Genomic DNA Isolation Micro Kit — Cat. # 52100

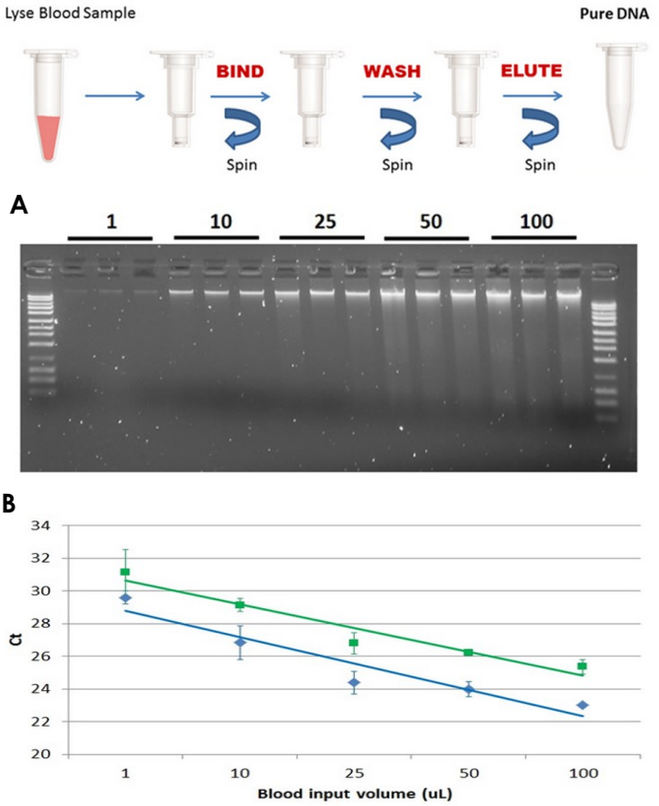


Figure 1. High Yields and amplification efficiency of Genomic DNA Isolated from 1 µL to 100 µL of Whole Blood. Genomic DNA was isolated from 1, 10, 25, 50 and 100 µL of whole blood using Norgen's Blood Genomic DNA Isolation Micro Kit. **(A)** 15 µL from each 100 µL elution was loaded on 1% TAE agarose gel. The kit demonstrated a good DNA yield and integrity. The used ladder is Norgen's UltraRanger 1kb DNA Ladder. **(B)** Five microliters of the DNA from each 100 µL of elution was used in a real-time PCR reaction (total reaction volume of 20 µL) with GAPDH TaqMan probe and primers. The real-time PCR was successful in amplifying the GAPDH gene from both the first elution (blue) and the second elution (green), with a linear decrease in Ct value with the increase in blood input volume, indicating that the DNA is of a high quality and can be used in sensitive downstream applications.

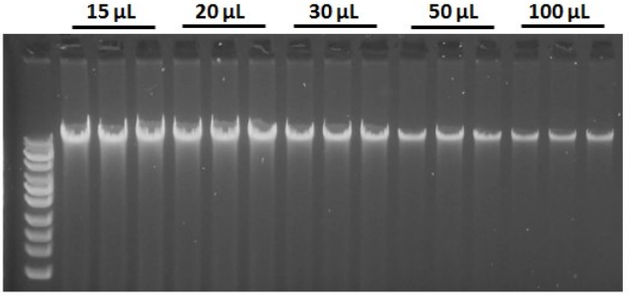


Figure 2. High Concentration of Genomic DNA Eluted in Different Elution Volumes. Genomic DNA was isolated from 100 µL of whole blood and eluted in 20, 30, 50 and 100 µL of Elution Buffer using Norgen's Blood Genomic DNA Isolation Micro Kit. Following isolation, 5 µL from each elution was loaded on 1% TAE agarose gel. Norgen's Blood Genomic DNA Isolation Micro Kit demonstrated a good DNA yield and integrity with increased concentration when using lower elution volumes (20 and 30 µL) compared to the larger elution volumes (50 and 100 µL). Lane M: Norgen's HighRanger 1kb DNA Ladder.

Rapid preparation of genomic DNA from 1 µL to 100 µL of whole blood.

The Blood Genomic DNA Isolation Micro Kit allows for the isolation of genomic DNA from the blood of various species, including humans. Purification is based on spin column technology without the use of organic solvents. Typical yields of genomic DNA will vary depending on the cell density of the blood sample. Preparation time for a single sample is less than 30 minutes, and each kit contains sufficient materials for 50 preparations. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with sensitive downstream applications.

Features and Benefits

- **Isolate DNA from small volumes of blood -** Isolate DNA from inputs of 1µL to 100 µL of blood
- **Isolate DNA from blood pathogens -** Isolate DNA from viral and bacterial blood pathogens
- **No Phenol-Chloroform extraction or alcohol precipitation** - Isolate genomic DNA without the use of harmful chemicals
- **Fast and easy processing** - Rapid spin-column format allows for the processing of multiple samples in 30 minutes.
- **Recovered genomic DNA is suitable for downstream applications** - Purified genomic DNA is fully compatible with restriction enzyme digestions, Southern Blot, PCR analysis, sequencing and microarray analysis.
- **High quality DNA with no RNA contamination** - No contamination or degradation of genomic DNA is observed.

Applications

- Quantitative PCR
- Genotyping
- SNP analysis
- Microarray analysis
- Next Generation Sequencing
- PCR-based pathogen detection

Feature	Specifications
Maximum Blood Input	100 µL
Column Binding Capacity	> 25 µg
Average Yield (100 µL of blood)	2-6 µg*
Elution Volume	20-100 µL
Analyte Purified	Genomic DNA, mitochondrial DNA, viral DNA
Format	Spin column
Time to Complete 10 Purifications	30 minutes

*Yield will vary depending on the type of blood processed

Ordering information

Cat #	Quantity
52100	50 preps

Single Preparation - DNA Isolation Kits

Blood Genomic DNA Isolation Mini Kit — Cat. # 46300

Blood Genomic DNA Isolation Mini Kit Dx — Cat. # Dx46300

CE

CE-certified kit for in-vitro diagnostic purposes - not available in all regions

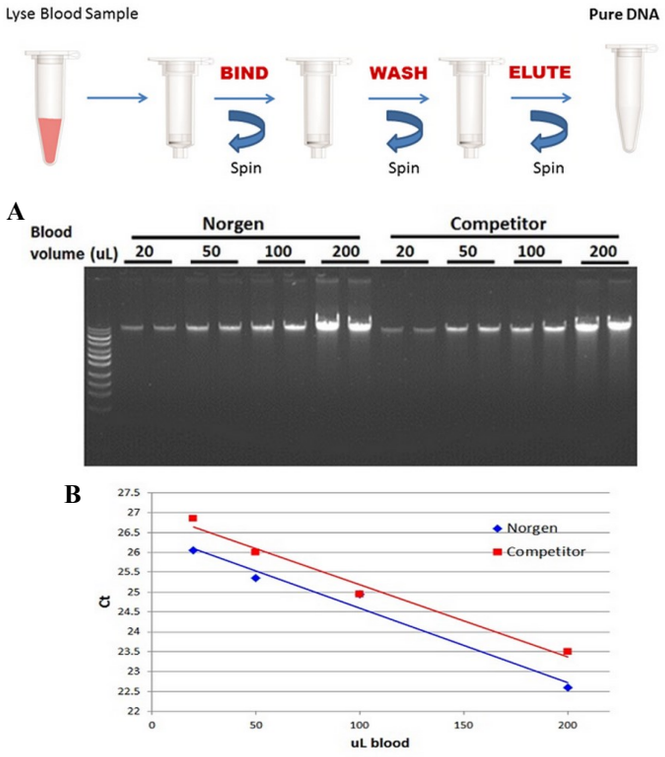


Figure 1. High Yields and amplification efficiency of Genomic DNA Isolated from 20 µL to 200 µL of Whole Blood. Genomic DNA was isolated from 20, 50, 100 and 200 µL of whole blood using Norgen's Blood Genomic DNA Isolation Mini Kit and a leading competitor's kit. **(A)** 15 µL from each 200 µL elution was loaded on 1% TAE agarose gel. Norgen's Blood Genomic DNA Isolation Mini Kit demonstrated a better DNA yield than the leading competitor's kit. The used ladder is Norgen's UltraRanger 1kb DNA Ladder. **(B)** Nine µL of the DNA from each 200 µL of elution was used in a real-time PCR reaction (total reaction volume of 20 µL) with GAPDH TaqMan probe and primers. The real-time PCR was successful in amplifying the GAPDH gene, with a linear decrease in Ct value with the increase in blood input volume. Furthermore, Norgen-isolated DNA was amplified with a lower Ct value from all DNA isolated from the different blood input volumes, indicating the higher yield and purity of DNA isolated using Norgen's kit.

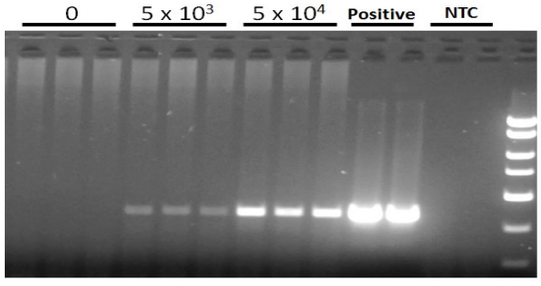


Figure 2. Detection of *Listeria monocytogenes* in DNA isolated with Norgen's Genomic DNA Isolation Mini Kit. DNA was isolated from blood spiked with 5 x 10^3 and 5 x 10^4 *L. Monocytogenes* cells. One microliter of the DNA from each 200 µL of elution was used in a real-time PCR reaction (total reaction volume of 20 µL) with *L. monocytogenes* specific primer set (Norgen Biotek). The real-time PCR was successful in detecting the pathogen from the two spiked amounts. NTC is the no template control and the used ladder is Norgen's FastRunner DNA Ladder.

Rapid preparation of genomic DNA from up to 200 µL of whole blood.

The Blood Genomic DNA Isolation Mini Kit allows for the isolation of genomic DNA from the blood of various species, including humans. Purification is based on spin column technology without the use of organic solvents. Typical yields of genomic DNA will vary depending on the cell density of the blood sample. Preparation time for a single sample is less than 30 minutes, and each kit contains sufficient materials for 50 preparations. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications.

Features and Benefits

- **Isolate DNA from small volumes of blood -** Isolate DNA from inputs of up to 200 µL of blood
- **Isolate DNA from blood pathogens -** Isolate DNA from viral and bacterial blood pathogens
- **No Phenol-Chloroform extraction or alcohol precipitation** - Isolate genomic DNA without the use of harmful chemicals
- **Fast and easy processing** - Rapid spin-column format allows for the processing of multiple samples in 30 minutes.
- **Recovered genomic DNA is suitable for downstream applications** - Purified genomic DNA is fully compatible with restriction enzyme digestions, Southern Blot, PCR analysis, sequencing and microarray analysis.
- **High quality DNA with no RNA contamination** - No contamination or degradation of genomic DNA is observed.

Applications

- Quantitative PCR
- Genotyping
- SNP analysis
- Microarray analysis
- Next Generation Sequencing
- PCR-based pathogen detection

Feature	Specifications
Maximum Blood Input	200 µL
Column Binding Capacity	> 50 µg
Average Yield (200 µL of blood)	4-12 µg*
Elution Volume	50-200 µL
Analyte Purified	Genomic DNA, mitochondrial DNA, viral DNA
Format	Spin column
Time to Complete 10 Purifications	30 minutes

*Yield will vary depending on the type of blood processed

Ordering information

Cat #	Quantity
46300	50 preps
Dx46300	50 preps

Application Note

Blood Genomic DNA Isolation Mini Kit **Cat. # 46300**

Genomic DNA Isolation from blood collected on different anticoagulants: Citrate, EDTA & Heparin

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[3]Centre for Biotechnology. Brock University, St. Catharines, Ontario, Canada

INTRODUCTION

Blood is an excellent sample for diagnostic purposes. It is rich in protein and nucleic acid-based biomarkers that can be used to detect a panel of diseases long before any physical symptoms become apparent. Studies have shown that cancer biomarkers, such as differentially methylated target genes, are detectable in blood samples and have proven to be extremely sensitive and specific for given cancer types[1]. These biomarkers can be cell associated, such as those found in leukocytes[2], or cell-free, such as those found in plasma or serum samples. For this reason, blood is often the sample of choice for biomarker or diagnostic research.

Investigators utilizing blood in their research have unique needs based on their downstream applications. Blood can be collected on different anticoagulants including citrate, EDTA or heparin. The type of anticoagulant used can affect the results from blood DNA isolation kits, and ultimately influence the results of research-based or diagnostic tests associated with blood. Investigators must ensure that their blood DNA isolation method is flexible, i.e. it can work efficiently on isolating blood from the specific anticoagulant used in blood collection. A robust blood DNA isolation kit eliminates sample processing biases, and increases data reproducibility.

The purpose of this study is to determine if Norgen's Blood Genomic DNA Isolation Mini Kit (Cat# 46300) can efficiently isolate blood genomic DNA from blood collected on different anticoagulants (Citrate, EDTA, Heparin).

MATERIALS AND METHODS

Sample Collection

Six milliliters of blood was collected in EDTA tubes, while 2.7 milliliters was collected in citrate tubes, and 3 milliliters was collected in heparin tubes from the same healthy individual, by a trained professional. The samples were frozen at -70°C until processed.

Blood DNA Extraction

DNA was extracted from the thawed blood sample using Norgen's Blood Genomic DNA Isolation Mini Kit (Cat# 46300), as per the manufacturer's instruction. Briefly, Proteinase K was added to a microcentrifuge tube, followed by 20µL of blood. Next, 300µL of Lysis Solution was then added and the samples were vortexed and incubated at 55°C for 10 minutes. Next, 250µL of ethanol was added to each sample, and samples were bound, washed and eluted as per the manufacturer's protocol.

Spectrophotometry

Blood DNA quantity was measured using the UltraSpec 2100 Pro (Fisher Scientific). Twenty five µL of each DNA elution was diluted with 475 µL of nuclease-free water, and OD measurements were taken using the cuvette-based spectrophotometry method.

Real-Time PCR

The purified DNA was then used as the template in a real-time TaqMan® PCR reaction. Briefly, 3 µL and 9 µL of isolated DNA was added to a real-time PCR reaction mixture containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007), 0.4 µL from a 25mM GAPDH primer pair mix and 0.2 µL of the TaqMan® probe. The volume was brought up to 20 µL using nuclease-free water. The PCR samples were amplified under the real-time program; 95°C for 3 minutes for an initial denaturation, 40 cycles of 95°C for 15 seconds for denaturation, 60°C for annealing and extension. The reaction was run on an iCycler iQ Realtime System (Bio-Rad).

RESULTS AND DISCUSSION

Blood is an excellent resource for research and diagnostic purposes. Systemic diseases can be detected through a simple blood test, and changes in DNA (both cellular and cell -free) can be detected from blood, giving rise to its high potential for screening for a panel of diseases.

The key to the success of any study utilizing blood is a reliable blood DNA isolation method that performs optimally from blood collected on any anticoagulant. One can determine the flexibility of a kit by performing DNA isolation on blood from the same donor but collected in tubes with different anticoagulants in order to see the kit robustness and consistency. In this study, DNA was isolated from blood collected from the same donor on three different anticoagulants: citrate, EDTA, and heparin, using Norgen's Blood Genomic DNA Isolation Mini Kit. Fifteen microliters of each 200 µL elution was then run on a 1X TAE 1.0% agarose gel to visually inspect the isolated gDNA (**Figure 1**). The three different anticoagulants demonstrated similar DNA yield and integrity when observed visually on the gel.

In order to determine the yield of DNA isolated from each blood sample collected on the different anticoagulants, samples were measured using spectrophotometric method **(Figure 2).** The highest DNA yield was obtained from blood collected on heparin, while the DNA yields from citrate and EDTA were very similar. The OD260/280 was similar between the three conditions and the OD260/230 is non-significantly higher in citrate than EDTA than heparin **(Figure 3).**

DNA quality was determined through the use of a TaqMan® Real-Time PCR method. In order to assess sample inhibition, 3 µL and 9 µL of each sample was used in the reaction. The Ct values were then graphed (**Figure 4)**. It was found that qPCR performance was optimal with EDTA with the 3uL input,

as seen by the lower Ct value. Similar Ct values were found for all with the 9uL input.

For all the blood samples processed, DNA isolated from all conditions consistently displayed a lower Ct value when increasing the DNA template amount from 3 µL to 9 µL.

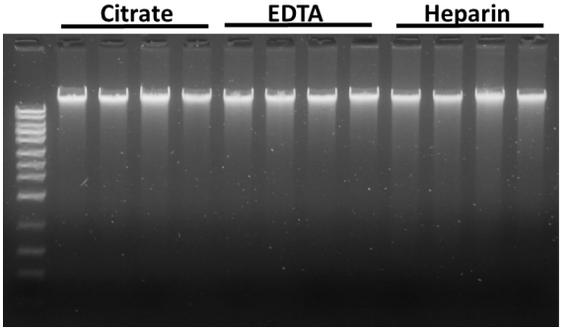


Figure 1. Resolution of DNA isolated from blood collected on citrate, EDTA and heparin tubes and processed using Norgen's Blood Genomic DNA Isolation Mini Kit. Fifteen microliters of each 200 µL elution was run on 1X TAE 1.0% agarose gel. Marker= Norgen's UltraRanger DNA Ladder.

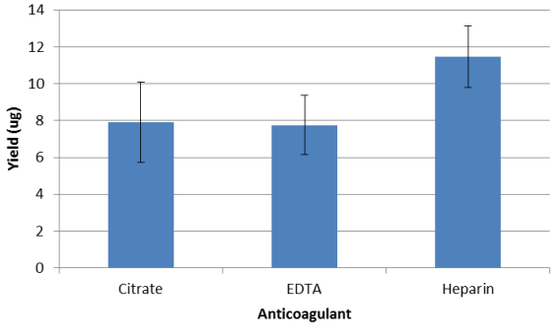


Figure 2. The difference in DNA yield from blood collected on citrate, EDTA, and heparin anticoagulants and processed using Norgen's Blood Genomic DNA Isolation Mini Kit. Twenty-five microliters of each sample was diluted in 475 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific). One elution was performed.

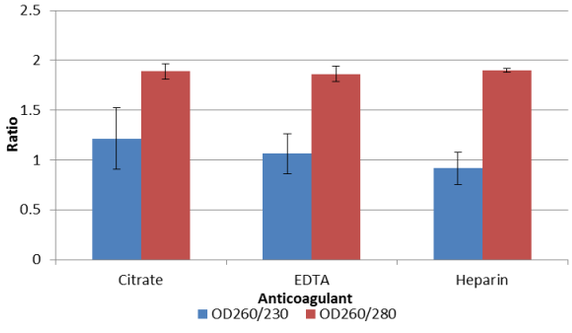


Figure 3. OD260/280 and OD260/230 ratios of DNA isolated from blood collected on citrate, EDTA, and heparin anticoagulants and processed using Norgen's Blood Genomic DNA Isolation Mini Kit. Twenty-five microliters of each sample was diluted in 475 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific). One elution was performed.

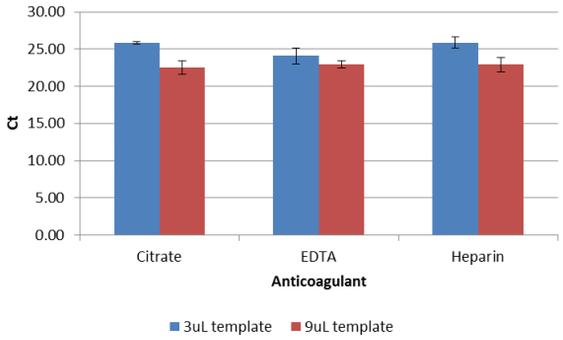


Figure 4. The difference in Ct values obtained from a Taqman® qPCR reaction performed on DNA isolated from blood collected on Citrate, EDTA, and Heparin anticoagulants. Three and nine microliters of each elution were used in a 20µL qPCR reaction involving GAPDH primers.

CONCLUSIONS

Norgen's Blood Genomic DNA Isolation Mini Kit can be used to isolate DNA from blood collected on citrate, EDTA or heparin, the 3 most commonly used anticoagulants. The highest yield was obtained from blood collected on heparin, and a similar DNA integrity, purity and qPCR performance was observed from the three anticoagulants.

REFERENCES

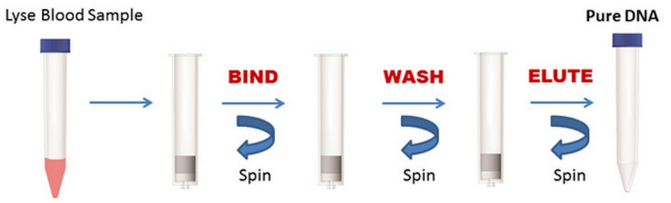
Warren JD, Xiong W, Bunker AM, Vaughn CP, Furtado LV, Roberts WL, et al. 2011. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. BMC Med; 9 (133).

Koestler DC, Marsit CJ, Christensen BC, Accomando W, Langevin SM, Houseman EA, et al. 2012. Peripheral blood immune cell methylation profiles are associated with nonhematopoietic cancers. Cancer Epidemiol Biomarkers Prev; 21(8):1293-302.

For an electronic version of this application note, please visit: http://norgenbiotek.com

Single Preparation - DNA Isolation Kits

Blood Genomic DNA Isolation Midi Kit — Cat. # 51400



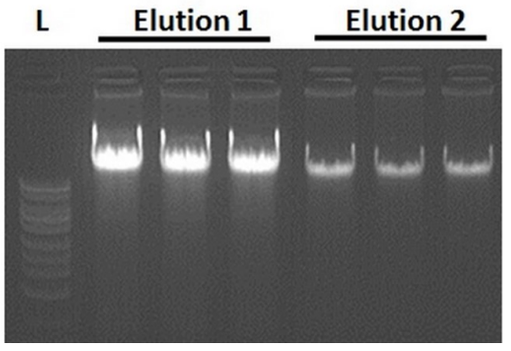


Figure 1. High Yields of Genomic DNA Isolated from 2 mL of Whole Blood. Genomic DNA was isolated from 2 mL of whole blood using Norgen's Blood Genomic DNA Isolation Midi Kit. Following isolation, 20 µL from 300 µL first and second elutions was loaded on 1% TAE agarose gel. Norgen's Blood Genomic DNA Isolation Kit demonstrated a high DNA yield and integrity. Lane L: Norgen's HighRanger 1kb DNA Ladder.

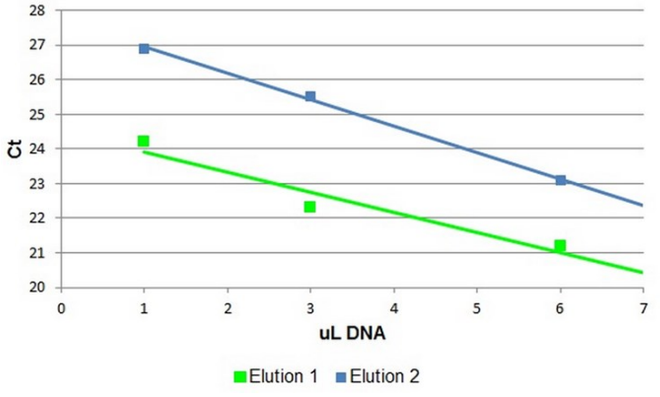


Figure 2. Purified DNA Can be Amplified in a Real-time PCR (TaqMan) Reaction. Genomic DNA was isolated from 2 mL of whole human blood using Norgen's Blood Genomic DNA Isolation Midi Kit. Different imput amounts (1, 3 & 6 µL) of the DNA from each of the 300 µL elutions was used in a real-time PCR reaction (total reaction volume of 20 µL) with GAPDH TaqMan probe and primers. The real-time PCR was successful in amplifying the GAPDH gene with a linear decrease in Ct value with the increased DNA template volume, indicating that the DNA is of a high quality and can be used in sensitive downstream applications.

Rapid preparation of genomic DNA from up to 0.3 to 2 mL of whole blood.

The Blood Genomic DNA Isolation Midi Kit allows for the isolation of genomic DNA from the blood of various species, including humans. Purification is based on spin column technology without the use of organic solvents. Typical yields of genomic DNA will vary depending on the cell density of the blood sample. Preparation time for a single sample is less than 45 minutes, and each kit contains sufficient materials for 20 preparations. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications.

Features and Benefits

- **No Phenol-Chloroform extraction or alcohol precipitation** - Isolate genomic DNA without the use of harmful chemicals
- **Isolate DNA from blood pathogens -** Isolate DNA from viral and bacterial blood pathogens
- **Fast and easy processing** - Rapid spin-column format allows for the processing of multiple samples in 45 minutes.
- **Recovered genomic DNA is suitable for downstream applications** - Purified genomic DNA is fully compatible with restriction enzyme digestions, Southern Blot, PCR analysis, sequencing and microarray analysis.
- **High quality DNA with no RNA contamination** - No contamination or degradation of genomic DNA is observed.

Applications

- Quantitative PCR
- Genotyping
- SNP analysis
- Microarray analysis
- Next Generation Sequencing
- PCR-based pathogen detection

Feature	Specifications
Maximum Blood Input	2 mL
Column Binding Capacity	> 100 µg
Average Yield	60 µg*
Elution Volume	500 µL
Analyte Purified	Genomic DNA, mitochondrial DNA, viral DNA
Format	Spin column
Time to Complete 10 Purifications	45 minutes

*Yield will vary depending on the type of blood processed

Ordering information

Cat #	Quantity
51400	20 preps

Blood Genomic DNA Isolation Maxi Kit — Cat. # 31200

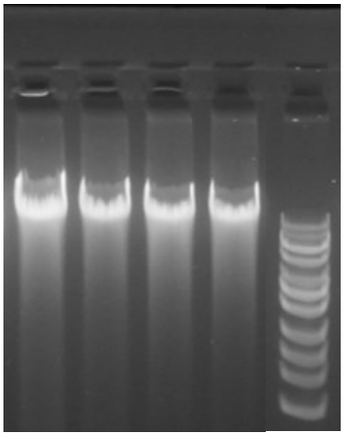


Figure 1. High Yields of Genomic DNA Isolated from Whole Blood. Genomic DNA was isolated from four different 10 mL whole blood samples using Norgen's Blood Genomic DNA Isolation Maxi Kit. Following isolation, 15 µL from each 2 mL elution was loaded on 1% TAE agarose gel. The used ladder is Norgen's UltraRanger 1kb DNA Ladder. Norgen's Blood Genomic DNA Isolation Maxi Kit demonstrated a high yield of intact genomic DNA.

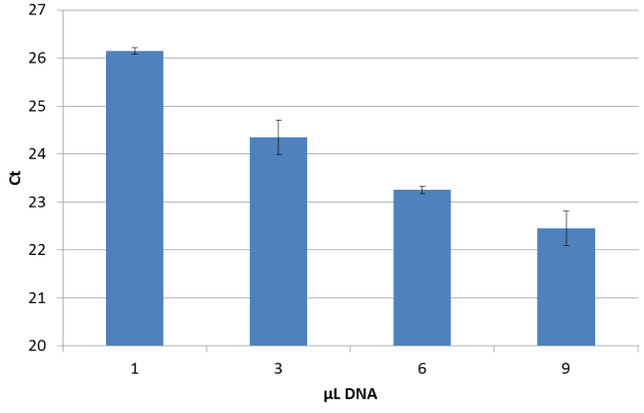


Figure 2. Purified DNA Can be Amplified in a Real-time PCR (TaqMan) Reaction at different template volumes. Genomic DNA was isolated from triplicate samples of 10 mL each of whole human blood using Norgen's Blood Genomic DNA Isolation Maxi Kit. Different DNA template volumes (1, 3, 6 & 9 µL) from each elution was used in a real-time PCR reaction (total reaction volume of 20 µL) with GAPDH TaqMan probe and primers. The real-time PCR was successful in amplifying the GAPDH gene, with a linear decrease in Ct value with the increase in DNA template volume. This indicates the DNA is of a high quality, free of PCR inhibitors and can be used in sensitive downstream applications.

Rapid preparation of genomic DNA from up to 3 to 10 mL of whole blood.

The Blood Genomic DNA Isolation Maxi Kit allows for the isolation of genomic DNA from the blood of various species, including humans. Purification is based on spin column technology without the use of organic solvents. Typical yields of genomic DNA will vary depending on the cell density of the blood sample. Preparation time for a single sample is less than 50 minutes, and each kit contains sufficient materials for 12 preparations. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications.

Features and Benefits

- **No Phenol-Chloroform extraction or alcohol precipitation** - Isolate genomic DNA without the use of harmful chemicals
- **Isolate DNA from blood pathogens -** Isolate DNA from viral and bacterial blood pathogens
- **Fast and easy processing** - Rapid spin-column format allows for the processing of multiple samples in 50 minutes.
- **Recovered genomic DNA is suitable for downstream applications** - Purified genomic DNA is fully compatible with restriction enzyme digestions, Southern Blot, PCR analysis, sequencing and microarray analysis.
- **High quality DNA with no RNA contamination** - No contamination or degradation of genomic DNA is observed.

Applications

- Quantitative PCR
- Genotyping
- SNP analysis
- Microarray analysis
- Next Generation Sequencing
- PCR-based pathogen detection

Feature	Specifications
Maximum Blood Input	10 mL
Column Binding Capacity	> 500 µg
Average Yield	200-600 µg*
Elution Volume	1-2 mL
Analyte Purified	Genomic DNA, mitochondrial DNA, viral DNA
Format	Spin column
Time to Complete 10 Purifications	50-70 minutes

*Yield will vary depending on the type of blood processed

Ordering information

Cat #	Quantity
31200	12 preps

Single Preparation - DNA Isolation Kits

Dried Blood Spot (DBS) Genomic DNA Isolation Kit Cat. # 36000

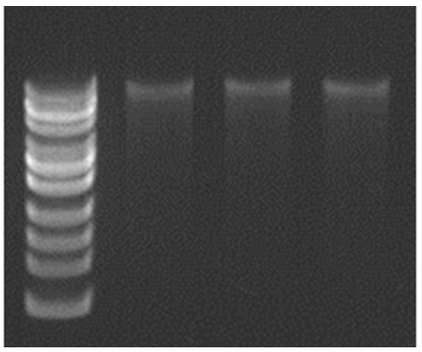


Figure 1. Genomic DNA Isolated from 3 x 3mm Diameter Circles. Blood collected on EDTA was applied to Whatman's 903 Protein Saver Card and allowed to dry for 1 week. DNA was isolated from 3 x 3 mm diameter circles per sample using Norgen's Dried Blood spot Genomic DNA Isolation Kit. Following isolation, 15 µL from each 150 µL elution was loaded on 1% TAE agarose gel. Norgen's Blood Genomic DNA Isolation Kit demonstrated a good DNA yield and integrity. The ladder corresonds to Norgen's UltraRanger 1kb DNA Ladder.

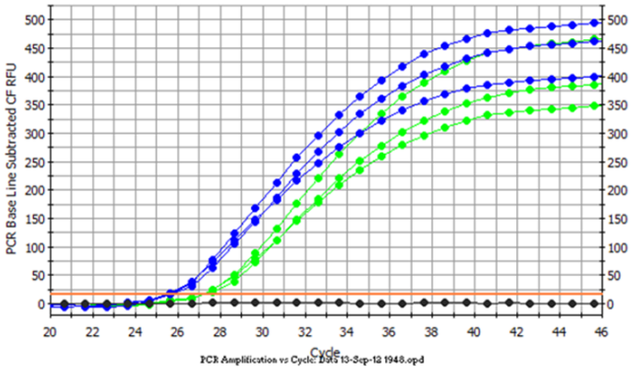


Figure 2. Purified DNA can be Amplified in a Real-time PCR (TaqMan) Reaction. Genomic DNA was isolated from 3 x 3mm diameter circles per sample using Norgen's Dried Blood Spot Genomic DNA Isolation Kit. Next, 3 µL (green line) & 9 µL (blue line) of the DNA from each of the 150 µL elutions was used in a real-time PCR reaction (total reaction volume of 20 µL) with GAPDH TaqMan probe and primers. The real-time PCR was successful in amplifying the GAPDH gene, indicating that the DNA is of a high quality and can be used in sensitive downstream applications. The black line is a no-template control.

Rapid preparation of genomic DNA from dried blood spots

The Dried Blood Spot (DBS) Genomic DNA Isolation Kit allows for the isolation of genomic DNA from the blood of various species, including humans. Purification is based on spin column technology without the use of organic solvents. The blood should be spotted and dried on suitable filter paper or specimen collection cards. Typical yields of genomic DNA will vary depending on the cell density of the blood sample. Preparation time for a single sample is 35 minutes. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications including Southern Blot analysis.

Features and Benefits

- **Isolate DNA from small volumes of blood -** Isolate DNA from dried blood spots, blood smears and blood spotted on most materials
- **No Phenol-Chloroform extraction or alcohol precipitation** - Isolate genomic DNA without the use of harmful chemicals
- **Fast and easy processing** - Rapid spin-column format allows for the processing of multiple samples in 35 minutes.
- **Recovered genomic DNA is suitable for downstream applications** - Purified genomic DNA is fully compatible with Southern Blot and PCR analysis.
- **High quality DNA with no RNA contamination** - No contamination or degradation of genomic DNA is observed.

Applications

- Quantitative PCR
- Genotyping
- SNP analysis
- PCR-based pathogen detection

Feature	Specifications
Input	3 x 3 mm diameter punches
Column Binding Capacity	> 25 µg
Average Yield	150 ng*
Elution Volume	20-100 µL
Analyte Purified	Genomic DNA, mitochondrial DNA, viral DNA
Format	Spin column
Time to Complete 10 Purifications	35 minutes

*Yield will vary depending on the type of blood processed

Ordering information

Cat #	Quantity
36000	50 preps

Application Note

Dried Blood Spot (DBS) Genomic DNA Isolation Kit **Cat. # 36000**

Isolation of Genomic DNA from Dried Blood Collected on Different Materials: Blood Cards, Cloth and Solid Surface using Norgen's Dried Blood Spot Genomic DNA Isolation Kit

L. Graziano[1], M. El-Mogy, PhD[2], Y. Haj-Ahmad, Ph.D[2,3]
[1]Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada.
[2]Norgen Biotek Corporation, Thorold, Ontario, Canada
[3]Centre for Biotechnology. Brock University, St. Catharines, Ontario, Canada

INTRODUCTION

Blood is an excellent sample for diagnostic purposes. It is rich in protein and nucleic acid-based biomarkers that can be used to detect a panel of diseases long before any physical symptoms become apparent. Studies have shown that cancer biomarkers, such as differentially methylated target genes, are detectable in blood samples and have proven to be extremely sensitive and specific for given cancer types[1]. These biomarkers can be cell associated, such as those found in leukocytes[2], or cell-free, such as those found in plasma or serum samples. For this reason, blood is often the sample of choice for biomarker or diagnostic research.

Blood DNA is used in a variety of lab procedures, from diagnostics to forensic applications. Dried blood spots can be found on almost any material, and investigators have unique needs based on the type of material that the dried blood spot has been recovered on. The type of material can affect the results of blood DNA isolation kits, ultimately influencing the results of research-based, diagnostic and identification tests associated with blood. Another factor that must be considered is the age of the dried blood. The amount of time that blood has been on a material can affect the quality and quantity of the DNA it contains. Investigators must ensure that their blood DNA isolation method is flexible, i.e. it can work efficiently on isolating blood from a range of materials.

The purpose of this study is to isolate blood genomic DNA from dried blood collected on blood cards, cloth and solid surfaces using Norgen's Dried Blood Spot Genomic DNA Isolation Kit (Cat# 36000) after one day and one week post-collection.

MATERIALS AND METHODS

Sample collection

Fresh blood was collected from 3 donors on blood cards, cloth, and as drops on a solid surface (petri dishes). The blood was then allowed to dry at room temperature.

Blood DNA extraction

DNA was extracted from the dried blood spot samples using Norgen's Dried Blood Spot Genomic DNA Isolation Kit, as per the manufacturer's instruction. Blood cards as well as cloth were excised as punches of 3x3mm diameter and placed into clean tubes, then 100 µL of digestion buffer was added to the tubes and vortexed for 10 seconds. Blood clots on the solid surface were collected by adding 50 µL of the kit's digestion buffer on the blood spot to dissolve then the liquid was collected by a swab and the swab tip with the liquid was placed into a clean tube containing another 50 µL of the digestion buffer, then the tube was vortexed for 10 seconds. All tubes were then incubated at 85°C for 10 minutes and spun briefly in a centrifuge. Next, Proteinase K was added to the microcentrifuge tube, plus 300 µL of the Lysis Solution was added, and samples were vortexed and incubated at 55°C for 10 minutes. Next, 250 µL ethanol was added to each sample, and samples were bound, washed and eluted as per the manufacturer's protocol, with elution of the DNA in a 50 µL volume.

Real-Time PCR

The purified DNA was then used as the template in a real-time TaqMan® PCR reaction. Briefly, 2 µL of isolated DNA was added to 20 µL of real-time PCR reaction mixture containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007), 0.4 µL of a 25 µM GAPDH primer pair mix and 0.2 µL of the TaqMan® probe. The volume was brought up to 20 µL using nuclease-free water. The PCR samples were amplified under the real-time program; 95°C for 3 minutes for an initial denaturation, 40 cycles of 95°C for 15 seconds for denaturation, 60°C for annealing and extension. The reaction was run on an iCycler iQ Realtime System (Bio-Rad).

RESULTS AND DISCUSSION

Blood is an excellent resource for diagnostic procedures, forensic applications and identification purposes. It is beneficial for studies utilizing dried blood spots to use a blood DNA isolation method that performs optimally for blood found on any material, as the samples available may be of different types. In this study, DNA was isolated from dried blood spots on cards, cloth and solid surfaces from three different donors using Norgen's Dried Blood Spot Genomic DNA Isolation Kit. Fifteen microliters of each 50 µL elution was then run on a 1X TAE 1.0% agarose gel to visually inspect the isolated gDNA from isolations done after one day and one week post-collection (**Figure 1 a**,**b**).

All the samples showed good DNA integrity with different yields, as the samples were collected from different donors. High integrity DNA was obtained from samples after one day as well as one week post-collection.

DNA quality was determined through the use of a TaqMan® Real-Time PCR method. Two µL of each sample was used in the reaction. The Ct values were then graphed from each donor from both the one day and one week post-collection isolations (**Figure 2 a,b)**. The average Ct value for blood isolated from each material is illustrated in **Figure 3 (a,b)**. Positive amplification was obtained from all samples regardless of the collection method or time post-collection (1 day or week).

Application Note

Dried Blood Spot (DBS) Genomic DNA Isolation Kit Cat. # 36000

The overall Ct average for each collection method (average Ct from the three donors per each collection method) shows non-significant differences between samples isolated after one day post collection and those isolated after one week post-collection.

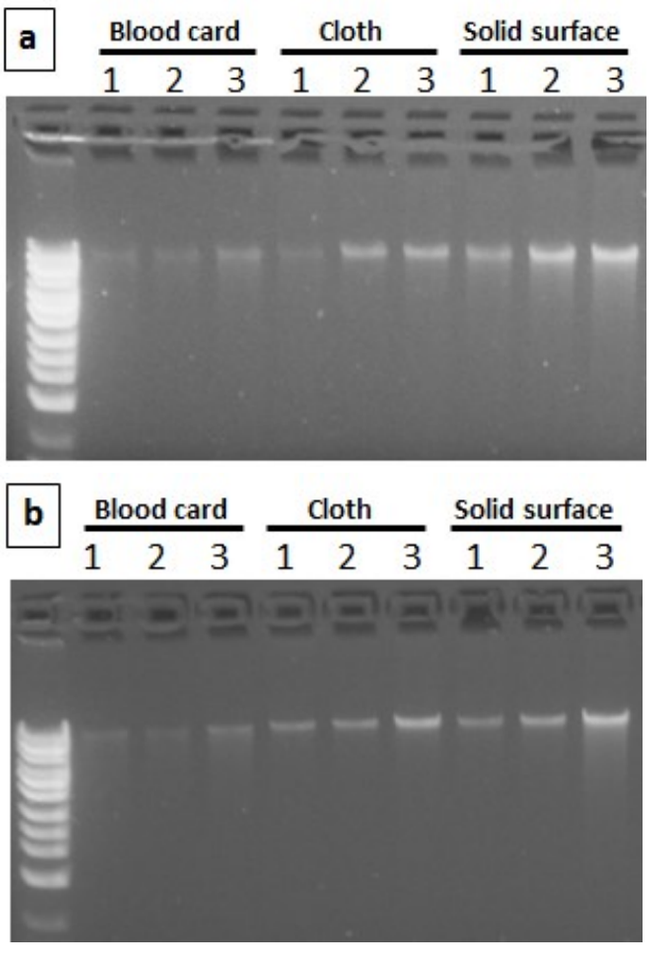


Figure 1. Resolution of DNA isolated from different materials using Norgen's Dried Blood Spot Genomic DNA Isolation Kit. Fifteen microliters of 50 µL elutions were run on 1X TAE 1.0% agarose gel. Panel (a) shows DNA isolated one day post-collection and Panel (b) shows DNA isolated after 1 week post-collection. Marker = Norgen's UltraRanger DNA Ladder

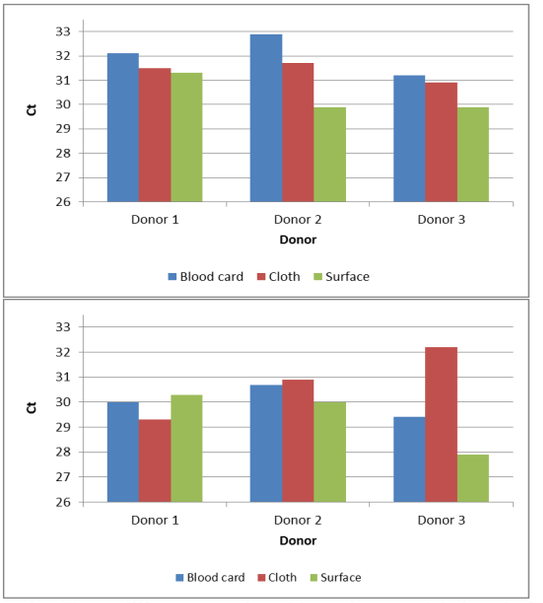


Figure 2. The difference in Ct values obtained from a Taqman® qPCR reaction performed on DNA isolated from different materials using Norgen's Dry Blood Spot Genomic DNA Isolation Kit. Two microliters of each elution were used in a 20 µl qPCR reaction involving GAPDH primers. Panel (a) corresponds to isolation done one day post-collection and Panel (b) corresponds to isolation done 1 week post-collection.

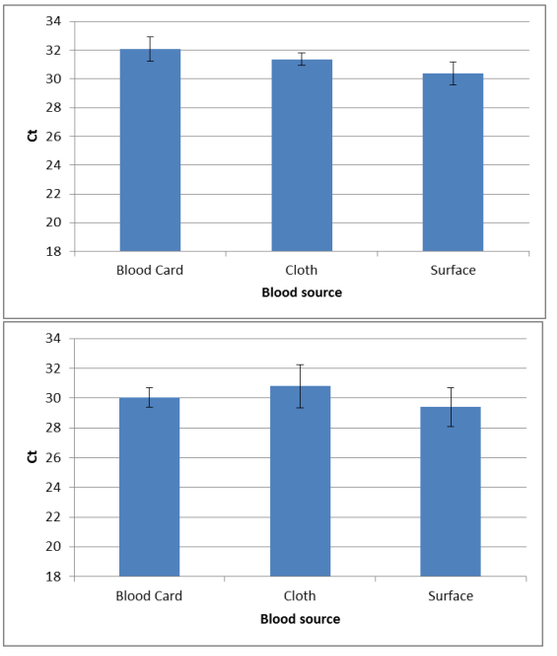


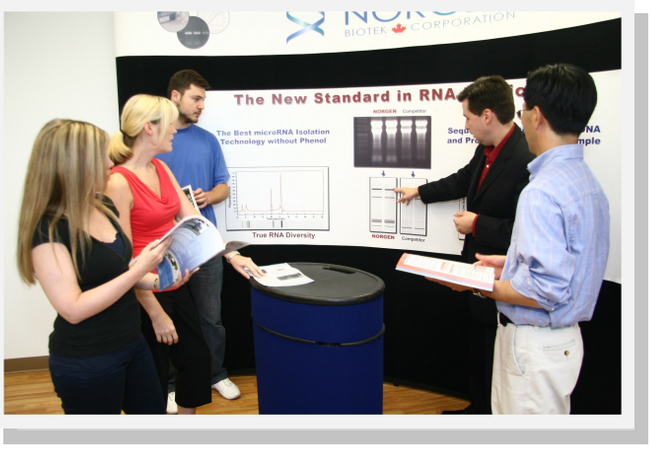
Figure 3. The average Ct value between DNA isolated from the different materials using a Taqman® qPCR reaction performed on DNA isolated from different materials using Norgen's Dry Blood Spot Genomic DNA Isolation Kit. Two microliters of each elution were used in a 20 µL qPCR reaction involving GAPDH primers. Panel (a) corresponds to solation done one day post-collection and Panel (b) corresponds to isolation done 1 week post-collection.

Dried Blood Spot (DBS) Genomic DNA Isolation Kit — Cat. # 36000

CONCLUSIONS

From the data presented in this report, the following can be concluded:

1. Norgen's Dried Blood Spot Genomic DNA Isolation Kit can isolate good integrity DNA from cloth as well as surface after one day or one week post-collection drying at room temperature.
2. All of the isolated DNA have high quality and free of PCR inhibitors.



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Warren JD, Xiong W, Bunker AM, Vaughn CP, Furtado LV, Roberts WL, et al. 2011. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. BMC Med; 9 (133).

Koestler DC, Marsit CJ, Christensen BC, Accomando W, Langevin SM, Houseman EA, et al. 2012. Peripheral blood immune cell methylation profiles are associated with nonhematopoietic cancers. Cancer Epidemiol Biomarkers Prev; 21(8):1293-302.

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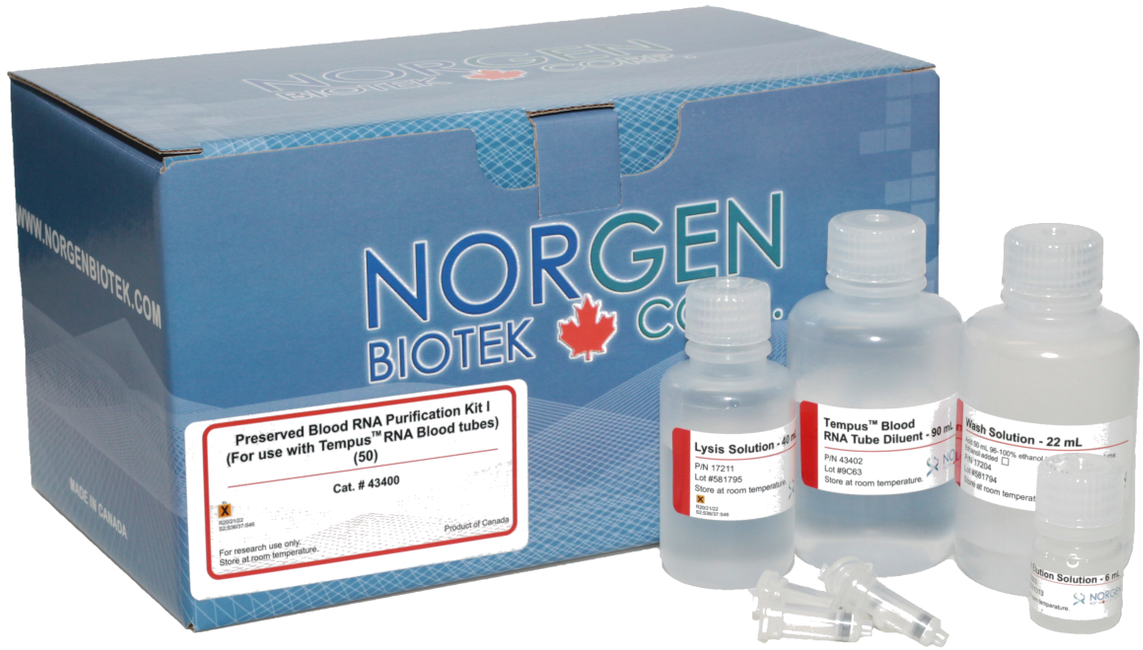


Blood DNA Purification Kit - 30 mL

Cat. # 52500

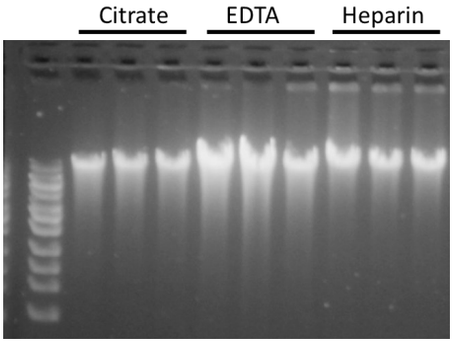


Figure 1. High Yields of Genomic DNA Isolated from 300 µL of Whole Blood. Genomic DNA was isolated from 300 µL of whole blood collected on different anticoagulants (Citrate, EDTA and Heparin, different donors) using Norgen's Blood Genomic DNA Purification Kit. Following isolation, 10 µL from each 100 µL elution was loaded on 1% TAE agarose gel. Purified DNA has a good yield and integrity. The used ladder is Norgen's UltraRanger 1kb DNA Ladder.

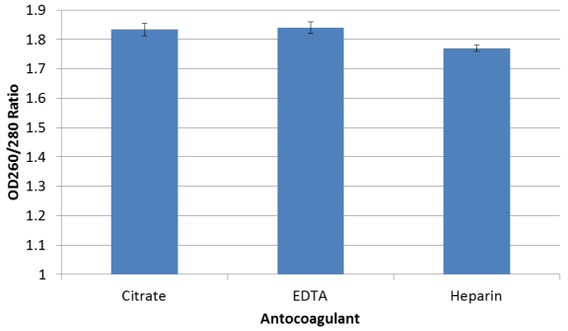


Figure 2. High purity of Genomic DNA Isolated from 300 µL of Whole Blood. Genomic DNA was isolated from 300 µL of whole blood collected on different anticoagulants (Citrate, EDTA and Heparin, different donors) using Norgen's Blood Genomic DNA Purification Kit. Following isolation, OD260/280 ratio was measured using spectrophotometric method. Purified DNA from blood collected on the different anticoagulants has a good OD260/280 ratio > 1.7.

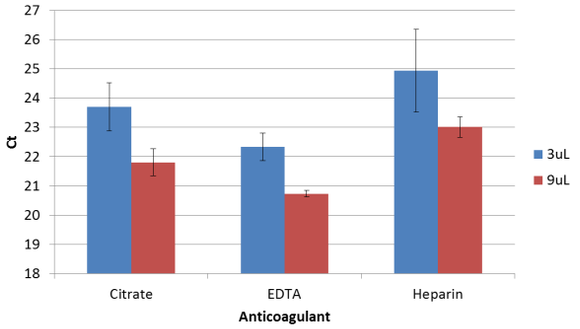


Figure 3. Purified DNA Can be Amplified in a Real-time PCR (TaqMan) reaction. Genomic DNA was isolated from 300 µL of whole blood collected on different anticoagulants (Citrate, EDTA and Heparin, different donors) using Norgen's Blood Genomic DNA Purification Kit. Three and nine µL of the DNA from each 100 µL elution was used in a real-time PCR reaction (reaction volume of 20 µL) with GAPDH TaqMan probe and primers. The real-time PCR was successful in amplifying the GAPDH gene, indicating that the DNA is of a high quality and can be used in sensitive downstream applications. Furthermore, the 9 µL template showed lower Ct value than the 3 µL template, indicating that purified DNA is free of PCR contaminants.

Fast and simple procedure for purifying high molecular weight genomic DNA from up to 10 mL of blood using alcohol-precipitation

The Blood DNA Purification Kit allows for the isolation of high molecular weight genomic DNA from the blood of various species, including humans. The kit can be used with blood inputs of 0.3 mL and up to 10 mL. Typical yields of genomic DNA will vary depending on the cell density of the blood sample. Preparation time for a single sample is less than 30 minutes, and each kit contains sufficient materials to process 30 mL of blood (100 preparations from 0.3 mL of blood). The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications.

Features and Benefits

- **Fast and Easy Processing** - Rapid alcohol precipitation format allows for the processing of multiple samples in 30 minutes.
- **Isolate a Diversity of DNA Species -** Isolate high quality and high molecular weight genomic DNA
- **Variable Volume Input -** Isolate genomic DNA from 300 µL up to 10 mL of blood
- **Recovered genomic DNA is suitable for downstream applications** - Purified genomic DNA is fully compatible with restriction enzyme digestions, Southern Blot, PCR analysis, sequencing and microarray analysis.
- **High quality DNA with no RNA contamination** - No contamination of genomic DNA is observed.

Applications

- Quantitative PCR
- Genotyping
- SNP analysis
- DNA archiving

Feature	Specifications
Minimum Blood Input	0.3 mL
Maximum Blood Input	10 mL
Average Yield from 1 mL of whole blood	24 µg*
DNA Size	Up to 200 kbp
Average Purity (OD260/280)	> 1.7
Format	Alcohol precipitation
Time to Complete 10 Purifications	30-60 minutes (+ DNA rehydration)

*Yield will vary depending on the type of blood processed

Ordering information

Cat #	Quantity
52500	100 preps (0.3 mL blood)

Single Preparation - RNA Isolation Kits

Total RNA Purification Kit — Cat. # 17200 & 37500

Specific protocol for total RNA isolation from different types of blood samples

Total RNA Purification Kit Dx — Cat. # Dx17200

CE

CE-certified kit for in-vitro diagnostic purposes - not available in all regions

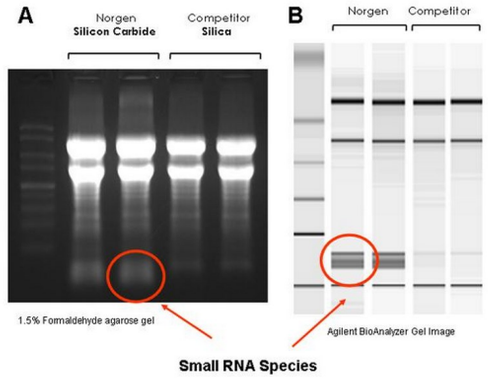


Figure 1. High Quality of Isolated RNA with Complete Size Range. Unlike most competitors' kits, Norgen's Total RNA Purification Kit allows for the isolation of all sizes of RNA, from the very large RNA down to the microRNA without the use of phenol. Total RNA was isolated from 1 x 10^9 *E. coli* cells using Norgen's Total RNA Purification Kit and a competitor's kit. **Panel A:** Five microliters of the 50 µL isolated RNA was analyzed on an agarose gel. **Panel B:** One microliters of the 50 µL isolated RNA was analyzed on the Agilent® 2100 BioAnalyzer RNA Nano 6000 chip. Note the presence of small RNA species (red circle) in the samples isolated via Norgen's kit and the absence of these RNA species in the competitor RNA preparation.

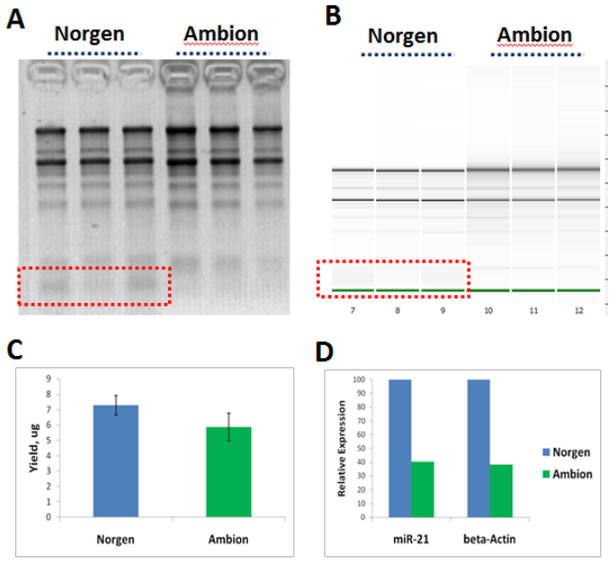


Figure 2. Recovery of True Total RNA including microRNA from 100 mL of Hamster Blood. Panel A is a 1X MOPS 1% agarose gel showing the RNA that was isolated from 3 different samples of 100 mL of Hamster using either Norgen's Total RNA Purification Kit or Ambion *RiboPure*™-Blood Kit. Both kits isolated large RNA (represented by 28S and 18S rRNA) with high integrity but Norgen's Total RNA Purification Kit provided the added benefit of recovering small RNA without any additional protocol (highlighted). **Panel B** is a result from a bioanalyzer resolution of the eluted RNA. Similar to the agarose gel, Norgen's Total RNA Purification Kit showed the added benefit of recovering small RNA. **Panel C** showed that Norgen's Total RNA Purification Kit recovered higher RNA yield. **Panel** D showed Norgen's Total RNA Purification Kit recovered high quality RNA for sensitive downstream application. that One microgram of RNA was used in RT-qPCR reactions for beta-Actin (for Large RNA) and miR-21 (for microRNA) genes. The RNA isolated by Norgen's Total RNA Purification Kit showed much better relative expression of the genes studied.

Rapid preparation of blood total RNA - including microRNA - without phenol

Principle

Purification is based on spin column using Norgen's proprietary resin as the separation matrix. Briefly, the blood sample of interest is first lysed using the provided Lysis Solution, ethanol is added and the RNA is bound to Norgen's column. Under these conditions only the RNA will bind to Norgen's resin while most of the contaminating cellular proteins are removed in the flowthrough or retained on top of the resin. The bound RNA is then washed to remove any remaining impurities. Lastly, the purified total RNA is eluted into 50 µL of the provided Elution Buffer.

Norgen's proprietary resin provides superior affinity to the full size range of RNA molecules, resulting in large and small RNA (miRNA) purification with better linearity and sensitivity. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time RT-PCR, RT-PCR, Northern blotting, RNase protection and primer extension, expression profiling, miRNA cloning and amplification and Next Generation Sequencing.

Performance

Norgen's Total RNA Purification Kit provides a rapid method for the isolation and purification of total RNA in as little as 20 minutes. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA), without the use of phenol or chloroform. Norgen's kit is the only kit on the market that isolates true total RNA, as other kits must use phenol to recover all sizes of RNA. Therefore Norgen's kit offers significant advantages in functionality, savings on cost, ease-of-use, no hazardous organic waste, and no inhibitory effect on PCR amplifications as a result of residual phenol. With this kit both miRNA and mRNA can be studied from the same sample without further purifications, thus offering considerable advantages when comparing and relating expression of miRNA to other RNA. Furthermore, this is an excellent kit for the extraction of miRNA from all samples including plasma.

Applications

- Quantitative, real-time RT-PCR for both large mRNA and small RNA including miRNA
- RT-PCR for both large mRNA and small RNA including miRNA
- Expression profiling
- Next Generation Sequencing for RNA and miRNA
- miRNA from plasma for discovery
- microRNA cloning and amplification
- PCR-based virus detection
- PCR-based viable bacteria detection
- Northern blotting
- RNase protection
- Primer extension

Single Preparation - RNA Isolation Kits

Total RNA Purification Kit — Cat. # 17200 & 37500

Specific protocol for total RNA isolation from different types of blood samples

Total RNA Purification Kit Dx — Cat. # Dx17200

CE

CE-certified kit for in-vitro diagnostic purposes - not available in all regions

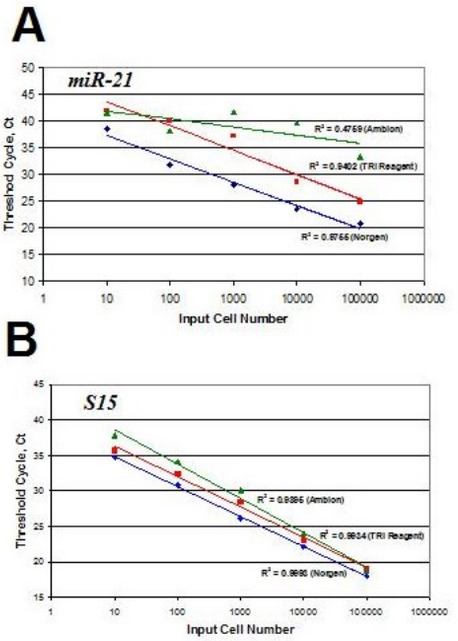


Figure 3. Linear and Sensitive Isolation of Both Large and Small RNA. Norgen's Total RNA Purification Kit allows consistent isolation of both large and small RNA from different input amounts. Total RNA was isolated from 10 to 100,000 HeLa cells using Norgen's Total RNA Purification Kit (**blue**), a competitor's silica-based kit (**green**) and a phenol-based RNA extraction method (**red**). **Panel A:** Relative expression of *miR-21* **Panel B:** Relative expression of *S15*. Both were determined by RT-qPCR of total RNA samples. In brief, 1 µL of the 50 µL isolated RNA was then subjected to a 20 µL reverse transcription using miR-21 stem-loop reverse primer or oligo dT primer. Two microliters of the reverse transcription was used in a 20 µL real-time PCR reaction with primers to detect the human *miR-21* (Panel A) and the *S15* transcripts (Panel B). The resulting threshold cycle (*Ct*) values were plotted against input cell number. RNA isolated using Norgen's Total RNA Purification had the best linearity (higher R^2) and sensitivity (lower *Ct*) for both large RNA (*S15*) and small RNA (*miR-21*).

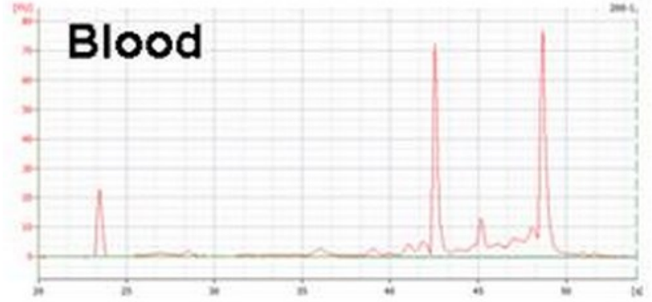


Figure 4. High Quality of RNA from Blood. Total RNA was isolated from 100 µL rat blood using Norgen's Total RNA Purification Kit. One microliter of the 50 µL isolated RNA was analyzed on the Agilent® 2100 BioAnalyzer using an RNA Nano 6000 chip. Note the integrity of RNA from all inputs with the presence of small RNA species. Norgen's Total RNA Purification Kit consistently isolates high quality RNA from various inputs that score a RIN value between 8 and 10.

Features and Benefits

- **No phenol:chloroform extractions** - Total RNA is isolated from blood without the use of harmful chemicals such as phenol or chloroform
- **Isolate total RNA from very small samples -** Total RNA has been isolated and detected from as little as 10 µL of blood
- **Extremely efficient isolation of low abundance microRNA -** Norgen's Total RNA Purification Kit has been shown to be extremely efficient at recovering low abundance microRNA from plasma samples
- **Isolate a diversity of RNA species** - All RNA species can be isolated, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA)
- **Fast and easy processing** - Rapid spin-column format allows for the processing of 10 samples in 20 minutes
- **Isolate total RNA from a broad input source -** Total RNA has been isolated from cultured animal cells, small tissue samples, LCM samples, bacteria, yeast, fungi, plants, viruses and various bodily fluids including blood, plasma, serum, saliva, nasal or throat swabs
- **No need for carrier RNA** - Isolate all sizes of RNA without the use of carrier RNA
- **Multiple kit sizes available** - This kit is available in both 50 prep and 100 prep sizes

Feature	Specifications
Column binding capacity	Up to 50 µg RNA
Maximum Loading Volume Per Spin Column	650 µL
Size of RNA Purified	All sizes, including < 200 nt
Time to Complete 10 Purifications	20 minutes
RNA Yield 100 µL human blood 100 µL hamster blood	 1 µg 5 µg
Format	Spin columns

Ordering information

Cat #	Quantity
17200	50 preps
Dx17200	50 preps
37500	100 preps

Leukocyte RNA Purification Kit

Cat. # 21200

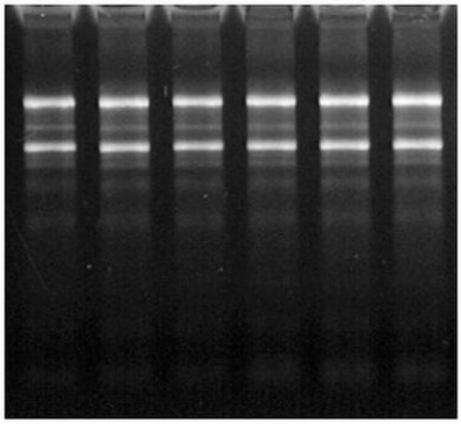


Figure 1. Consistent Isolation of High Quality Leukocyte RNA. Norgen's Leukocyte RNA Purification Kit isolates leukocyte RNA of high quality with great consistency. Total leukocyte RNA was isolated from 100 µL of hamster blood using Norgen's Leukocyte RNA Purification Kit. A total of 6 replicates were performed, and 7 µL of the 50 µL purified RNA was then resolved on a 1.2% formaldehyde-agarose gel. As it can be seen, Norgen's kit not only isolated high and consistent yields of total RNA, but the RNA was also of high quality as evidenced by intactness of the major 28S and 18S rRNA.

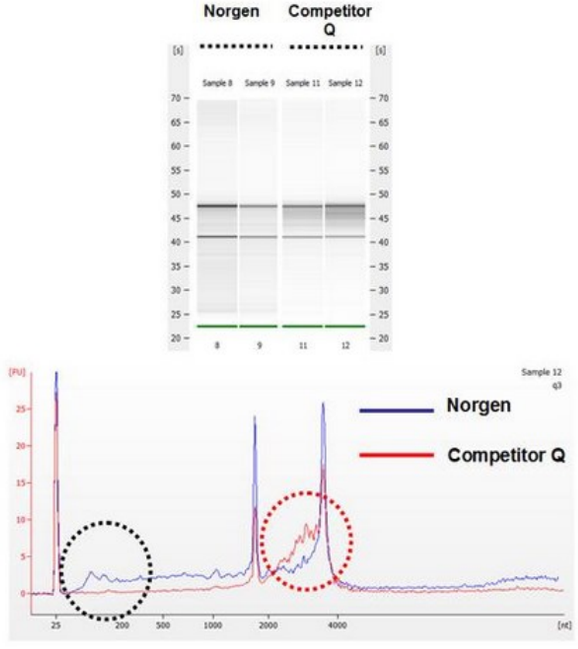


Figure 2. Higher Yield and Quality of Leukocyte RNA Isolated by Norgen's Leukocyte RNA Purification Kit. Norgen's Leukocyte RNA Purification Kit isolates Leukocyte RNA that exceeds the yield and quality of competitors. Total RNA was isolated from 200 µL of hamster blood using Norgen's Leukocyte RNA Purification Kit and a leading competitor's kit. One microliter of the 50 µL purified RNA was resolved on an Agilent RNA Nano 6000 chip. The gel diagram (Upper panel) and the electropherogram (Lower panel) showed better quality of RNA isolated by Norgen's kit. In particular, RNA isolated by Norgen's Leukocyte RNA Purification Kit did not have any evidence of the RNA degradation that was present in RNA isolated by the competitor's kit (Red Circle). In addition, Norgen's kit isolated higher amounts of RNA (Blue Line) with the additional recovery of small RNAs including miRNA (Black Circle) which were not present in RNA isolated by the competitor's kit.

Rapid extraction and purification of total RNA from leukocytes

Principle

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. Briefly, the red blood cells are first removed from the sample through differential red blood cell lysis, and the leukocytes are recovered through centrifugation. The leukocytes are lysed, and the leukocyte RNA is bound to Norgen's column. Under these conditions only the RNA will bind to Norgen's resin, while the DNA, proteins and other contaminants will be removed in the flowthrough. The bound RNA is then washed to remove any remaining impurities. Lastly, the purified RNA will be eluted into 50 µL of the provided Elution Buffer or water.

Performance

Norgen's Leukocyte RNA Purification Kit provides a rapid method for the isolation and purification of total leukocyte RNA from mammalian blood samples in 40 minutes. Selective isolation of leukocyte RNA results in improved expression profiling and other downstream applications by removing the masking effects of some RNAs which are very abundant in whole blood, such as globin mRNAs. The kit is able to isolate total leukocyte RNA, including both large mRNA and all small RNA species containing microRNA (miRNA) and small silencing RNA (siRNA). The purified RNA is of the highest quality and can be used in a number of downstream applications.

Features and Benefits

- **Fast and easy processing -** Rapid spin-column format allows for the processing of 10 samples in 40 minutes.
- **No phenol:chloroform extractions** - Norgen's Leukocyte RNA Purification Kit isolates RNA without the use of harmful chemicals such as phenol or chloroform.
- **Recovered RNA is suitable for downstream applications -** Purified RNA can be used in a number of downstream applications including real-time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array analysis requiring the use of intact RNA.
- **Isolate total leukocyte RNA** - All leukocyte RNA species are isolated, from large mRNA down to microRNA.
- **Fractionate leukocytes from whole blood in minutes -** Rapid removal of red blood cells from whole blood samples using differential red blood cell lysis.

Single Preparation - RNA Isolation Kits

Leukocyte RNA Purification Kit — Cat. # 21200

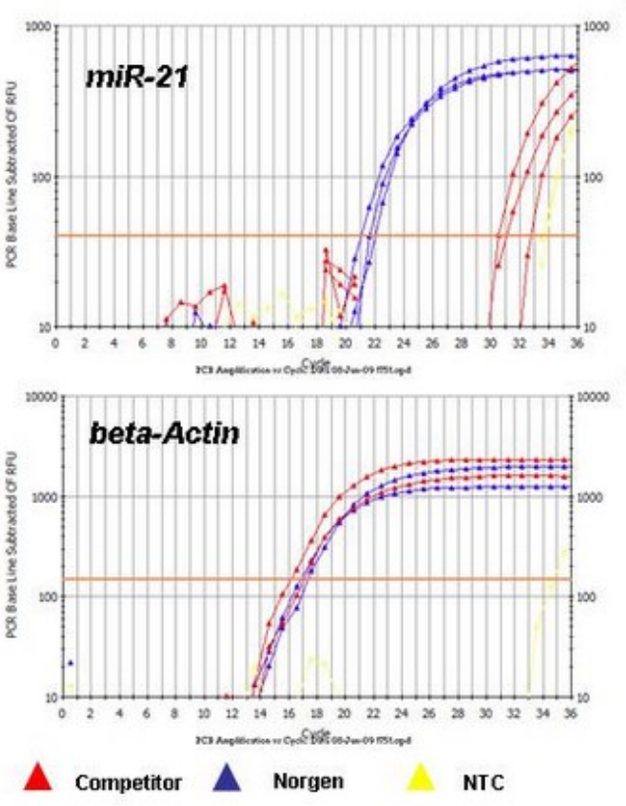


Figure 3. High Yield of a Diversity of RNA Species. Norgen's Leukocyte RNA Purification Kit effectively recovers all sizes of RNA from large mRNA to small RNA including microRNAs. Total RNA was isolated from 200 µL of hamster blood sample using Norgen's Leukocyte RNA Purification Kit and a leading competitor's kit as illustrated in Figure 2. Two hundred nanograms of the purified RNA (both 50 µL elution volumes) was then used as the template in an RT-qPCR for detecting miR-21(Upper Panel) and for detecting the beta-actin gene (Lower Panel). In both graphs the blue lines correspond to Norgen isolated-RNA and the red lines correspond to competitor-isolated RNA.
Upper Panel: detection of the miR-21. Norgen's kit isolated higher yields of microRNA, as indicated by the lower Ct values of the blue lines.
Lower Panel: detection of the beta-actin gene. Norgen's kit successfully isolated a similar amount of the large RNA compared to the competitor's kit indicating the full diversity of RNA species isolated.

Applications

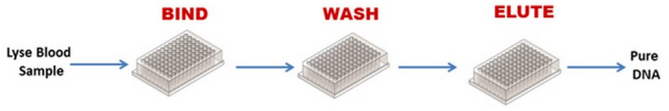
- Bioanalyzer
- Quantitative, real-time RT-PCR for large RNA and small RNA including miRNA
- RT-PCR for large RNA and small RNA including miRNA
- Northern blotting
- RNase protection
- Primer extension
- Expression array assays
- Next Generation Sequencing
- microRNA Cloning

Feature	Specifications
Column binding capacity	Up to 50 µg RNA
Maximum Loading Volume Per Spin Column	650 µL
Size of RNA Purified	All sizes, including < 200 nt
Minimum Blood Input Volume	10 µL
Maximum Blood Input Volume	2 mL or 3 x 10^6 leukocytes
Time to Complete 10 Purifications	20 minutes
Input Type	Blood from Human, Hamster, Mouse, Rabbit and Rat
Yield from Human Blood (500 µL)	1.5 µg
Yield from Hamster Blood (100 µL)	2.5 µg
Format	Spin columns

Ordering information

Cat #	Quantity
21200	50 preps

Blood Genomic DNA Isolation 96-Well Kit — Cat. # 46350



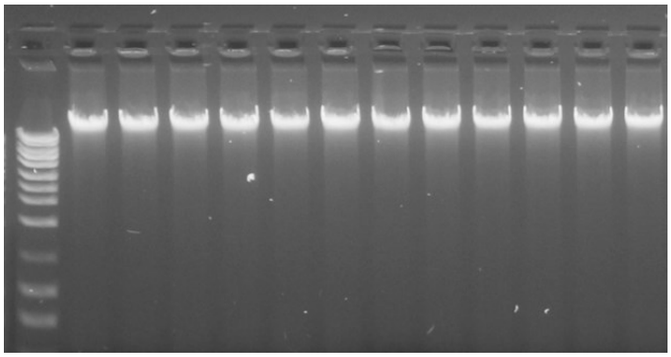


Figure 1. High Yields of Genomic DNA Isolated from 200 µL of Whole Blood. Genomic DNA was isolated from 200 µL of whole blood using Norgen's Blood Genomic DNA Isolation 96-Well Kit. Following isolation of 12 samples, 15 µL from each 200 µL elution was loaded on 1% TAE agarose gel. Norgen's Blood Genomic DNA Isolation 96-Well Kit demonstrated a good and consistent DNA yield and integrity. The used ladder is Norgen's HighRanger 1kb DNA Ladder.

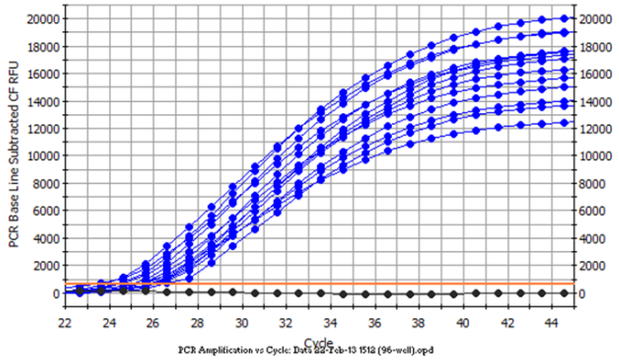


Figure 2. Purified DNA Can be Amplified in a Real-time PCR (TaqMan) Reaction. Genomic DNA was isolated from 200 µL of whole human blood using Norgen's Blood Genomic DNA Isolation 96-Well Kit. Five µL of the DNA from each 200 µL elution was used in a real-time PCR reaction (total reaction volume of 20 µL) with GAPDH TaqMan probe and primers. The real-time PCR was successful in amplifying the GAPDH gene from all the isolated 12 samples (blue). This indicates that the isolated DNA from all samples is of a high quality and can be used in sensitive downstream applications. The black line is a no-template control.

Rapid high-throughput preparation of genomic DNA from up to 200 µL of whole blood.

The Blood Genomic DNA Isolation 96-Well Kit allows for the isolation of genomic DNA from the blood of various species, including humans. Purification is based on 96-well spin column technology without the use of organic solvents. Typical yields of genomic DNA will vary depending on the cell density of the blood sample. Preparation time for 96 samples is about 45 minutes, and each kit contains sufficient materials for 192 preparations. The purified genomic DNA is compatible with sensitive downstream applications.

Features and Benefits

- **No Phenol-Chloroform extraction or alcohol precipitation** - Isolate genomic DNA without the use of harmful chemicals
- **Isolate DNA from blood pathogens -** Isolate DNA from viral and bacterial blood pathogens
- **Fast and easy processing** - Rapid spin-column format allows for the processing of 96 samples in 45 minutes.
- **Recovered genomic DNA is suitable for downstream applications** - Purified genomic DNA is fully compatible with restriction enzyme digestions, Southern Blot, PCR analysis, sequencing and microarray analysis.
- **High quality DNA with no RNA contamination** - No contamination or degradation of genomic DNA is observed.

Applications

- Quantitative PCR
- Genotyping
- SNP analysis
- Microarray analysis
- Next Generation Sequencing
- PCR-based pathogen detection

Feature	Specifications
Maximum Blood Input	200 µL
Binding Capacity	> 50 µg
Average Yield (200 µL of blood)	2-8 µg*
Elution Volume	50-200 µL
Analyte Purified	Genomic DNA, mitochondrial DNA, viral DNA
Format	96-Well plate
Time to Complete 96 Purifications	45 minutes

*Yield will vary depending on the type of blood processed

Ordering information

Cat #	Quantity
46350	2 x 96-well plates

Blood DNA Preservation

Blood DNA Preservation Buffer (3X) Cat. # 29111, 29112

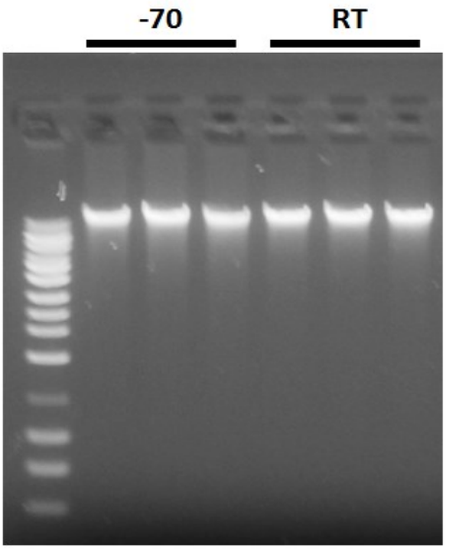


Figure 1. High Yields and integrity of Genomic DNA Isolated from 200 µL of preserved Whole Blood after 1 week preservation at room temperature. Blood was collected on EDTA and either directly stored at -70 or mixed with Norgen's Blood DNA Preservation Buffer (2 volumes of blood + 1 volume of preservation buffer). Genomic DNA was isolated from 133 µL of blood stored at -70 or 200 µL of preserved blood, using Norgen's Blood Genomic DNA Isolation Mini Kit (# 46300). Following isolation of 3 samples from each condition, 15 µL from each 200 µL elution was loaded on 1% TAE agarose gel. The isolated DNA after 8 week preservation at room temperature shows equivalent yield and integrity to that stored at -70°C. The used ladder is Norgen's UltraRanger 1kb DNA Ladder.

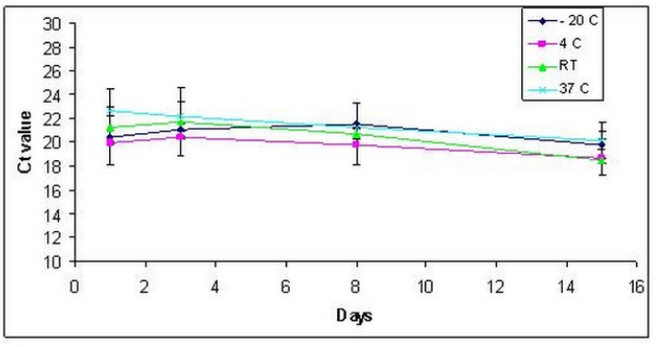


Figure 2. Stability of DNA Isolated from Preserved Blood Samples Stored up to 2 Weeks at Different Temperatures. A 0.5 mL sample of blood was mixed with 0.25 mL of Norgen's Blood DNA Preservation Buffer (3X) and stored at different temperatures (-20 °C, 4 °C, room temperature and 37 °C) for 2 weeks. Aliquots of the stored blood were removed at various time points (1 day, 3 days, 8 days and 15 days) and the DNA was isolated using Norgen's Blood Genomic DNA Isolation Mini Kit (Cat #46300). The purified DNA was then used as the template in a real-time PCR using 5S specific primers. As indicated from the Ct value, all the DNA templates were successfully amplified without significant change in the Ct values over the 15 days.

Rapid and simple preservation of blood DNA at ambient temperatures

Norgen's Blood DNA Preservation Buffer (3X) is a 3X aqueous storage buffer designed for rapid cellular lysis and subsequent preservation of DNA from fresh blood samples. The buffer prevents the growth of Gram-negative and Gram-positive bacteria and fungi, and also inactivates viruses allowing the resulting non-infectious samples to be handled and shipped safely. In addition, the buffer eliminates the need to immediately process or freeze samples and allows the samples to be shipped to centralized testing facilities at ambient temperature. The components of the buffer allow samples to be stored for one week under conditions where DNA degradation would occur normally. The buffer is intended to be used in clinical laboratories with the ability to preserve samples for use in downstream diagnostic assays.

Performance

- Norgen's Blood DNA Preservation Buffer (3X) is a 3X solution. One volume of the buffer is added to two volumes of fresh blood followed by mixing by inversion for ten times.
- Specimens may be held or shipped to the testing laboratory at room temperature for up to 8 weeks. Specimens held longer should be kept at -20°C or lower until testing.
- Storage at -20°C or lower is recommended for archival samples and will provide optimal preservation. The preservation buffer will freeze at -20°C. Samples can be stored indefinitely at -80°C.
- Samples can be stored at room temperature (22°C) for up to 8 weeks without significant loss of DNA quality.
- DNA has also been successfully isolated from samples stored at 37°C for 2 week.

Features and Benefits

- **No need to immediately process samples** - The buffer eliminates the need to immediately process or freeze samples
- **DNA preservation for 2 weeks at room temperature** – Intact, biologically active blood DNA has been isolated from samples stored in the Blood DNA Preservation Buffer (3X) for 8 weeks.
- **Ship blood samples at room temperature** – Blood samples stored in the Blood DNA Preservation Buffer (3X) can be safely shipped at room temperature with no signs of DNA degradation
- **Compatible with most DNA isolation methods** – Blood DNA can be isolated from the preserved samples using a number of different methods, including Norgen's Blood Genomic DNA Isolation Kits.

Ordering information

Cat #	Quantity
29111	25 mL
29112	100 mL

C. Plasma/Serum Sample Preparation Kits

Norgen's Plasma/Serum Circulating DNA and RNA Purification Kits provide an efficient method for the purification of fragmented free-circulating nucleic acids from variable volumes of human plasma or serum. The kits are able to isolate all sizes of circulating DNA or RNA. The slurry format provides an advantage over other available kits in that it does not require extension tubes for the purification of free-circulating DNA or RNA from large sample volumes. The purified plasma/serum free-circulating DNA or RNA are eluted in an elution solution that is compatible with PCR, qPCR, methylation-sensitive PCR, Southern blot analysis, reverse transcription PCR, Northern blotting, RNase protection, primer extension and expression array assays.

Sample Preparation Selection Table:

Kit	Cat. #	Kit size	Sample Size
Single Preparation - DNA Isolation Kits			
Plasma/Serum Circulating DNA Purification Mini Kit (Slurry Format)	50600, Dx50600*	50 preps	50-400 µL
Plasma/Serum Circulating DNA Purification Midi Kit (Slurry Format)	51200	20 preps	0.4-2 mL
Plasma/Serum Circulating DNA Purification Maxi Kit (Slurry Format)	51300	10 preps	2-10 mL
Single Preparation - RNA Isolation Kits			
Total RNA Purification Kit Specific protocol for plasma/serum samples	17200, 37500, Dx17200*	50, 100 preps	Up to 200 µL
Plasma/Serum Circulating and Exosomal RNA Purification Kit (Slurry Format)	42800, Dx42800*	50 preps	0.25-5 mL
Plasma/Serum Circulating and Exosomal RNA Purification Mini Kit (Slurry Format)	51000	50 preps	0.25-2 mL
Plasma/Serum Circulating and Exosomal RNA Purification Maxi Kit (Slurry Format)	50900	25 preps	2-5 mL
Single Preparation - Nucleic Acid Isolation Kits			
Plasma/Serum Circulating Nucleic Acid Purification Mini Kit (Slurry Format)	53300	50 preps	0.25-2 mL
Plasma/Serum Circulating and Exosomal RNA Purification Maxi Kit (Slurry Format)	53400	25 preps	2-5 mL
High Throughput RNA Preparation			
Plasma/Serum Circulating RNA Purification 96-Well Kit (Slurry Format)	29500	1 x 96-well plate	0.25-2 mL

*CE-certified kit for in-vitro diagnostic purposes - not available in all regions - very similar protocol to non-Dx kit.

Single Preparation - DNA Isolation Kits

Plasma/Serum Circulating DNA Purification Mini Kit (Slurry Format) Cat. # 50600

Plasma/Serum Circulating DNA Purification Mini Kit Dx Cat. # Dx50600

CE-certified kit for in-vitro diagnostic purposes - not available in all regions

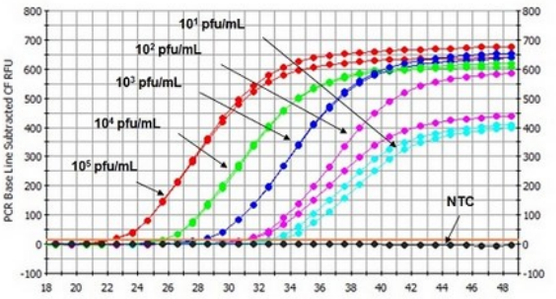


Figure 1. Highly Sensitive Isolation of Viral DNA from 0.4 mL of Plasma. A serial dilution of Adenovirus 5 (10^5, 10^4, 10^3, 10^2 and 10^1) was spiked into 0.4 mL of plasma, and adenoviral DNA was then isolated using Norgen's Plasma/Serum Circulating DNA Purification Mini Kit (Slurry Format; Cat# 50600). Three microlitres of each 100 µL elution was then used as the template in a 20 µL PCR reaction using Ad5-specific primers, an Ad5-specific probe and Norgen's 2X PCR Master Mix (Cat #28007). The results were quantified using a standard curve that was plotted using standard adenoviral DNA of known concentration. As few as 80 pfu/mL could be isolated and detected from 0.4 mL of plasma.

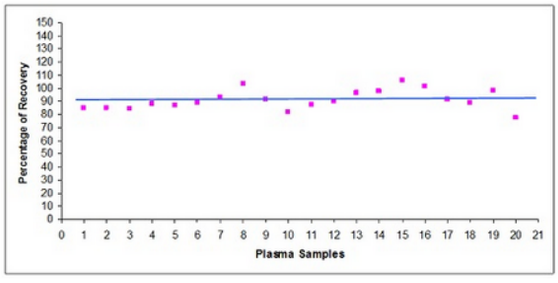
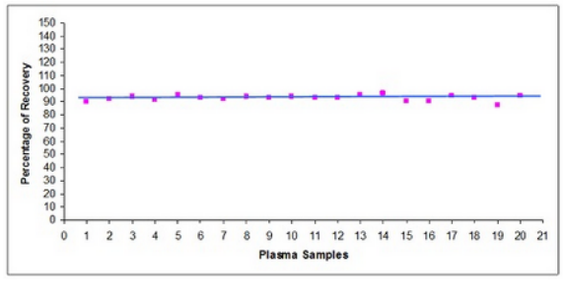


Figure 2. Reproducible Recovery of Circulating DNA. Norgen's Plasma/Serum DNA Purification Mini Kit (Slurry Format) (Cat# 50600) was used to isolate equal amounts of DNA fragments (300 bp) added to 20 plasma samples. DNA was purified from 0.4mL Plasma using Norgen's Plasma/Serum DNA Purification Mini Kit (Slurry Format) (Cat# 50600) with an elution of 100 µL. DNA yield was quantified using real-time PCR targeting the two added DNA fragments. The blue line indicates the average percentage of recovery ± SD (91% ± 7).



Rapid and simple isolation of circulating DNA from plasma/serum samples

Norgen's Plasma/Serum Circulating DNA Purification Mini Kit (Slurry Format) provides an efficient method for the purification of fragmented free-circulating DNA from 50 µL - 400 µL of human plasma or serum. Purification is based on spin column technology without the use of organic solvents. The kit is able to isolate all sizes of circulating DNA. The slurry format provides an advantage over other available kits in that it does not require extension tubes for the purification of free-circulating DNA from large sample volumes. DNA can be isolated from either fresh or frozen samples using this kit. Typical yields of purified free-circulating DNA will vary depending on the input sample (1-100ng/mL circulating DNA in human plasma), with more concentrated samples tending to yield more free-circulating nucleic acids. Preparation time for a single sample is less than 30 minutes. The purified genomic DNA is compatible with sensitive downstream applications.

Features and Benefits

- **Fast and easy processing** - Rapid format allows for the processing of multiple samples in less than 30 minutes.
- **Versatile plasma and serum input volumes** - Isolate circulating DNA from 50 µL - 400 µL of plasma/serum. No requirement for extension tubes when processing larger sample volumes.
- **Concentrate circulating DNA** – Circulating DNA present in input volumes of 50 µL - 400 µL are concentrated into final elution volumes of 100 µL.
- **Isolate inhibitor-free DNA** - Purified DNA can be used in a number of sensitive downstream applications including PCR, qPCR, methylation-sensitive PCR and Southern Blot analysis.
- **Isolate all sizes of circulating DNA**- The kit allows for the isolation of all sizes of fragmented circulating DNA, ranging from high molecular weight fragments down to fragments as small as 50bps.

Applications

- Quantitative PCR
- Genotyping
- SNP analysis
- Microarray analysis
- Next Generation Sequencing

Feature	Specifications
Minimum Plasma/Serum Input	50 µL
Maximum Plasma/Serum Input	400 µL
Minimum Elution Volume	100 µL
Time to Complete Purifications	< 30 minutes

Ordering information

Cat #	Quantity
50600	50 preps
Dx50600	50 preps

Plasma/Serum Circulating DNA Purification Midi Kit
(Slurry Format)

Cat. # 51200

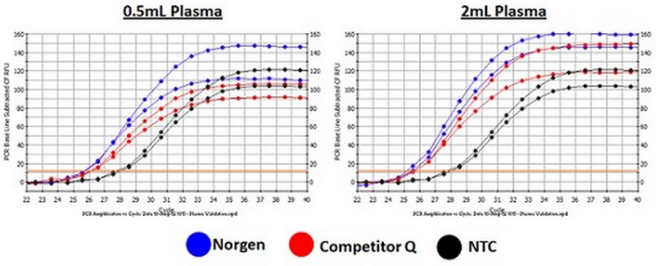


Figure 1. Detection of Human 5S Gene from 0.5mL and 2mL of Plasma. Norgen's Plasma/Serum Circulating DNA Purification Midi Kit was compared to a leading Competitor's kit for their ability to isolate high quality plasma DNA ready for sensitive downstream applications such as qPCR. Norgen's samples (blue) were found to amplify sooner than competitor Q's samples (red), when both 0.5mL and 2mL of plasma were processed, indicating a higher recovery of high quality circulating DNA present in Norgen's samples.

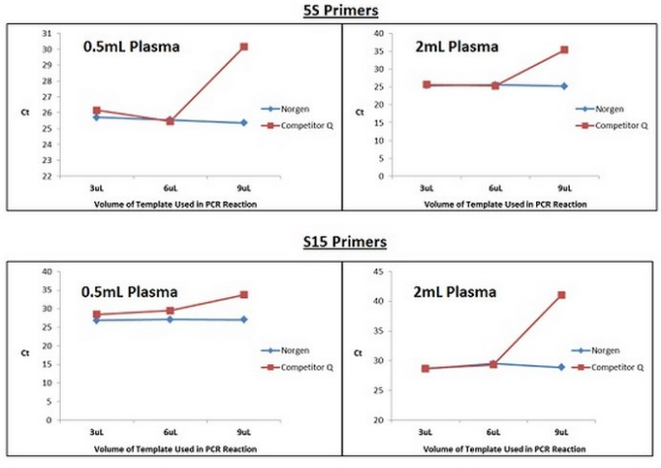


Figure 2. Determination of the Rate of Inhibition Present in Plasma DNA Samples. A common issue with processing high volumes of plasma is that inhibitors naturally present in plasma samples are often co-eluted, and with higher volumes of plasma comes higher concentrations of these inhibitors. Therefore, DNA was isolated from 0.5 and 2mL of plasma using Norgen's Plasma/Serum Circulating DNA Purification Midi Kit and a leading Competitor's kit. Increasing volumes of elution (3, 6 and 9 µL) were then used in a 20 µL reaction containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007) spiked with SYBR green, 5 mM of either the 5S or S15 primer pair, and nuclease-free water. The PCR samples were amplified under the real-time program; 95°C for 3 minutes for an initial denaturation, 40 cycles of 95°C for 15 seconds for denaturation, 60°C for annealing and 72°C for extension. The reaction was run on an iCycler iQ Realtime System (Bio-Rad). It was found that an increase in elution volume used in the PCR did not greatly affect the Ct value generated from Norgen samples. Competitor samples, on the other hand, were found to show a higher degree of PCR inhibition as 9µL of elution led to a drastic increase in Ct value. This trend was apparent for both 0.5mL and 2mL, as well as for 5S and S15 primers.

Rapid and simple isolation of circulating DNA from plasma/serum samples

Norgen's Plasma/Serum Circulating DNA Purification Midi Kit (Slurry Format) provides an efficient method for the purification of fragmented free-circulating DNA from 0.4 mL to 2 mL of human plasma or serum. Purification is based on spin column technology without the use of organic solvents. The kit is able to isolate all sizes of circulating DNA. The slurry format provides an advantage over other available kits in that it does not require extension tubes for the purification of free-circulating DNA from large sample volumes. DNA can be isolated from either fresh or frozen samples using this kit. Typical yields of purified free-circulating DNA will vary depending on the input sample (1-100ng/mL circulating DNA in human plasma), with more concentrated samples tending to yield more free-circulating nucleic acids. The purified genomic DNA is compatible with sensitive downstream applications.

Features and Benefits

- **Fast and easy processing** - Rapid format allows for the processing of multiple samples in less than 45 minutes.
- **Versatile plasma and serum input volumes** - Isolate circulating DNA from 400 µL - 2mL of plasma/serum. No requirement for extension tubes when processing larger sample volumes.
- **Concentrate circulating DNA** – Circulating DNA present in input volumes of 400 µL - 2 mL are concentrated into final elution volumes of 100 µL.
- **Isolate inhibitor-free DNA** - Purified DNA can be used in a number of sensitive downstream applications including PCR, qPCR, methylation-sensitive PCR and Southern Blot analysis.
- **Isolate all sizes of circulating DNA**- The kit allows for the isolation of all sizes of fragmented circulating DNA, ranging from high molecular weight fragments down to fragments as small as 50bps.

Applications

- Quantitative PCR
- Genotyping
- SNP analysis
- Microarray analysis
- Next Generation Sequencing

Feature	Specifications
Minimum Plasma/Serum Input	400 µL
Maximum Plasma/Serum Input	2 mL
Minimum Elution Volume	100 µL
Time to Complete Purifications	< 45 minutes

Ordering information

Cat #	Quantity
51200	20 preps

Plasma/Serum Circulating DNA Purification Maxi Kit
(Slurry Format)

Cat. # 51300

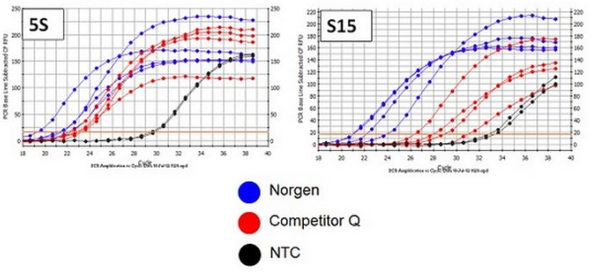


Figure 1. Detection of Human 5S and S15 Genes from 5mL of Plasma. Norgen's Plasma/Serum Circulating DNA Purification Maxi Kit (Slurry Format) was compared to a leading competitor's kit for their ability to isolate high quality plasma DNA ready for sensitive downstream applications such as qPCR. Three microliters of each elution was used in a 20 µL reaction containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007) spiked with SYBR green, 5 mM of either the 5S or S15 primer pair, and nuclease-free water. As it can be seen, Norgen's samples (blue) were found to amplify sooner than the competitor samples (red), for both 5S and S15, indicating a higher recovery of high quality circulating DNA present in Norgen's samples.

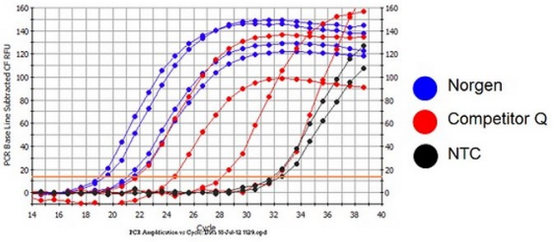


Figure 2. Detection of Spiked Viral DNA from 5mL of Plasma. Norgen's Plasma/Serum Circulating DNA Purification Maxi Kit (Slurry Format) was compared to a leading competitor kit for the ability to isolate viral DNA from 1 x 10^6 spiked viral particles. Adenovirus (AdV, 1 x 10^6) was spiked into 5mL of plasma, and total DNA was isolated from the plasma using Norgen's Plasma/Serum Circulating DNA Purification Maxi Kit (Slurry Format) or the competitor's kit. Detection of the viral DNA was then based on the AdV DNA Binding Protein (DBP). Three microliters of each elution was used in a 20µl reaction containing 10µL of Norgen's 2X PCR Mastermix (Cat# 28007) spiked with SYBR green, 5 mM of the DBP primer pair, and nuclease-free water. Norgen's samples (blue) were found to amplify sooner than the competitor's samples (red), indicating a higher recovery of viral DNA present in Norgen's samples.

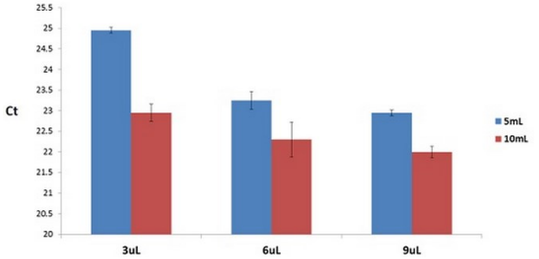


Figure 3. Determination of the Rate of Inhibition Present in Plasma DNA Samples. A common issue with processing high volumes of plasma is that inhibitors naturally present in plasma samples are often co-eluted, and with higher volumes of plasma comes higher concentrations of these inhibitors. To test for the presence of inhibitors, DNA was isolated from 5 and 10 mL of plasma using Norgen's Plasma/Serum Circulating DNA Purification Maxi Kit (Slurry Format). Increasing volumes of elution (3, 6 and 9 µL) were then used in a 20 µL PCR reaction containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007) spiked with SYBR green, 5 mM of the 5S primer pair, and nuclease-free water. It was found that as the volume of elution used in the PCR reaction increased, the Ct values subsequently decreased in a linear fashion, indicating minimal PCR inhibition present in these plasma DNA samples.

Rapid and simple isolation of circulating DNA from plasma/serum samples

Norgen's Plasma/Serum Circulating DNA Purification Midi Kit (Slurry Format) provides an efficient method for the purification of fragmented free-circulating DNA from 2 mL to 10 mL of human plasma or serum. Purification is based on spin column technology without the use of organic solvents. The kit is able to isolate all sizes of circulating DNA. The slurry format provides an advantage over other available kits in that it does not require extension tubes for the purification of free-circulating DNA from large sample volumes. DNA can be isolated from either fresh or frozen samples using this kit. Typical yields of purified free-circulating DNA will vary depending on the input sample (1-100ng/mL circulating DNA in human plasma), with more concentrated samples tending to yield more free-circulating nucleic acids. The purified genomic DNA is compatible with sensitive downstream applications.

Features and Benefits

- **Fast and easy processing** - Rapid format allows for the processing of multiple samples in less than 45 minutes.
- **Versatile plasma and serum input volumes** - Isolate circulating DNA from 2mL - 10 mL of plasma/serum. No requirement for extension tubes when processing larger sample volumes.
- **Concentrate circulating DNA** – Circulating DNA present in input volumes of 2 mL - 10 mL are concentrated into final elution volumes of 100 µL.
- **Isolate inhibitor-free DNA -** Purified DNA can be used in a number of sensitive downstream applications including PCR, qPCR, methylation-sensitive PCR and Southern Blot analysis.
- **Isolate all sizes of circulating DNA**- The kit allows for the isolation of all sizes of fragmented circulating DNA, ranging from high molecular weight fragments down to fragments as small as 50bps.

Applications

- Quantitative PCR
- Genotyping
- SNP analysis
- Microarray analysis
- Next Generation Sequencing

Feature	Specifications
Minimum Plasma/Serum Input	2 mL
Maximum Plasma/Serum Input	10 mL
Minimum Elution Volume	100 µL
Time to Complete Purifications	< 45 minutes

Ordering information

Cat #	Quantity
51300	10 preps

Total RNA Purification Kit — Cat. # 17200 & 37500

Specific protocol for total RNA isolation from different types of plasma or serum samples

Total RNA Purification Kit Dx — Cat. # Dx17200

CE

CE-certified kit for in-vitro diagnostic purposes - not available in all regions

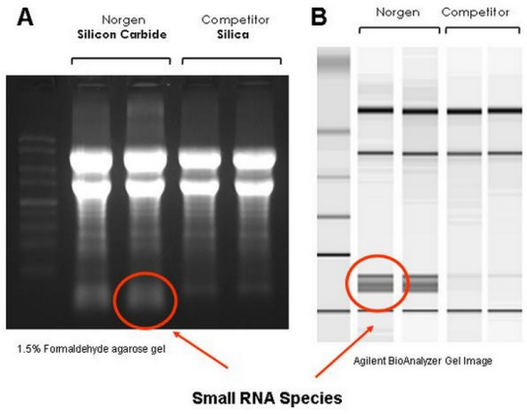
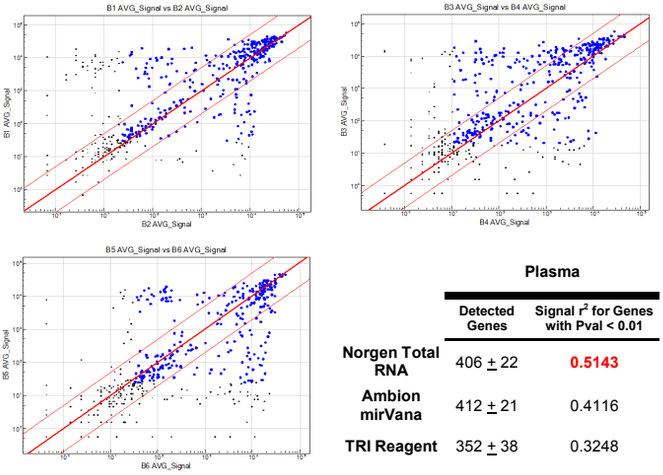


Figure 1. High Quality of Isolated RNA with Complete Size Range. Unlike most competitors' kits, Norgen's Total RNA Purification Kit allows for the isolation of all sizes of RNA, from the very large RNA down to the microRNA without the use of phenol. Total RNA was isolated from 1 x 10^9 *E. coli* cells using Norgen's Total RNA Purification Kit and a competitor's kit.
Panel A: Five microliters of the 50 µL isolated RNA was analyzed on an agarose gel
Panel B: One microliters of the 50 µL isolated RNA was analyzed on the Agilent® 2100 BioAnalyzer RNA Nano 6000 chip.
Note the presence of small RNA species (red circle) in the samples isolated via Norgen's kit and the absence of these RNA species in the competitor RNA preparation.



Plasma

	Detected Genes	Signal r^2 for Genes with Pval < 0.01
Norgen Total RNA	406 ± 22	**0.5143**
Ambion mirVana	412 ± 21	0.4116
TRI Reagent	352 ± 38	0.3248

Figure 2. Recovering Diverse miRNA Species from Plasma with Better Consistency. Total RNA including miRNA was isolated from 100 mL of mouse plasma using Norgen Total RNA Purification Kit, Ambion mirVana miRNA Kit or Sigma TRI Reagent and applied to Illumina microRNA expression profiling kit. Scatter plots displaying better consistency (better clustering) of Norgen replicate signal of Plasma RNA. Gene with Pval < 0.01 for both replicates were in blue. Norgen Protocol recovered same diversity of miRNA with better consistency (higher r^2 value).

Rapid preparation of plasma or serum total RNA - including microRNA - without phenol

Principle

Purification is based on spin column using Norgen's proprietary resin as the separation matrix. Briefly, the blood sample of interest is first lysed using the provided Lysis Solution, ethanol is added and the RNA is bound to Norgen's column. Under these conditions only the RNA will bind to Norgen's resin while most of the contaminating cellular proteins are removed in the flowthrough or retained on top of the resin. The bound RNA is then washed to remove any remaining impurities. Lastly, the purified total RNA is eluted into 50 µL of the provided Elution Buffer.

Norgen's proprietary resin provides superior affinity to the full size range of RNA molecules, resulting in large and small RNA (miRNA) purification with better linearity and sensitivity. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time RT-PCR, RT-PCR, Northern blotting, RNase protection and primer extension, expression profiling, miRNA cloning and amplification and Next Generation Sequencing.

Performance

Norgen's Total RNA Purification Kit provides a rapid method for the isolation and purification of total RNA in as little as 20 minutes. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA), without the use of phenol or chloroform. Norgen's kit is the only kit on the market that isolates true total RNA, as other kits must use phenol to recover all sizes of RNA. Therefore Norgen's kit offers significant advantages in functionality, savings on cost, ease-of-use, no hazardous organic waste, and no inhibitory effect on PCR amplifications as a result of residual phenol. With this kit both miRNA and mRNA can be studied from the same sample without further purifications, thus offering considerable advantages when comparing and relating expression of miRNA to other RNA. Furthermore, this is an excellent kit for the extraction of miRNA from all samples including plasma.

Applications

- Quantitative, real-time RT-PCR for both large mRNA and small RNA including miRNA
- RT-PCR for both large mRNA and small RNA including miRNA
- Expression profiling
- Next Generation Sequencing for RNA and miRNA
- miRNA from plasma for discovery
- microRNA cloning and amplification
- PCR-based virus detection
- PCR-based viable bacteria detection
- Northern blotting
- RNase protection
- Primer extension

Single Preparation - RNA Isolation Kits

Total RNA Purification Kit — Cat. # 17200 & 37500

Total RNA Purification Kit Dx — Cat. # Dx17200

CE-certified kit for in-vitro diagnostic purposes - not available in all regions

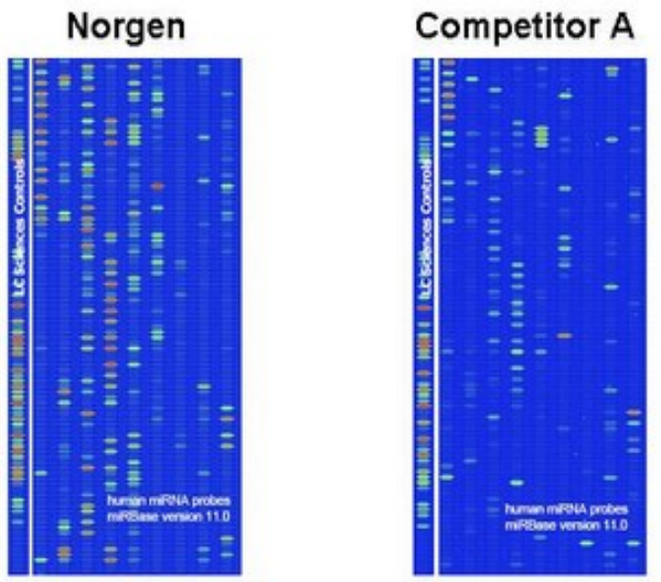


Figure 3: Better Diversity of miRNA Detected from Plasma. Norgen's Total RNA Purification Kit isolates miRNA from plasma with better diversity than a leading competitor. Total RNA including miRNA was isolated from 100 µL of plasma using Norgen's Total RNA Purification Kit or 625 µL of plasma using Competitor A's leading miRNA Kit, and was applied to an NCode expression profiling kit. Microarray images suggested that Norgen's Total RNA Purification Kit (left) isolates a better diversity of miRNA from a smaller input amount of plasma than the competitor's miRNA kit (right). Image courtesy of LC Sciences, Houston. (www.lcsciences.com).

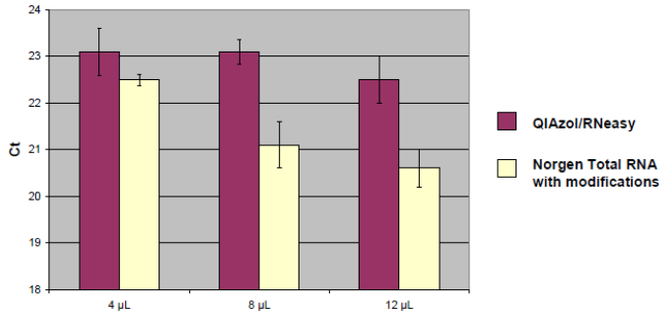


Figure 4. Comparison of Relative PCR Inhibition Observed Between Norgen's Total RNA Kit (With Modifications) and the QIAzol/RNeasy Phenol-Based Method for Plasma microRNA Isolation. Total RNA including microRNA was isolated from 200 µL of sheep plasma collected in EDTA tubes and spiked with human microRNAs. Equal portions (4, 8 or 12 µL) of isolated RNA were then used for detection of miR-16 using RT-qPCR.

Features and Benefits

- **No phenol:chloroform extractions** - Total RNA is isolated without the use of harmful chemicals such as phenol or chloroform
- **Isolate total RNA from very small samples -** Total RNA has been isolated and detected from as little as 10 µL of plasma or serum
- **Extremely efficient isolation of low abundance microRNA -** Norgen's Total RNA Purification Kit has been shown to be extremely efficient at recovering low abundance microRNA from plasma samples
- **Isolate a diversity of RNA species** - All RNA species can be isolated, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA)
- **Fast and easy processing** - Rapid spin-column format allows for the processing of 10 samples in 20 minutes
- **Isolate total RNA from a broad input source -** Total RNA has been isolated from cultured animal cells, small tissue samples, LCM samples, bacteria, yeast, fungi, plants, viruses and various bodily fluids including blood, plasma, serum, saliva, nasal or throat swabs
- **No need for carrier RNA** - Isolate all sizes of RNA without the use of carrier RNA
- **Multiple kit sizes available** - This kit is available in both 50 prep and 100 prep sizes

Feature	Specifications
Column binding capacity	Up to 50 µg RNA
Maximum Loading Volume Per Spin Column	650 µL
Size of RNA Purified	All sizes, including < 200 nt
Time to Complete 10 Purifications	20 minutes
RNA Yield 100 µL plasma or serum	1-100 ng
Format	Spin columns

Ordering information

Cat #	Quantity
17200	50 preps
Dx17200	50 preps
37500	100 preps

Application Note

Total RNA Purification Kit **Cat. # 17200 & 37500**

Effect of RNA Isolation Method on microRNA Quantity and Quality in Plasma: A Comparative Study

M. Abdalla[1], B. Lam[1], M. Simkin[1], N. Rghei[1], Y. Haj-Ahmad[1,2].
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[2]Centre for Biotechnology. Brock University, St. Catharines, Ontario, Canada

INTRODUCTION

MicroRNAs (miRNAs) are small, non-protein coding RNAs that post-transcriptionally regulate gene expression by suppression of target mRNAs (1 and 5). It has recently been demonstrated that miRNAs circulate within blood in a highly stable, cell-free form and can be detected in plasma and serum (3, 4, 5, 6, 7, 9 and 12). In addition, tumour cells have been shown to release miRNAs into the circulation (9) and profiles of miRNAs in plasma and serum have been found to be altered in cancer and other diseases (3, 8 and 12). These results indicate the potential for the use of circulating miRNAs as blood-based markers for molecular diagnostics.

In order to develop circulating miRNA-based diagnostics, the miRNAs must be measured from plasma or serum with sufficient sensitivity and precision to be clinically relevant. The small size of the mature miRNA sequence (~22 nt) and sequence homology between the mature and precursor miRNA forms has required advances in the PCR-based detection methods used for the quantitative analysis of miRNAs. These challenges have been solved by innovative solutions based on real-time, reverse-transcriptase PCR (RTqPCR) (2, 10 and 11). Adapting the RT-qPCR technologies to the detection of circulating miRNAs, however, requires modified methods of RNA extraction to permit the use of plasma or serum as a source of these biomarkers. There are also difficulties in quantifying the minute amounts of RNA typically recovered from plasma and serum, as well as the requirement for data normalization to correct for technical variations in the RNA isolation procedure.

The objective of this study is to compare two commercially available plasma miRNA kits: 1) Norgen's Total RNA Kit (Blood protocol, and modified high ethanol concentration protocol optimized for plasma), and 2) Qiagen's QIAzol extraction followed by a modified Qiagen's RNeasy Mini Kit cleanup. Comparisons were made based on:

- **miRNA Recovery**
- **Consistency**
- **PCR Inhibition**

MATERIALS AND METHODS

1) Blood Collection and Plasma Preparation

Human Plasma. Human blood was drawn directly into Citrate, EDTA and Heparin tubes in one single seating from the same individual. Two individuals were tested. Plasma was prepared according to standard procedure. No additional RNA was spiked in. Hence only endogenous microRNAs were detected.

Sheep Plasma. In order to test the robustness of the procedure, blood from a non-human species was tested. Sheep whole blood was collected in EDTA blood tubes. Sheep plasma used in experiments were spiked with microRNA fraction (~100 ng per purification) of HeLa cells isolated using Norgen's microRNA Purification Kit (Cat# 21300).

2) Plasma microRNA Purification

Silicon Carbide-Based Method with No Organic Extraction. RNA was isolated from 200 µL of plasma using Norgen's Total RNA Purification Kit (Cat# 17200) protocol for blood. A modified version was also used, where the percentage of ethanol used in the binding and washing steps was increased.

Organic Extraction. RNA was isolated from 200 µL of plasma using Qiagen's QIAzol extraction followed by a modified Qiagen's RNeasy Mini Kit cleanup (Exiqon's protocol: http://www.exiqon.com/ls/Documents/Scientific/ serum-plasma-RNA-isolation.pdf)

3) microRNA Detection

All microRNAs were converted into cDNA using Exiqon's miRCURY LNA™ Universal RT cDNA Synthesis Kit (Cat# 203300). Equal portions of purified RNA (4 µL of a 50 µL elution) were used in a 20 µL cDNA reaction. The synthesized DNA were then tested with multiple LNA microRNA primer sets from Exiqon using the Exiqon SYBR Green Master Mix (Cat# 203450). All PCRs were carried out on a Bio-Rad iCycler Real-Time PCR system.

RESULTS AND DISCUSSION

A) miRNA Recovery. Total RNA including microRNA was isolated from human plasma collected in Citrate, EDTA or Heparin tubes using Norgen's Total RNA Purification Kit either with or without modification and compared to a phenol-based method. Isolated RNA was then used for the detection of various microRNAs using RT-qPCR. **Figure 1** shows the RT-qPCR results of all three methods, with 8 different miRNA targets used, with plasma collected in Citrate. Similarly, **Figure 2** shows the RT-qPCR results of all three methods with plasma collected in EDTA. In both cases, the non-modified Norgen protocol was equivalent to, or in some cases performed better than, the phenol-based method, currently considered the gold standard. There was superior recovery of microRNA when using Norgen's modified protocol, with an average reduction in Ct values of 2-3 on all types of microRNAs, including those which are highly expressed (miR-21; miR-16) or those with very low amounts of endogenous transcripts (miR-192; miR423-5p). In addition, there was superior consistency in microRNA isolation by the modified protocol, as indicated by the small standard deviations (red arrows).

Application Note

Total RNA Purification Kit — Cat. # 17200 & 37500

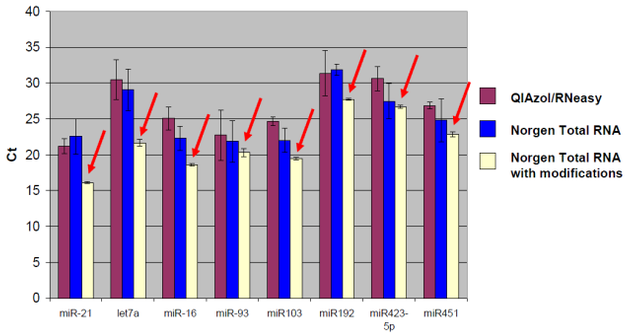

Figure 1. Plasma microRNA Recovery from Human Plasma Collected from **Citrate** Blood Tubes by Norgen's Total RNA Kit (With and Without Modifications) Compared to the QIAzol/ RNeasy Clean Up Method.

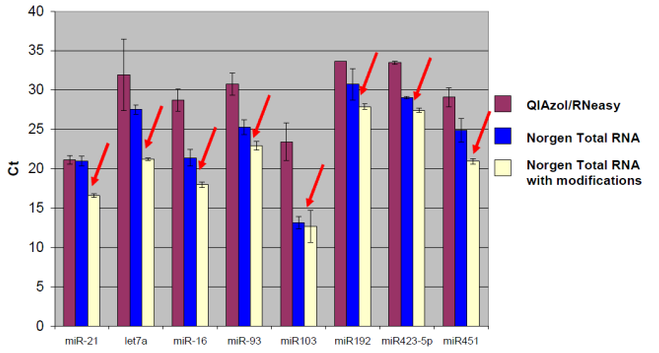

Figure 2. Plasma microRNA Recovery from Human Plasma collected from **EDTA** Blood Tubes by by Norgen's Total RNA Kit (With and Without Modifications) Compared to the QIAzol/ RNeasy Clean Up Method.

On the other hand, when Heparin was used as the anticoagulant, superior recovery was obtained when plasma microRNAs were isolated using Norgen's unmodified blood protocol (**Figure 3**). On average a 2-3 Ct value reduction (when compared to the phenol-based method) was observed on all types of microRNAs being assayed (red arrows). Norgen's unmodified protocol is also highly consistent as indicated by the small standard deviation. It was found that Norgen's modified protocol is not recommended for use with Heparin tubes. This is greatly beneficial, as most recently-reported studies (that used phenol:chloroform extractions) suggested the difficulties in using heparin plasma for any expression studies due to PCR-inhibiting heparin carryover.

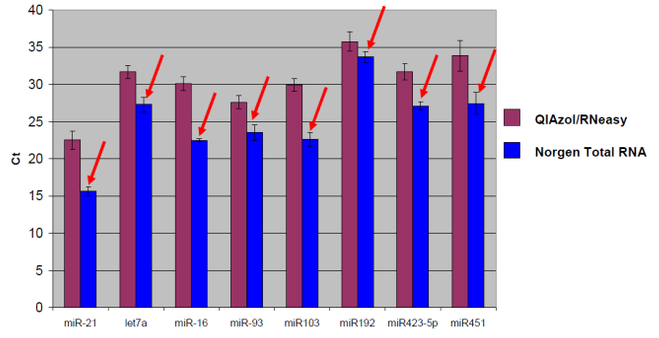

Figure 3. Plasma microRNA Recovery from Human Plasma collected from **Heparin** Blood Tubes by Norgen's Total RNA Kit (With and Without Modifications) Compared to the QIAzol/ RNeasy Clean Up Method

Next, human plasma was compared to sheep plasma for both kits (**Figure 4**). Once again, for both human plasma (**Figure 4A**) and sheep plasma (**Figure 4B**), an overall superior recovery was obtained when using Norgen's modified protocol. This indicates the robustness of the sample preparation method (see red arrows).

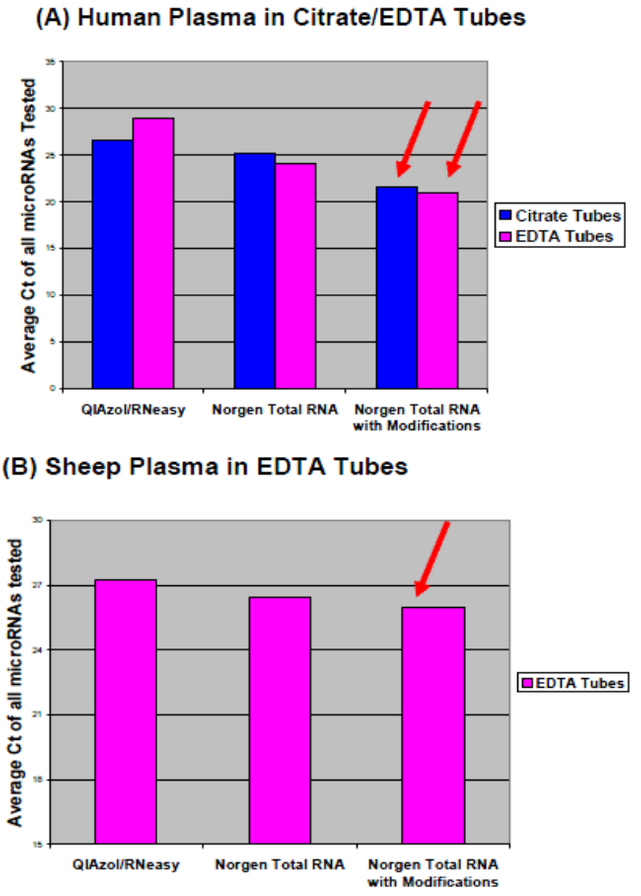

Figure 4. Recovery of Plasma microRNA from Different Species Collected in Different Blood Tubes by Norgen's Total RNA Kit (With and Without Modifications) Compared to the QIAzol/RNeasy Clean Up Method. A) Human plasma; B) Sheep plasma.

B) Consistency. Detection in changes in microRNAs in biological fluids such as plasma may contribute greatly to the diagnosis of various diseases including cancers. In order to obtain biologically meaningful results from various forms of gene expression studies (such as RT-qPCR or microarrays), it is important that the plasma microRNA isolation method is consistent. In **Figure 5**, we showed the comparison of the consistency of plasma microRNA isolated from 200 µL of human plasma collected in Citrate (**Figure 5A**), or EDTA tubes (**Figure 5B**), or sheep plasma collected in EDTA tubes (**Figure 5C**) using the various aforementioned methods. An overall superior isolation consistency was obtained using Norgen's modified protocol for all Citrate and EDTA tubes. In particular, significantly smaller standard deviations were obtained with Norgen's protocol that does not require the use of organic extraction. In contrast, due to the requirement for a two-phase separation for the phenol-based method, the inconsistency in isolation was expected.

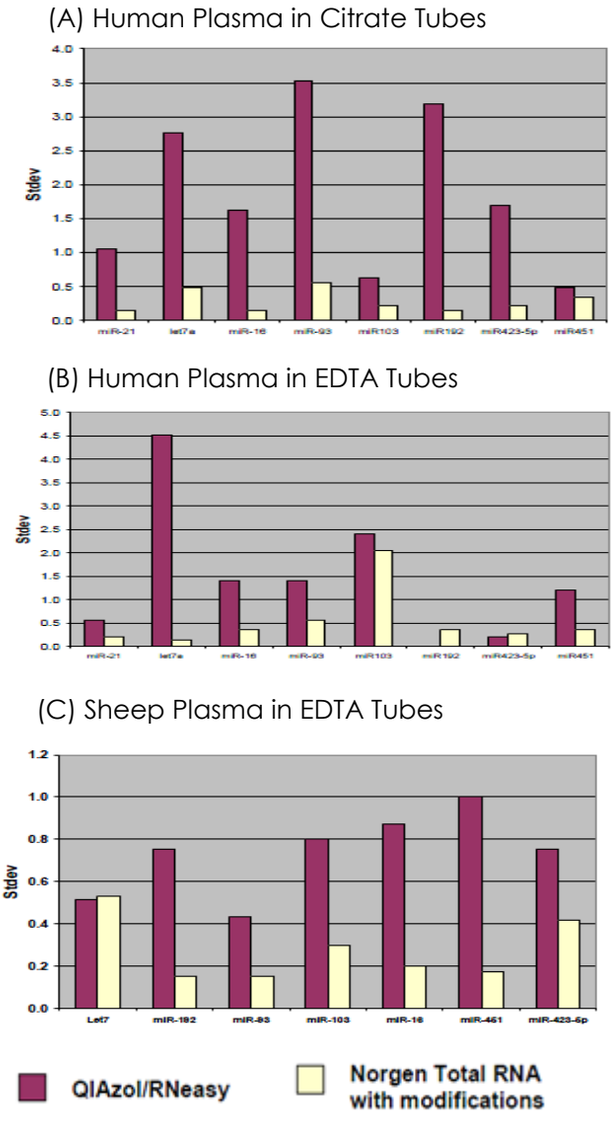

Figure 5. Consistency of Plasma microRNA Recovery from Different Species Collected in Different Blood Tubes by Norgen's Total RNA Kit (With Modifications) Compared to the QIAzol/ RNeasy Clean-Up Method.

C) PCR Inhibition. Increasing the starting amount of sample input in the RT-qPCR reaction can accurately determine which samples have higher amounts of PCR inhibitors. When Ct values do not correlate with sample input amount, this indicates PCR inhibition. **Figure 6** shows the results of increasing the starting sample volume in an RT-qPCR reaction involving Norgen-isolated and QIAzol-isolated plasma miRNA. Four, 8 and 16 µL of purified RNA were used. Superior detection of miR-16 was obtained using Norgen's modified protocol with a smaller standard deviation. Moreover, Ct values obtained using Norgen's protocol were correlated with the amount of input (i.e. higher input = lower Ct value) indicating the RNA was of high purity and lacked PCR inhibitors. In contrast, the phenol-based method had a higher average Ct value, and did not correlate with input volume, indicating possible PCR inhibition.

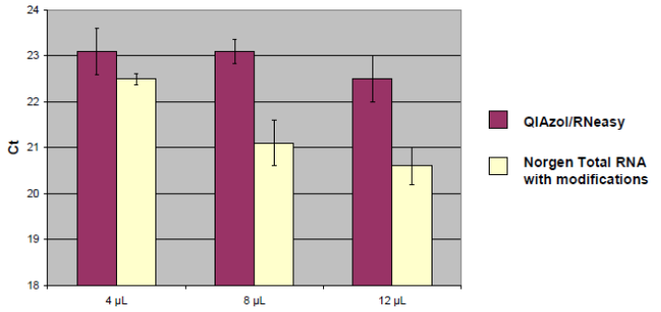

Figure 6. Comparison of Relative PCR Inhibition Observed Between Norgen's Total RNA Kit (With Modifications) and the QIAzol/RNeasy Phenol-Based Method for Plasma microRNA Isolation. Total RNA including microRNA was isolated from 200 µL of sheep plasma collected in EDTA tubes and spiked with human microRNAs. Equal portions (4, 8 or 12 µL) of isolated RNA were then used for detection of miR-16 using RT-qPCR.

CONCLUSIONS

From the data presented in this report, the following can be concluded:

Norgen's silicon carbide technology provides an efficient and effective way of isolating plasma miRNA without the use of hazardous organic extraction. The resulting advantages includes:

- **Better Consistency**
- **Better Recovery**
- **Faster Protocol**
- **High-Throughput (such as 96-wells)**

Norgen's silicon carbide technology provides a solution to overcome the main problem associated with plasma miRNA purification - sample source. A consistent microRNA isolation procedure, without the use of phenol is provided for plasma samples collected into various anticoagulants such as Citrate, EDTA and Heparin.

No PCR inhibition was observed with Norgen's silicon carbide-based plasma microRNA protocol, even for samples purified from blood tubes containing heparin, a known PCR inhibitor.

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Single Preparation - RNA Isolation Kits

Plasma/Serum Circulating and Exosomal RNA Purification Kit (Slurry Format) Cat. # 42800

Plasma/Serum Circulating and Exosomal RNA Purification Kit Cat. # Dx42800

CE-certified kit for in-vitro diagnostic purposes - not available in all regions

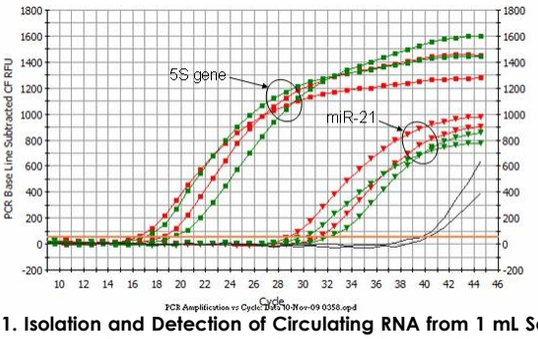

Figure 1. Isolation and Detection of Circulating RNA from 1 mL Samples of Serum and Plasma Norgen's Plasma/Serum Circulating RNA Purification Kit (Slurry Format) was used to isolate circulating RNA from 1 mL samples of serum and plasma. Five microlitres of the purified RNA was then used as the template in RT-qPCR reactions to detect the human 5S gene and the miR-21 gene. Both the 5S housekeeping gene and the miR-21 gene could be detected from plasma (red) and serum (green), indicating the high quality of the purified RNA. The black line corresponds to the no template control.

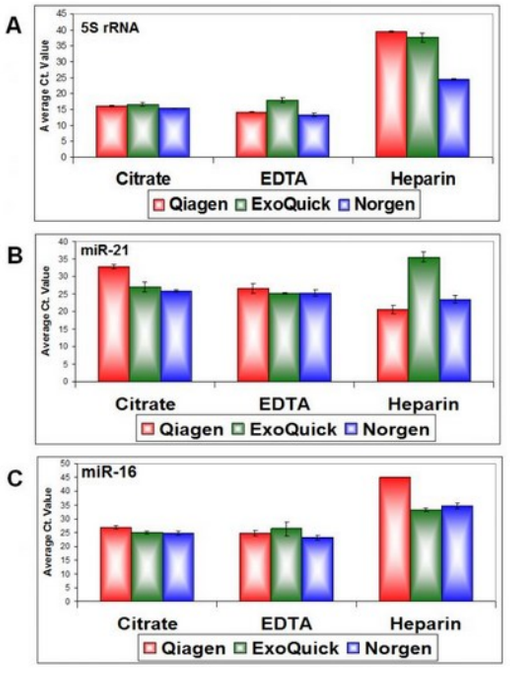

Figure 2. Effective and Consistent Detection of Plasma Exosome RNA. Norgen's Plasma/Serum Circulating and Exosomal RNA Isolation Kits can effectively isolate RNA from plasma. Plasma Exosome RNA was isolated from 500 µL of human plasma prepared from blood collected on citrate, EDTA or Heparin in triplicates using Norgen's Plasma/Serum RNA Isolation Kit (blue), a ExoQuick Exosome Precipitation Reagent (green) and Qiagen's QIAzol extraction followed by a modified Qiagen's RNeasy Mini Kit cleanup (red). Stem loop RT-qPCR using primers specific to miR-21 and miR-16 as well as the housekeeping 5S rRNA was performed. In brief, three microliters of the 100 µL isolated RNA was then subjected to a 20 µL reverse transcription using 5S rRNA, miR-21 and miR-16 stem-loop reverse primer or reveres primer. Three microliters of the reverse transcription was used in a 20 µL real-time PCR reaction with primers to detect the human miR-21, human miR-16 and the 5S rRNA. Norgen's Plasma/Serum Exosome RNA Isolation Kit is the only product that showed consistent detection of all tested transcripts with the highest quality regardless of the type of anti-coagulant used for blood collection.

Rapid and simple isolation of circulating RNA including exosomal RNA from plasma/serum samples

Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit (Slurry Format) provides a fast, reliable and simple procedure for isolating circulating RNA and exosomal RNA from plasma and serum samples ranging from 0.25 mL to 5 mL. Purification is based on spin column technology without the use of organic solvents. The kit is able to isolate all sizes of circulating and exosomal RNA, including microRNA, without the use of phenol or chloroform. The slurry format provides an advantage over other available kits in that it does not require extension tubes for the purification of free-circulating and exosomal RNA from large sample volumes. RNA can be isolated from either fresh or frozen samples using this kit. This kit is suitable for the isolation of RNA from serum or plasma prepared from blood collected only on either EDTA or citrate. Plasma samples prepared from blood collected on heparin should not be used as heparin can significantly interfere with many downstream applications including RT-PCR. The purified RNA is compatible with downstream applications.

Features and Benefits

- **Isolate all sizes of circulating RNA** - The kit allows for the isolation of all sizes of fragmented circulating RNA and exosomal RNA, including microRNA.
- **Fast and easy processing** - Rapid spin column format allows for the processing of multiple samples in under 40 minutes.
- **Versatile input volume** - Isolate circulating RNA from 0.25 - 5 mL of plasma/serum. No requirement for extension tubes when processing larger sample volumes.
- **Concentrate circulating RNA** – Circulating and exosomal RNA present in input volumes of 0.25 - 5 mL are concentrated into final elution volume of 100 µL.
- **Isolate inhibitor-free RNA** - Purified RNA can be used in a number of sensitive downstream applications.

Applications

- Quantitative, real-time RT-PCR for both large mRNA and small RNA including miRNA
- RT-PCR for both large mRNA and small RNA including miRNA
- Expression profiling
- Next Generation Sequencing for RNA and miRNA
- PCR-based pathogen detection

Feature	Specifications
Minimum Plasma/Serum Input	0.25 mL
Maximum Plasma/Serum Input	5 mL
Size of RNA Purified	All sizes, including microRNA
Time to Complete Purifications	< 40 minutes

Ordering information

Cat #	Quantity
42800	50 preps
Dx42800	50 preps

Application Note

Plasma/Serum Circulating and Exosomal RNA Purification Kit (Slurry Format) **Cat. # 42800**

Effect of RNA GC Content on RNA Purification Efficiency

Bernard Lam[1], Taha Haj-Ahmad[2], Vanja Misic[2], Moemen Abdalla[1,3], Mohamed El-Mogy[1,4], Won-Sik Kim[1], Nezar Rhgei[1] and Yousef Haj-Ahmad[1]

Unlike messenger RNA and large RNA species, a slight change in base composition in microRNA (miRNA) will drastically alter the molecules GC content and its binding preference to targets. It has been commonly assumed that standard RNA isolation methods work equally well for all miRNAs without bias. In this study, we have shown that GC content does indeed bias purification efficiency in most phenol-based methods tested, and that the best non-biased isolation product is the Silicon Carbide-based technology from Norgen Biotek. Among the advantages of this technology are the following:

1) Simple and Fast Procedure with no lengthy phenol:chloroform extractions, filtration or precipitation

2) Minimal GC Content Bias of RNA Isolation from various inputs including cells, bodily fluids and tissues

3) Sensitive Isolation of RNA from biological inputs with low RNA content including plasma, serum or urine

INTRODUCTION

MicroRNAs (miRNAs) are small RNAs (~ 22 nucleotides) that play important roles in gene regulatory networks by binding to and repressing the activity of specific target messenger RNAs (mRNAs). A large volume of basic research has been done to characterize miRNAs biology in various tissues and cell lines of model organisms. Moreover, the potential utilization of miRNAs as biomarkers in molecular diagnostics has been extensively studied. Expression profiling of miRNAs has been tested with various clinical specimens including urine, blood, serum, plasma, tumor biopsies and formalin-fixed paraffin-embedded (FFPE) tissues.

A number of procedures have been developed to purify total RNA including miRNAs from these aforementioned samples. These protocols are generally divided into two main categories; 1) traditional, phenol:chloroform extraction followed by alcohol precipitation; and 2) column-based purification (with affinity resin including silica and silicon carbide). The majority of these methods are modifications or derivatives of existing protocols for large mRNA purification which assume that all biomolecules purified are highly homogenous. However, in small molecules such as miRNAs, parameters such as GC content could vary drastically, with published data suggesting a range of 20 - 80%. The large variation of GC content could result in a diverse surface property of the molecule. Such diversity may not only affect endogenous function of the molecule but it may also impact sample preparation efficiency. In fact, it has been previously shown that there was an insufficient recovery of miRNA with low GC content using certain isolation procedures, including the traditional phenol:chloroform extraction.

As miRNA expression study has become an important part of many basic biological research areas, as well as biomarker discovery, the choice of a sample preparation method that has the best coverage (and hence least bias) is critical. Here, we evaluated the recovery of different miRNA by numerous commercially available RNA extraction methods from several important clinical sample types.

Methods of Total RNA (including miRNA) Purification Vary Drastically in Processing Time and Complexity

MicroRNAs of different GC content that were previously studied (**ref. 1**) were synthesized. The synthesized microRNAs were reconstituted in water and mixed in equal molar fashion. An input of 50 fmoles of microRNA mix was used in various RNA isolation methods including standard phenol:chloroform extraction coupled with alcohol precipitation; silica column purification (which requires phenol:chloroform separation); or Norgen's silicon carbide column purification (no phenol required). As depicted in **Figure 1**, methods involving phenol are lengthy and require multiple steps while Norgen's method using silicon carbide is simple, does not require the use of phenol and can be completed in less than 15 minutes.

Intrinsic Variation in microRNA Recovery Efficiency and GC Bias by Different RNA Isolation Methods

In order to study how each purification method recovers microRNA under the best optimized conditions, without interference from components of the sample input (such as contaminants and other biomolecules), we tested the miRNA spike-in recovery using each isolation method without any biological input (Figure **2A**). Recovery of low amounts of miRNA, particularly with low GC%, is very poor using phenol:chloroform extraction coupled with alcohol precipitation. Given the low amount of miRNA spike-in used (50 fmoles), it was not surprising that the RNA recovery by phenol:chloroform extraction was very low as the effectiveness of RNA precipitation by alcohol is concentration-dependent. Recovery of

Application Note

Plasma/Serum Circulating and Exosomal RNA Purification Kit (Slurry Format) **Cat. # 42800**

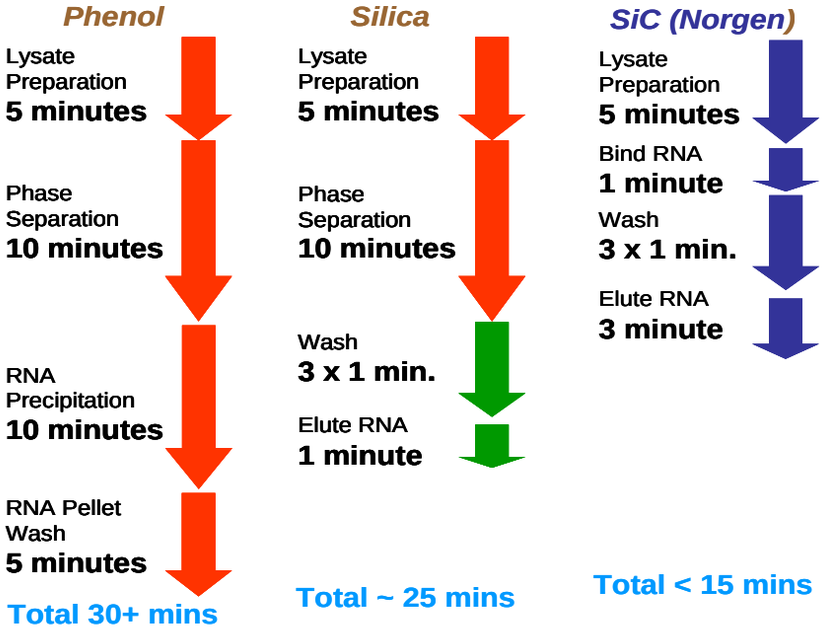

Figure 1. Outline of Different Isolation Methods for Total RNA (microRNA included).

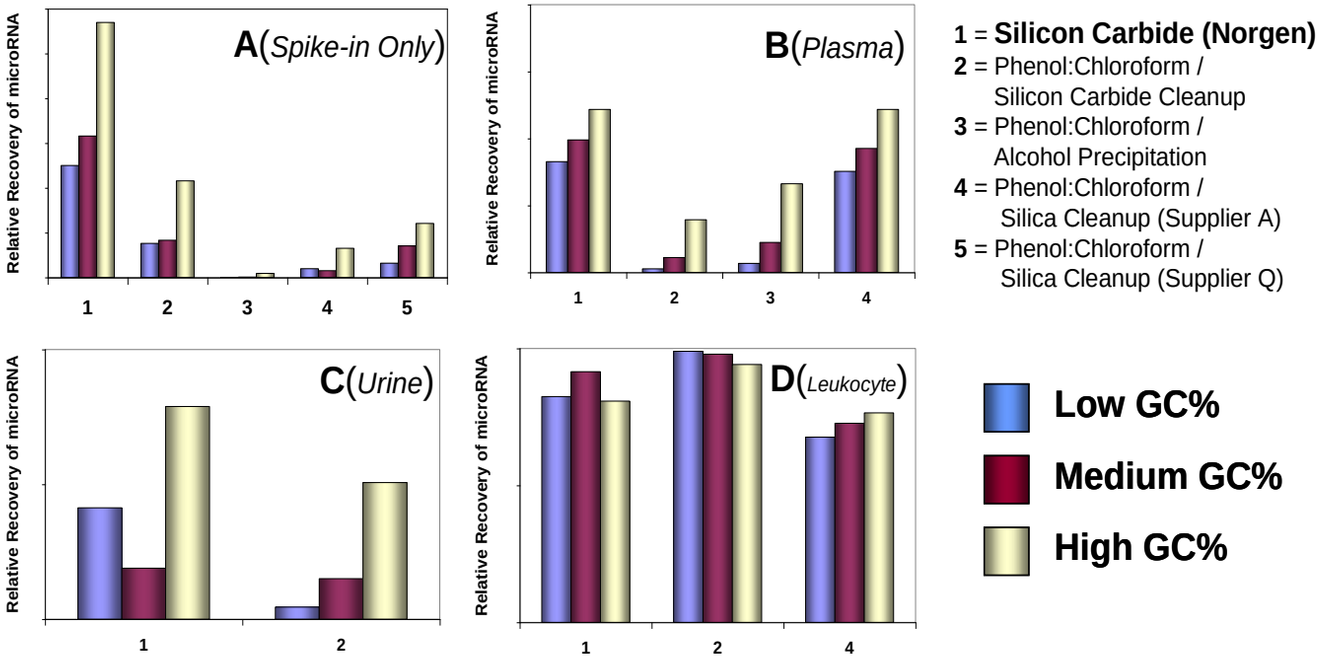

Figure 2. Recovery of microRNA of Different GC Content from Different Biological Fluids Input using Different Total RNA Isolation Methods

Plasma/Serum Circulating and Exosomal RNA Purification Kit (Slurry Format) Cat. # 42800

miRNA extracted by phenol:chloroform could be improved by using either silicon carbide or silica columns in place of precipitation, however there was some bias towards the high GC content miRNAs. More importantly, non-phenol purification of miRNA spike-in using silicon carbide columns resulted in the best recovery with minimal bias by GC content.

Difference in Recovery Efficiency of microRNA with Different GC Content from Biological Fluids (Blood, Plasma and Urine)

A tremendous amount of research has been done in blood and plasma microRNA profiling. Urine, while not as frequently studied, could be a good non-invasive alternative source. Unlike other inputs such as tissues and cells, the challenge in these biological fluids is that the RNA content is usually very low (< 1 to 100 ng per 100 mL). Here, we tested the recovery of the various spiked-in miRNAs from human plasma (Figure **2B**), human urine (Figure **2C**) and leukocyte of human blood (Figure **2D**). Good recovery with minimal GC bias was observed when input RNA content was high (such as in leukocyte). Very poor isolation was observed with phenol:chloroform extraction coupled with alcohol precipitation, particularly in plasma and urine. In contrast, the best recovery of RNA from plasma and urine was achieved by using Norgen's silicon carbide column without phenol. All methods tested for plasma and urine showed relatively lower recovery of microRNA with lower GC content. However, the non-phenol method using silicon carbide columns showed slightly better low GC microRNA recovery, mainly due to the overall higher recovery.

Reference

1. Davis et al. 2008. Computational Biology and Chemistry. **32**:222–226.

Single Preparation - RNA Isolation Kits

Plasma/Serum Circulating and Exosomal RNA Purification Mini Kit
(Slurry Format) **Cat. # 51000**

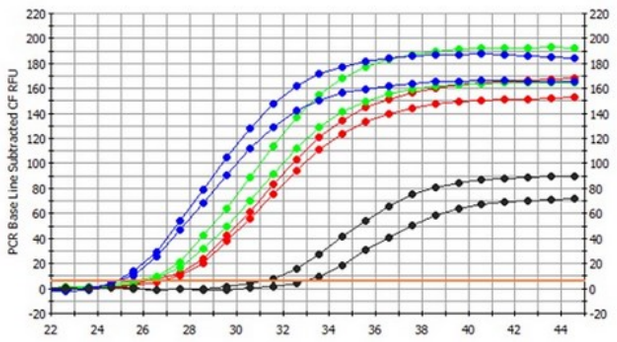

Figure 1. Isolation and Detection of Circulating RNA from Different Plasma Volumes. Norgen's Plasma/Serum Circulating RNA Purification Mini Kit (Slurry Format) was used to isolate circulating RNA from 0.5mL, 1mL and 2mL plasma. Three microlitres of the purified RNA was then used as the template in RT-qPCR reactions to detect the human 5S gene. The 5S housekeeping gene was detected from all plasma sample volumes used. The amplification of the 5S rRNA showed an increasing amount of RNA with increasing the sample input volume. This is represented by the decrease of the Ct value with increasing the sample input volume. RNA isolated from 0.5mL plasma is represented by the Red line, RNA isolated from 1mL plasma is represented by the Green line whereas RNA isolated from 2mL plasma is represented by the Blue line. The black line corresponds to the no template control.

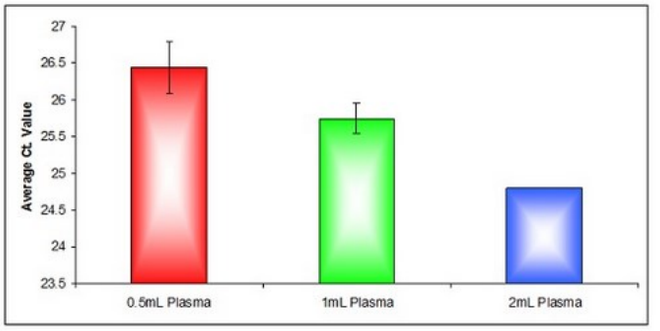

Figure 2. Effective Isolation of Plasma Circulating RNA from Different Volumes. Norgen's Plasma/Serum Circulating RNA Purification Mini Kit (Slurry Format) was used to isolate circulating RNA from 0.5mL, 1mL and 2mL plasma. Three microliters of the purified RNA was then used as the template in RT-qPCR reactions to detect the human 5S gene. The 5S housekeeping gene was detected from all plasma sample volumes used. The amplification of the 5S rRNA showed an increasing amount of RNA with increasing sample input volume. This is represented by the decrease of the Ct value with increasing the sample input volume. The average Ct value for the amplification of the 5S rRNA isolated from 0.5mL plasma is represented by the Red bar, Average Ct values for the amplification of the 5S rRNA isolated from 1mL plasma is represented by the Green bar, whereas the average Ct values for the amplification of the 5S rRNA isolated from 2mL plasma is represented by the Blue bar.

Rapid and simple isolation of circulating RNA including exosomal RNA from plasma/serum samples

Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Mini Kit (Slurry Format) provides a fast, reliable and simple procedure for isolating circulating and exosomal RNA from plasma and serum samples ranging from 0.25 mL to 2 mL. Purification is based on spin column technology without the use of organic solvents. The kit is able to isolate all sizes of circulating RNA and exosomal RNA, including microRNA, without the use of phenol or chloroform. The slurry format provides an advantage over other available kits in that it does not require extension tubes for the purification of free-circulating and exosomal RNA from large sample volumes. RNA can be isolated from either fresh or frozen samples using this kit. This kit is suitable for the isolation of RNA from serum or plasma prepared from blood collected only on either EDTA or citrate. Plasma samples prepared from blood collected on heparin should not be used as it can interfere with many downstream applications including RT-PCR. The purified RNA is compatible with downstream applications.

Features and Benefits

- **Isolate all sizes of circulating and exosomal RNA** - The kit allows for the isolation of all sizes of fragmented circulating and exosomal RNA, including microRNA.
- **Fast and easy processing -** Rapid spin column format allows for the processing of multiple samples in under 40 minutes.
- **Versatile input volume** - Isolate circulating and exosomal RNA from 0.25 - 2 mL of plasma/serum. No requirement for extension tubes when processing larger sample volumes.
- **Concentrate circulating and exosomal RNA** – Circulating and exosomal RNA present in input volumes of 0.25 - 2 mL are concentrated into final elution volume of 100 µL.
- **Isolate inhibitor-free RNA** - Purified RNA can be used in a number of sensitive downstream applications.

Applications

- Quantitative, real-time RT-PCR for both large mRNA and small RNA including miRNA
- RT-PCR for both large mRNA and small RNA including miRNA
- Expression profiling
- Next Generation Sequencing for RNA and miRNA
- PCR-based pathogen detection

Feature	Specifications
Minimum Plasma/Serum Input	0.25 mL
Maximum Plasma/Serum Input	2 mL
Size of RNA Purified	All sizes, including microRNA
Time to Complete Purifications	< 40 minutes

Ordering information

Cat #	Quantity
51000	50 preps

Plasma/Serum Circulating and Exosomal RNA Purification Maxi Kit
(Slurry Format) **Cat. # 50900**

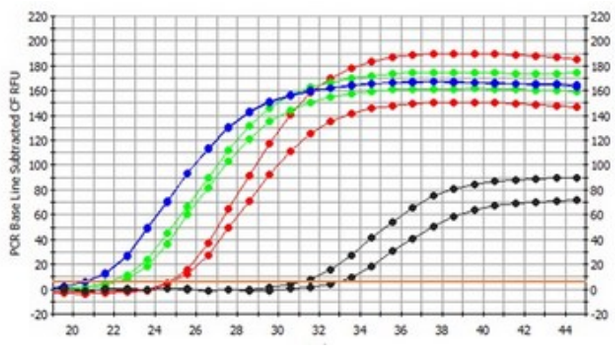

Figure 1. Isolation and Detection of Circulating RNA from Different Plasma Volumes. Norgen's Plasma/Serum Circulating RNA Purification Maxi Kit (Slurry Format) was used to isolate circulating RNA from 2mL, 3mL and 5mL plasma. Three microlitres of the purified RNA was then used as the template in RT-qPCR reactions to detect the human 5S gene. The 5S housekeeping gene was detected from all plasma sample volumes used. The amplification of the 5S rRNA showed an increasing amount of RNA with increasing the sample input volume. This is represented by the decrease of the Ct value with increasing sample input volume. RNA isolated from 2mL plasma is represented by the Red line, RNA isolated from 3mL plasma is represented by the Green line whereas RNA isolated from 5mL plasma is represented by the Blue line. The black line corresponds to the no template control.

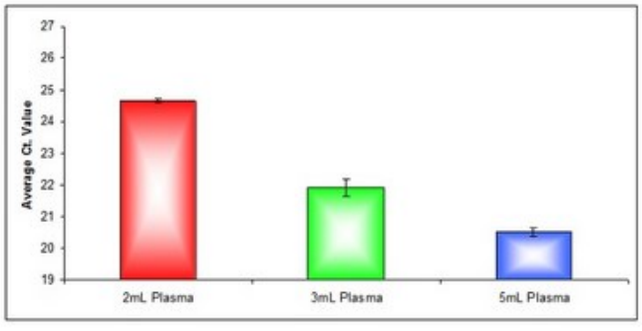

Figure 2. Effective Isolation of Plasma Circulating RNA from Different Volumes. Norgen's Plasma/Serum Circulating RNA Purification Maxi Kit (Slurry Format) was used to isolate circulating RNA from 2mL, 3mL and 5mL plasma. Three microlitres of the purified RNA was then used as the template in RT-qPCR reactions to detect the human 5S gene. The 5S housekeeping gene was detected from all plasma sample volumes used. The amplification of the 5S rRNA showed an increasing amount of RNA with increasing the sample input volume. This is represented by the decrease of the Ct value with increasing sample input volume. The average Ct value for the amplification of the 5S rRNA isolated from 2mL plasma is represented by the Red bar, the average Ct value for the amplification of the 5S rRNA isolated from 3mL plasma is represented by the Green bar, whereas the average Ct. values for the amplification of the 5S rRNA isolated from 5mL plasma is represented by the blue bar.

Rapid and simple isolation of circulating RNA including exosomal RNA from plasma/serum samples

Norgen's Plasma/Serum Circulating and Exosomal RNA Purification Kit (Slurry Format) provides a fast, reliable and simple procedure for isolating circulating and exosomal RNA from plasma and serum samples ranging from 2 mL to 5 mL. Purification is based on spin column technology without the use of organic solvents. The kit is able to isolate all sizes of circulating and exosomal RNA, including microRNA, without the use of phenol or chloroform. The slurry format provides an advantage over other available kits in that it does not require extension tubes for the purification of free-circulating and exosomal RNA from large sample volumes. RNA can be isolated from either fresh or frozen samples using this kit. This kit is suitable for the isolation of RNA from serum or plasma prepared from blood collected only on either EDTA or citrate. Plasma samples prepared from blood collected on heparin should not be used as heparin can significantly interfere with many downstream applications including RT-PCR. The purified RNA is compatible with downstream applications.

Features and Benefits

- **Isolate all sizes of circulating and exosomal RNA** - The kit allows for the isolation of all sizes of fragmented circulating and exosomal RNA, including microRNA.
- **Fast and easy processing** - Rapid spin column format allows for the processing of multiple samples in under 40 minutes.
- **Versatile input volume** - Isolate circulating and exosmal RNA from 2 - 5 mL of plasma/serum. No requirement for extension tubes when processing larger sample volumes.
- **Concentrate circulating and exosomal RNA** – Circulating and exosomal RNA present in input volumes of 2 - 5 mL are concentrated into final elution volume of 100 µL.
- **Isolate inhibitor-free RNA** - Purified RNA can be used in a number of sensitive downstream applications.

Applications

- Quantitative, real-time RT-PCR for both large mRNA and small RNA including miRNA
- RT-PCR for both large mRNA and small RNA including miRNA
- Expression profiling
- Next Generation Sequencing for RNA and miRNA
- PCR-based pathogen detection

Feature	Specifications
Minimum Plasma/Serum Input	2 mL
Maximum Plasma/Serum Input	5 mL
Size of RNA Purified	All sizes, including microRNA
Time to Complete Purifications	< 40 minutes

Ordering information

Cat #	Quantity
50900	25 preps

Plasma/Serum Circulating Nucleic Acid Purification Mini Kit
(Slurry Format)

Cat. # 53300

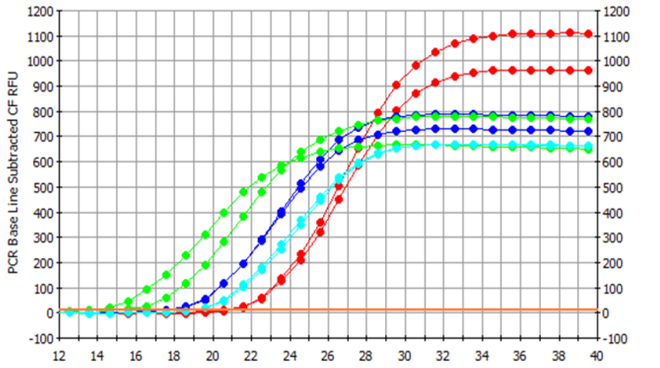

Figure 1. Isolation and Detection of Circulating RNA from Different Plasma Volumes. Norgen's Plasma/Serum Circulating Nucleic Acid Purification Mini Kit (Slurry Format) was used to isolate circulating RNA from 0.25mL, 0.5mL, 1mL and 2mL plasma. Three microlitres of the purified RNA was then used as the template in RT-qPCR reactions to detect the human 5S gene. The 5S housekeeping gene was detected from all plasma sample volumes used. The amplification of the 5S rRNA showed an increasing amount of RNA with increasing the sample input volume. This is represented by the decrease of the Ct value with increasing the sample input volume.

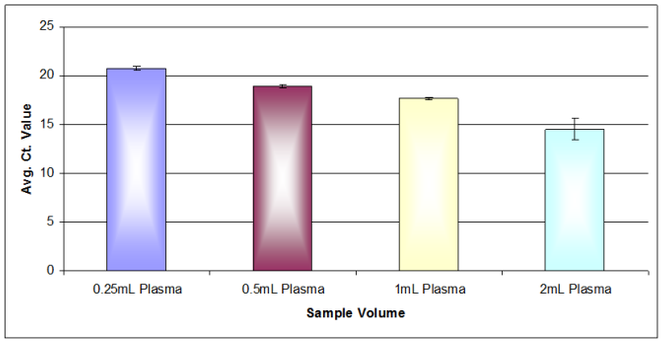

Figure 2. Effective Isolation of Plasma Circulating RNA from Different Volumes. Norgen's Plasma/Serum Circulating Nucleic Acid Purification Mini Kit (Slurry Format) was used to isolate circulating RNA from 0.25mL, 0.5mL, 1mL and 2mL plasma. Three microlitres of the purified RNA was then used as the template in RT-qPCR reactions to detect the human 5S gene. The 5S housekeeping gene was detected from all plasma sample volumes used. The amplification of the 5S rRNA showed an increasing amount of RNA with increasing the sample input volume. This is represented by the decrease of the Ct value with increasing the sample input volume.

Rapid and simple isolation of circulating nucleic acid from plasma/serum samples

Principle

Purification is based on the use of Norgen's proprietary resin as the separation matrix. The kit is able to isolate all sizes of circulating RNA, including microRNA, as well as all sizes of exosomal RNA and circulating DNA. Norgen's Plasma/Serum Circulating Nucleic Acid Purification Mini Kit provides an advantage over other available kits in that it does not require extension tubes for the purification of free-circulating NA from large sample volumes. Free-Circulating NA can be isolated from either fresh or frozen samples using this kit, and the kit allows for the concentration of NA that is present in low concentrations (1-100ng/mL in human plasma). Typical yields of free-circulating NA will vary depending on the input sample, as the amount of NA present in plasma and serum will depend upon the health status of the individual and the level of nucleases present in the blood.

Performance

Norgen's Plasma/Serum Circulating Nucleic Acid Purification Mini Kit provides a fast, reliable and simple procedure for isolating circulating Nucleic Acid (mRNA, miRNA, exosomal RNA, Viral RNA, DNA and Viral DNA) from various amounts of plasma/serum ranging from 0.25 mL to 2 mL. Free-circulating nucleic acids in plasma and serum have the potential to provide biomarkers for certain cancers and disease states, and include tumor-specific extracellular nucleic acid fragments and fetal DNA in maternal blood. Free-circulating DNA is usually present as short fragments of less than 1000 bp, whereas free-circulating RNA and exosomal RNA in plasma and serum are usually present as short fragments of less than 1000 nt. In addition, plasma or serum also contains free-circulating miRNA that are as small as 21 nt. Furthermore, circulating viral DNA or viral RNA may be present in the plasma or the serum of viral-infected patients. The Kit provides an efficient method for the purification of all sizes of these fragmented free-circulating Nucleic Acid from human plasma or serum. It is suitable for the purification of Nucleic Acid from serum or plasma prepared from blood collected on either EDTA or citrate. Plasma samples prepared from blood collected on heparin should not be used as heparin can significantly interfere with many downstream applications such as RT-PCR.

Preparation time for 10 samples is less than 40 minutes. The purified plasma/serum free-circulating Nucleic Acid are eluted in an elution solution that is compatible with PCR, qPCR, reverse transcription qPCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, expression array assays, methylation-sensitive PCR and Southern Blot analysis

Plasma/Serum Circulating Nucleic Acid Purification Mini Kit
(Slurry Format)

Cat. # 53300

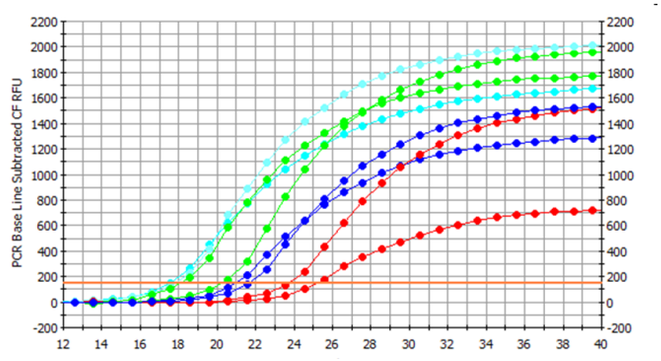

Figure 3. Isolation and Detection of Circulating DNA from Different Plasma Volumes. Norgen's Plasma/Serum Circulating Nucleic Acid Purification Mini Kit (Slurry Format) was used to isolate circulating DNA from 0.25mL, 0.5mL, 1mL and 2mL plasma. Three microlitres of the purified DNA was then used as the template in qPCR reactions to detect the human 5S rRNA gene. The 5S housekeeping gene was detected from all plasma sample volumes used. The amplification of the 5S rRNA gene showed an increasing amount of DNA with increasing the sample input volume. This is represented by the decrease of the Ct value with increasing the sample input volume.

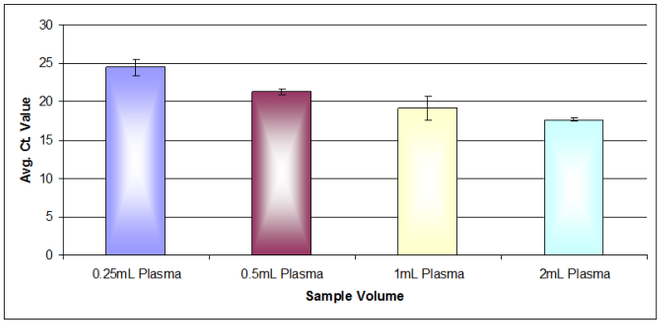

Figure 4. Effective Isolation of Plasma Circulating DNA from Different Volumes. Norgen's Plasma/Serum Circulating Nucleic Acid Purification Mini Kit (Slurry Format) was used to isolate circulating DNA from 0.25mL, 0.5mL, 1mL and 2mL plasma. Three microlitres of the purified DNA was then used as the template in qPCR reactions to detect the human 5S rRNA gene. The 5S housekeeping gene was detected from all plasma sample volumes used. The amplification of the 5S rRNA gene showed an increasing amount of DNA with increasing the sample input volume. This is represented by the decrease of the Ct value with increasing the sample input volume.

Features and Benefits

- **Isolate all sizes of circulating RNA and DNA in one elution** - The kit allows for the isolation of all sizes of fragmented circulating RNA and exosmal RNA, including microRNA as well as all sizes of circulating DNA
- **Fast and easy processing** - Rapid slurry format allows for the processing of multiple samples in under 40 minutes.
- **Versatile input volume** - Isolate circulating RNA from 0.25 - 2 mL of plasma/serum. No requirement for extension tubes when processing larger sample volumes.
- **Concentrate circulating Nucleic Acid**– Circulating and exosomal Nucleic Acid present in input volumes of 0.25 - 2 mL are concentrated into final elution volume of 100 µL.
- **Isolate inhibitor-free Nucleic Acid** - Purified Nucleic Acid can be used in a number of sensitive downstream applications including PCR, qPCR, reverse transcription qPCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, expression array assays, methylation-sensitive PCR and Southern Blot analysisapplications

Feature	Specifications
Minimum Plasma/Serum Input	0.25 mL
Maximum Plasma/Serum Input	2 mL
Size of RNA Purified	All sizes, including microRNA
Time to Complete Purifications	< 40 minutes
Elution Volume	50-100 µL

Ordering information

Cat #	Quantity
53300	50 preps

Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit
(Slurry Format)

Cat. # 53400

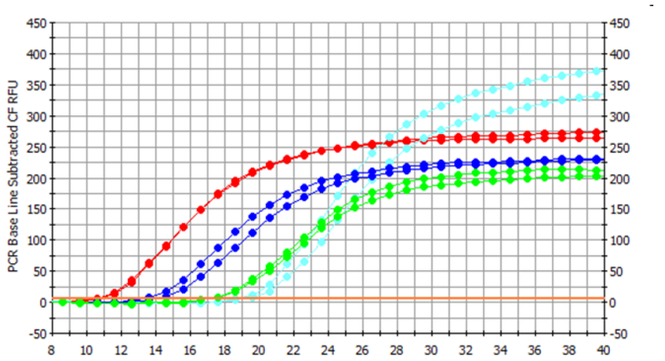

Figure 1. Isolation and Detection of Circulating RNA from Different Plasma Volumes. Norgen's Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit (Slurry Format) was used to isolate circulating RNA from 2mL, 3mL, 4mL and 5mL plasma. Three microlitres of the purified RNA was then used as the template in RT-qPCR reactions to detect the human 5S gene. The 5S housekeeping gene was detected from all plasma sample volumes used. The amplification of the 5S rRNA showed an increasing amount of RNA with increasing the sample input volume. This is represented by the decrease of the Ct value with increasing the sample input volume.

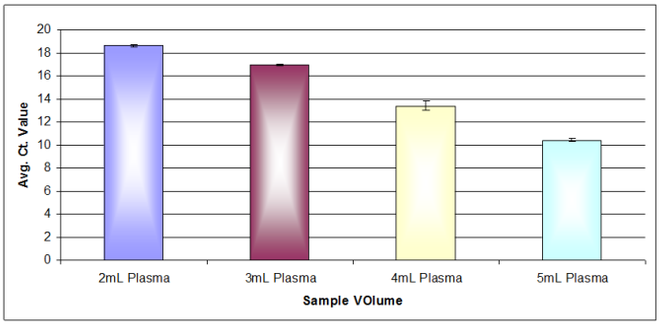

Figure 2. Effective Isolation of Plasma Circulating RNA from Different Volumes. Norgen's Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit (Slurry Format) was used to isolate circulating RNA from 2mL, 3mL, 4mL and 5mL plasma. Three microlitres of the purified RNA was then used as the template in RT-qPCR reactions to detect the human 5S gene. The 5S housekeeping gene was detected from all plasma sample volumes used. The amplification of the 5S rRNA showed an increasing amount of RNA with increasing the sample input volume. This is represented by the decrease of the Ct value with increasing the sample input volume.

Rapid and simple isolation of circulating nucleic acid from plasma/serum samples

Principle

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Performance

Norgen's Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit provides a fast, reliable and simple procedure for isolating circulating Nucleic Acid (mRNA, miRNA, exosomal RNA, Viral RNA, DNA and Viral DNA) from various amounts of plasma/serum ranging from 2 mL to 5 mL. Free-circulating nucleic acids in plasma and serum have the potential to provide biomarkers for certain cancers and disease states, and include tumor-specific extracellular nucleic acid fragments and fetal DNA in maternal blood. Free-circulating DNA is usually present as short fragments of less than 1000 bp, whereas free-circulating RNA and exosomal RNA in plasma and serum are usually present as short fragments of less than 1000 nt. In addition, plasma or serum also contains free-circulating miRNA that are as small as 21 nt. Furthermore, circulating viral DNA or viral RNA may be present in the plasma or the serum of viral-infected patients. Norgen's Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit provides an efficient method for the purification of all sizes of these fragmented free-circulating Nucleic Acid from human plasma or serum. This kit is suitable for the purification of Nucleic Acid from serum or plasma prepared from blood collected on either EDTA or citrate. Plasma samples prepared from blood collected on heparin should not be used as heparin can significantly interfere with many downstream applications such as RT-PCR.

Preparation time for 10 samples is less than 40 minutes. The purified plasma/serum free-circulating Nucleic Acid are eluted in an elution solution that is compatible with PCR, qPCR, reverse transcription qPCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, expression array assays, methylation-sensitive PCR and Southern Blot analysis.

Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit
(Slurry Format)

Cat. # 53400

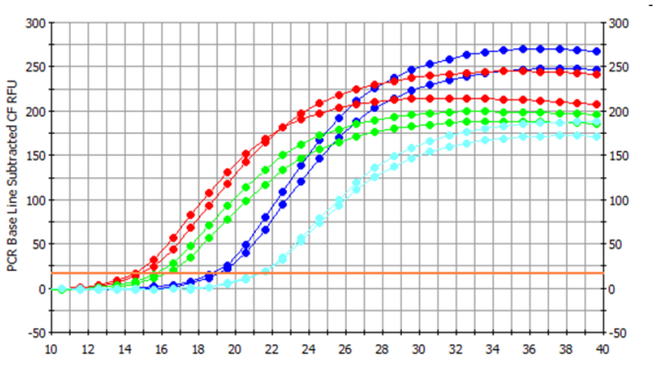


Figure 3. Isolation and Detection of Circulating DNA from Different Plasma Volumes. Norgen's Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit (Slurry Format) was used to isolate circulating DNA from 2mL, 3mL, 4mL and 5mL plasma. Three microlitres of the purified DNA was then used as the template in qPCR reactions to detect the human 5S rRNA gene. The 5S housekeeping gene was detected from all plasma sample volumes used. The amplification of the 5S rRNA gene showed an increasing amount of DNA with increasing the sample input volume. This is represented by the decrease of the Ct value with increasing the sample input volume.

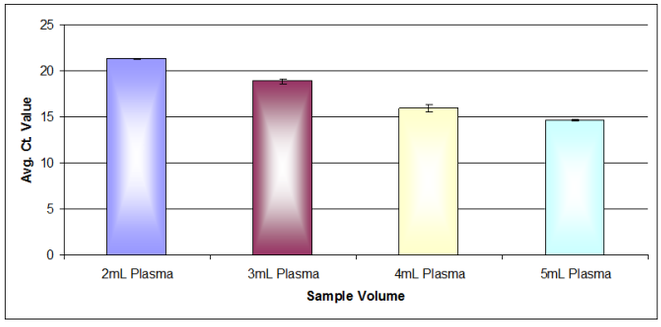


Figure 4. Effective Isolation of Plasma Circulating DNA from Different Volumes. Norgen's Plasma/Serum Circulating Nucleic Acid Purification Maxi Kit (Slurry Format) was used to isolate circulating DNA from 2mL, 3mL, 4mL and 5mL plasma. Three microlitres of the purified DNA was then used as the template in qPCR reactions to detect the human 5S rRNA gene. The 5S housekeeping gene was detected from all plasma sample volumes used. The amplification of the 5S rRNA gene showed an increasing amount of DNA with increasing the sample input volume. This is represented by the decrease of the Ct value with increasing the sample input volume.

Features and Benefits

- **Isolate all sizes of circulating RNA and DNA in one elution** - The kit allows for the isolation of all sizes of fragmented circulating RNA and exosmal RNA, including microRNA as well as all sizes of circulating DNA
- **Fast and easy processing** - Rapid slurry format allows for the processing of multiple samples in under 40 minutes.
- **Versatile input volume** - Isolate circulating RNA from 2 - 5 mL of plasma/serum. No requirement for extension tubes when processing larger sample volumes.
- **Concentrate circulating Nucleic Acid**– Circulating and exosomal Nucleic Acid present in input volumes of 2 - 2 mL are concentrated into final elution volume of 100 µL.
- **Isolate inhibitor-free Nucleic Acid** - Purified Nucleic Acid can be used in a number of sensitive downstream applications including PCR, qPCR, reverse transcription qPCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, expression array assays, methylation-sensitive PCR and Southern Blot analysis

Feature	Specifications
Minimum Plasma/Serum Input	2 mL
Maximum Plasma/Serum Input	5 mL
Size of RNA Purified	All sizes, including microRNA
Time to Complete Purifications	< 40 minutes
Elution Volume	50-100 µL

Ordering information

Cat #	Quantity
53400	25 preps

High Throughput RNA Preparation

Plasma/Serum Circulating RNA Purification 96-Well Kit
(Slurry Format)

Cat. # 29500

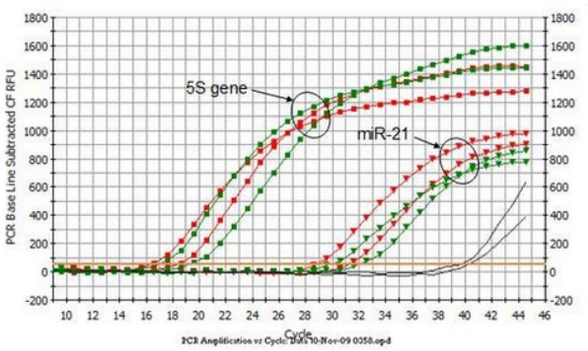


Figure 1. Isolation and Detection of Circulating RNA from 1 mL Samples of Serum and Plasma Norgen's Plasma/Serum Circulating RNA Purification 96-Well Kit was used to isolate circulating RNA from 1 mL samples of serum and plasma. Five microlitres of the purified RNA was then used as the template in RT-qPCR reactions to detect the human 5S gene and the miR-21 gene. Both the 5S housekeeping gene and the miR-21 gene could be detected from plasma (red) and serum (green), indicating the high quality of the purified RNA. The black line corresponds to the no template control.

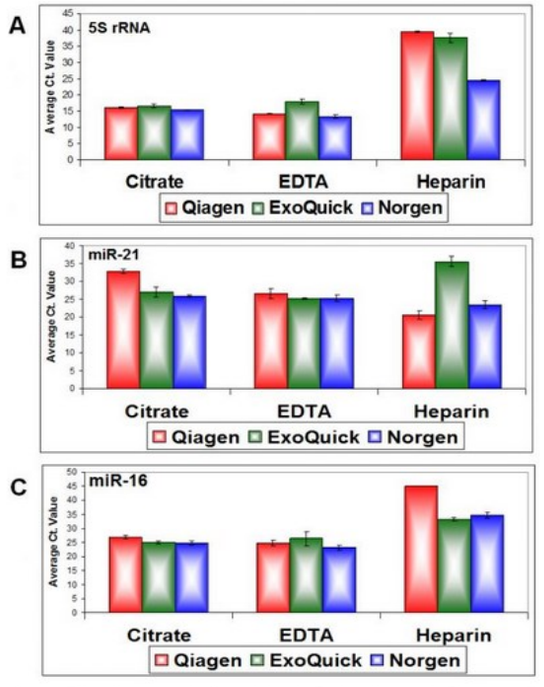


Figure 2. Effective and Consistent Detection of Plasma Exosome RNA. Norgen's Plasma/Serum Circulating and Exosomal RNA Isolation Kits can effectively isolate RNA from plasma. Plasma Exosome RNA was isolated from 500 µL of human plasma prepared from blood collected on citrate, EDTA or heparin in triplicates using Norgen's Plasma/Serum RNA Isolation Kit (blue), a ExoQuick Exosome Precipitation Reagent (green) and Qiagen's QIAzol extraction followed by a modified Qiagen's RNeasy Mini Kit cleanup (red). Stem loop RT-qPCR using primers specific to miR-21 and miR-16 as well as the housekeeping 5S rRNA was performed. In brief, 3 µL of the 100 µL isolated RNA was then subjected to a 20 µL reverse transcription using 5S rRNA, miR-21 and miR-16 stem-loop reverse primer or reveres primer. Three microliters of the reverse transcription was used in a 20 µL real-time PCR reaction with primers to detect the human miR-21, human miR-16 and the 5S rRNA. Norgen's Plasma/Serum Exosome RNA Isolation Kit is the only product that showed consistent detection of all tested transcripts with the highest quality regardless of the type of anti-coagulant used for blood collection.

High-throughput isolation of circulating RNA including exosomal RNA from plasma/serum samples

Norgen's Plasma/Serum Circulating RNA Purification 96-Well Kit (Slurry Format) provides a fast, reliable and simple procedure for isolating circulating and exosomal RNA from plasma and serum samples ranging from 0.25 mL to 2 mL. Purification is based on 96-well spin column technology without the use of organic solvents. The kit is able to isolate all sizes of circulating and exosomal RNA, including microRNA, without the use of phenol or chloroform. The slurry format provides an advantage over other available kits in that it does not require extension tubes for the purification of free -circulating and exosomal RNA from large sample volumes. RNA can be isolated from either fresh or frozen samples using this kit. The kit allows for the concentration of RNA that is present in low concentrations (1-100ng/mL circulating RNA in human plasma). The purified RNA is compatible with downstream applications.

Features and Benefits

- **Isolate all sizes of circulating RNA** - The kit allows for the isolation of all sizes of fragmented circulating and exosomal RNA, including microRNA.
- **Fast and easy processing** - Rapid format allows for the processing of 96 samples in 1 hour.
- **Versatile input volume** - Isolate circulating and exosomal RNA from 0.25 - 2 mL of plasma/serum. No requirement for extension tubes when processing larger sample volumes.
- **Concentrate circulating and exosomal RNA** – Circulating and exosmal RNA present in input volumes of 0.25 - 2 mL are concentrated into final elution volumes of 100 µL.
- **Isolate inhibitor-free RNA** - Purified RNA can be used in a number of sensitive downstream applications.

Applications

- Quantitative, real-time RT-PCR for both large mRNA and small RNA including miRNA
- RT-PCR for both large mRNA and small RNA including miRNA
- Expression profiling
- Next Generation Sequencing for RNA and miRNA
- PCR-based pathogen detection

Feature	Specifications
Minimum Plasma/Serum Input	0.25 mL
Maximum Plasma/Serum Input	2 mL
Size of RNA Purified	All sizes, including microRNA
Time to Complete Purifications	< 60 minutes

Ordering information

Cat #	Quantity
29500	1 x 96-well plate

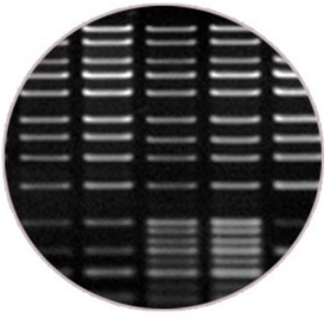
Contract R&D and Custom Purification Services

Professional and experienced scientists

High Quality **Buffers and Chemicals**

Quality assured products prepared in a sterile environment

Quality control to verify the purity and functionality of our reagents



Convenient. Ready-to-Use. High Quality Buffers and Reagents. Reduce

1. DNA and RNA Solutions and Reagents

DNA and RNA Gel Electrophoresis Solutions	Size	Catalogue #
50X TAE	500 mL, 1 L	28010, 28011
10X TBE	500 mL, 1 L	28012, 28013
10X MOPS (RNAse-Free)	500 mL, 1 L	28123, 28124
10 mg/mL Ethidium Bromide	10 mL	28033
40% Acrylamide/Bis-Acrylamide (19:1)	500 mL	28159

DNA and RNA Electrophoresis Loading Dyes	Size	Catalogue #
6X Orange-G DNA Loading Dye	5 x 1 mL	28129
6X Cresol-Red DNA Loading Dye	5 x 1 mL	28130
6X Bromophenol Blue DNA Loading Dye	5 x 1 mL	13526
6X Xylene Cyanol Dye DNA Loading Dye	5 x 1 mL	28132
6X Orange-G/Cresol-Red DNA Loading Dye	5 x 1 mL	28131
6X Bromophenol Blue/Xylene Cyanol DNA Loading Dye	5 x 1 mL	28151
2X TBE-Urea Sample Buffer	5 x 1 mL	28194
2X RNA Sample Loading Buffer	0.5 mL	15096

DNA and RNA Blotting Solutions	Size	Catalogue #
20X SSC Solution (RNase-free)	500 mL	28157
20X SSPE Solution (RNase-free)	500 mL	28158
50X Denhardt's Reagent	100 mL	28195
1X BLOTTO	100 mL	28196

DNA and RNA Utility Solutions	Size	Catalogue #
0.5M EDTA pH 8.0 (RNase-free)	100 mL	28140
0.1 mM EDTA pH 7.0 (RNase-free)	100 mL	28147
3M Potassium Acetate pH 5.5 (RNase-free)	100 mL	28141
3M Sodium Acetate pH 5.5 (RNase-free)	100 mL	28139
5M Ammonium Acetate (RNase-free)	100 mL	28142
7.5M LiCl (RNase-free)	100 mL	28152
10% SDS (RNase-free)	100 mL	28143
20% SDS (RNsae-free)	100 mL	28144
1M Tris pH 7.0 (RNase-free)	100 mL	28148
1M Tris pH 8.0 (RNase-free)	100 mL	28149
TE Buffer pH 7.0 (RNase-free)	100 mL	28145
TE Buffer pH 8.0 (RNase-free)	100 mL	28146
Adenosine 5'-triphosphate (ATP) Solution, 100 mM	0.25 mL, 10 x 0.25 mL	28284, 28285

Enzymes	Size	Catalogue #
RNase-free DNase I Kit	1 x 6mL (20mg/mL)	25710
Proteinase K in Storage Buffer	5 x 1mL (20mg/mL)	28229
Lysozyme	1 g, 5 g	41800, 41805

Save Time. Standardize Gel Runs. Reliability and Consistency. Quality Controlled Lots.

Powdered Chemicals	Size	Catalogue #
Tris Base - Biotechnology Grade	500 g, 1 kg	28052, 28029
EDTA - Disodium Salt, Dihydrate, Biotechnology Grade	100 g, 500 g	28053, 28030
Agarose - Biotechnology Grade	100 g, 500 g	28034, 28035
Urea	500 g	28054

2. Protein Solutions and Reagents

Gel Electrophoresis Reagents	Size	Catalogue #
10X Tris-Glycine SDS Running Buffer	1 L, 4 L	28120, 28207
10X Tris-Glycine Running Buffer (Native Gels)	1 L, 4 L	28122, 28208
40% Acrylamide/Bis-Acrylamide (37.5:1)	500 mL	28161
40% Acrylamide/Bis-Acrylamide (29:1)	500 mL	28160
10X Tris-Tricine SDS Running Buffer	500 mL	28162
Gel Loading Dyes	**Size**	**Catalogue #**
3X SDS-PAGE Sample Buffer	5 x 1 mL	28156
3X SDS-PAGE Sample Buffer (Dual Dye)	5 x 1 mL	28168
Gel Staining Solutions	**Size**	**Catalogue #**
Coomassie Blue R-250 Staining Solution	500 mL, 1 L	28133, 28134
Coomassie Blue R-250 Destain Solution	500 mL, 1 L	28135, 28136
Blotting Reagents	**Size**	**Catalogue #**
10X Tris Glycine Western Blotting Buffer	100 mL, 500 mL	28153, 28154
10X Carbonate Western Transfer Buffer	100 mL, 500 mL	28166, 28176
Powdered Chemicals	**Size**	**Catalogue #**
SDS - Biotechnology Grade	100 g	28031
Glycine - Biotechnology Grade	500 g, 1 kg	28116, 28032
Protein Quantification	**Size**	**Catalogue #**
Protein Standard Solution (0.5mg/mL BSA)	4 x 1.5mL	28236
Protein Expression	**Size**	**Catalogue #**
IPTG Solution (100 mM)	6 x 1.5 mL	28232
Detergents	**Size**	**Catalogue #**
Triton X-100	100 mL	41810

3. Cell Lysis Solutions

Cell Lysis Solutions	Size	Catalogue #
STET Lysis Solution	100 mL, 500 mL	28189, 28190
RIPA Lysis Solution	100 mL, 500 mL	28191, 28192
RBC Lysis Solution	90 mL	21201

4. ELISA Solutions and Reagents

ELISA Solution	Size	Catalogue #
10X Tris Buffered Saline	500 mL	28173
10X Tris Buffered Saline with Tween 20 (TBST)	500 mL	28163
10X Phosphate Buffered Saline	500 mL	28174
10X Phosphate Buffered Saline with Tween 20 (PBST)	500 mL	28175
ELISA Coating Buffer	100 mL	28164
ELISA Coating Buffer for Plant	100 mL	28165
ELISA Blocking Buffer (BSA in PBS)	100 mL, 500 mL	28177, 28178
ELISA Blocking Buffer (Skim Milk Powder in PBS)	100 mL, 500 mL	28179, 28180
ELISA Blocking Buffer (Casein in PBS)	100 mL, 500 mL	28181, 28182
ELISA Blocking Buffer (BSA in TBS)	100 mL, 500 mL	28183, 28184
ELISA Blocking Buffer (Skim Milk Powder in TBS)	100 mL, 500 mL	28185, 28186
ELISA Blocking Buffer (Casein in TBS)	100 mL, 500 mL	28187, 28188
ELISA Loading Buffer	100 mL	28169
ELISA Plant Loading Buffer	100 mL	28170
ELISA Substrate Buffer for AP	100 mL	28171
ELISA Substrate Buffer for HRP	100 mL	28172
o-Phenylenediamine	50 g	28206
p-Nitrophenyl Phosphate	5 g	28037

5. Microbiology Media

General Bacterial Growth Media	Size	Catalogue #
Luria-Bertani Media (LB)	500 mL	28200
Terrific Broth (TB)	500 mL	28201
Peptone Water	500 mL	28199
Tryptic Soy Broth (TSB)	500 mL	28198

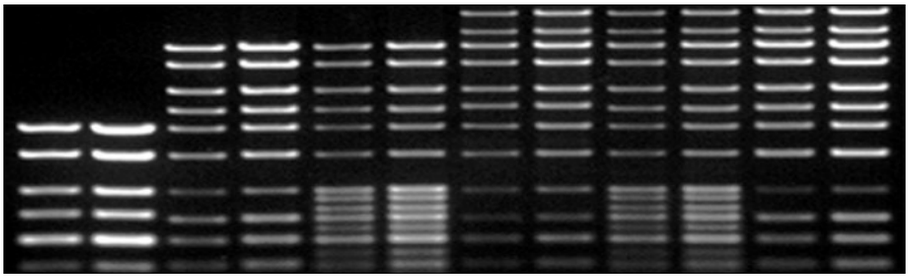
Yeast and Fungi Growth Media	Size	Catalogue #
Potato Dextrose Broth	500 mL	28204
YPD	500 mL	28202

Selective Bacterial Enrichment Media	Size	Catalogue #
Salmonella enterica Enrichment Media	500 mL	28197
Listeria monocytogenes Enrichment Broth	500 mL	28203
Listeria monocytogenes Supplement for Selective Enrichment	105 mg	28205

Antibiotics	Size	Catalogue #
Ampicillin (50 mg/mL)	6 x 1.5 mL	28233
Chloramphenicol (50 mg/mL)	6 x 1.5 mL	28230
Kanamycin (10 mg/mL)	6 x 1.5 mL	28234
Tetracycline (12.5 mg/mL)	6 x 1.5 mL	28237
Carbenicillin (20 mg/mL)	6 x 1.5 mL	28235

Assay Reagents	Size	Catalogue #
X-Gal Solution (40 mg/mL)	6 x 1.5 mL	28231

Best-in-Class
Molecular Weight Ladders



DNA Ladders		
11200	Mini Sizer 50bp DNA ladder	25bp to 650bp
13525	50bp DNA Ladder	50bp to 500bp
11300	PCR Ranger 100bp DNA ladder	50bp to 1,000bp
11400	PCR Sizer 100bp DNA ladder	100bp to 1,000bp
12800	FastRunner DNA Ladder	50bp to 2,000bp
11500	LowRanger 100bp DNA Ladder	100bp to 2,000bp
11600	Clone Sizer 100bp DNA Ladder	100bp to 2,000bp
11700	MidRanger 1kb DNA Ladder	300bp to 5,000bp
11800	FullRanger 100bp DNA Ladder	100bp to 5,000bp
11900	HighRanger 1kb DNA Ladder	300bp to 10,000bp
12000	HighRanger Plus 100bp DNA Ladder	100bp to 10,000bp
12100	UltraRanger 1kb DNA Ladder	300bp to 24,000bp

RNA Ladders		
15002	100 b RNA Ladder	100b to 1,000b
15003	1kb RNA Ladder	200b to 4,000b
15006	200 b to 4000 b RNA Ladder (Lyophilized)	200b to 4,000b

Protein Ladders		
12310	ProteoLadder 100	14.7 kDa - 100 kDa
12510	ProteoLadder 125	14.7 kDa - 125 kDa
12710	ProteoLadder 150	14.7 kDa - 150 kDa

Commitment to Quality

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