cf-DNA/cf-RNA Preservative 10 mL Tube WARNING H302



# LIQUID BIOPSIES

# cfc-DNA/cfc-RNA Preservation & Purification PLASMA/SERUM | URINE





# About Norgen

# NORGEN BIOTEK CORP.

### Our capabilities will meet every step of your workflow.



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 provide dedicated and expert support services to our customers and commercial partners worldwide. **Our products and services span the complete workflow from sample collection and preservation to purification and analysis.** 

Norgen is an ISO 9001: 2015 and ISO 13485: 2016 registered company, indicating our commitment to quality.

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**To Order by Fax:** (905) 227-1061

To Order by Email: orders@norgenbiotek.com

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**To Order by Mail:** Norgen Biotek Corp. 3430 Schmon Parkway Thorold, Ontario L2V 4Y6 CANADA



NORGEN BIOTEK

# LIQUID BIOPSIES

# cfc-DNA/cfc-RNA Preservation and Purification PLASMA/SERUM | URINE

Plasma/Serum cf-DNA/cf-RNA Preservation &

Plasma/Serum

Urine

Urine

Clean-Up &

Related

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### **cf-DNA/cf-RNA Preservative Tubes** Cat No. 63950, Dx63950 **€**



### Whole Blood Sample Collection and Preservation of Cell-Free Circulating DNA and Cell-Free Circulating RNA

Norgen Biotek's impressive cf-DNA/cf-RNA Preservative Tubes are easy-to-use tubes for collection, preservation, storage and transportation of whole blood. The tubes preserve cf-DNA and cf-RNA for up to 30 days and can be stored and shipped at room temperature. Compatible with commercially available DNA and RNA purification methods and automation.

- Preserve cf-DNA & cf-RNA for 30 days at ambient temperature
- Preserve Circulating Tumor Cells (CTCs) for 14 days at ambient temperature
- Formaldehyde-free preservative, no crosslinking of DNA
- Prevent apoptosis of blood cells and fragmentation of genomic DNA

- Prevent hemolysis
- Produce high quality/quantity of plasma cf-DNA/cf-RNA
- No plasma volume loss during shipping
- Vacuumed to draw 8.4 mL of blood in 10 mL tubes
- PET tubes to avoid breakage during transit



### cf-DNA Stable for 30 days at Ambient Temperature

Figure 1. Effect of ambient temperature storage on cf-DNA (pDNA), exemplified by the short Alu (115bp) fragment, and genomic DNA (gDNA), exemplified by the large Alu (247 bp) fragment. Blood samples were drawn into either: 1) EDTA tubes, 2) Competitor tubes or 3) Norgen's cf-DNA/cf-RNA Preservative Tubes and stored at room temperature. Levels of the ALU gene (115 bp) representing the pDNA and the ALU gene (247 bp) representing the gDNA should stay the same during the stabilization period indicating proper stabilization and no hemolysis. No stabilization of cf-DNA and extensive hemolysis was observed in plasma collected on EDTA tubes and stored at room temperature. Competitor showed no significant stabilization after 14 days, whereas cf-DNA was stable for 30 days at room temperature for Norgen's cf-DNA/cf-RNA Preservative Tubes.

### cf-RNA Stable for 30 days at Ambient Temperature







Stability of miR-21 in Preserved Plasma

Figure 2. Effect of ambient temperature storage on cf-RNA, exemplified by the 18S rRNA transcript, HPRT1 mRNA transcript and miR-21. Blood samples were drawn into either: 1) Competitor tubes or 2) Norgen's cf-DNA/cf-RNA Preservative Tubes. Competitor's tubes were stored at room temperature for 14 days whereas Norgen's cf-DNA/cf-RNA Preservative Tubes were stored for 30 days at room temperature. Levels of the 18S rRNA transcript, HPRT1 mRNA transcript and miR-21 should stay the same during the stabilization. Competitor showed significantly higher Ct values for the three targets after 7 days indicating cf-RNA degradation, whereas cf-RNA was stable for 30 days at room temperature for Norgen's cf-DNA/cf-RNA Preservative Tubes as observed by the stable Ct value during the stabilization period.

5

### Liquid Biopsies

### High cf-DNA Quantity from Norgen's Preserved Blood



Figure 3. High Quantity of cf-DNA purified from plasma preserved using Norgen's cf-DNA/cf-RNA Preservative Tubes. Blood sample from the same donor was drawn into either Competitor tubes or Norgen's cf-DNA/cf-RNA Preservative Tubes and stored at room temperature for 7 days. The cf-DNA was then isolated from the entire plasma volume recovered from Norgen's tube (6.5mL) and from the Competitor tubes (3.5mL) using Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Kits. As can be observed from the Agilent Bio-analyzer High Sensitivity DNA Chip trace, Norgen yielded more cf-DNA (Blue peak) as compared to the cf-DNA recovered from the competitor's preservative tube.

### **Prevent Hemolysis**



**Figure 4. Hemolysis of collected blood measured over time.** Blood samples drawn into three different tubes and stored up to 30 days. The amount of free hemoglobin, as measured at 414 nm, increased rapidly with each additional storage day in the EDTA tubes and Competitor tubes, and remained relatively constant in Norgen's cf-DNA/cf-RNA Preservative Tubes indicating that Norgen's tubes prevent hemolysis

### Prevent gDNA Release into Plasma during Shipping/Transportation



Figure 5. Prevent cell lysis and the release of gDNA and accumulation of apoptotic ladder in plasma. Blood samples were drawn into three different tubes (Norgen's, EDTA, and Competitor) and shipped. Norgen's cf-DNA/cf-RNA Preservative Tubes help prevent the release of high molecular weight gDNA into plasma while also minimizing the accumulation of contaminating apoptotic ladder from dying peripheral blood leukocytes during shipping as compared to both the competitor and EDTA tubes.

#### Stable at High Shipping Temperatures (37°C)



**Figure 6. Effect of high temperature (37°C) storage for 8 days.** Blood samples were drawn into either EDTA tubes, Competitor tubes or Norgen's cf-DNA/cf-RNA Preservative Tubes and stored at 37°C. cf-DNA was then isolated from processed plasma. cf-DNA stabilization was determined by real-time PCR using a long ALU gene (247 bp). Norgen's cf-DNA/cf-RNA Preservative Tubes stabilized samples for 8 days at 37°C as compared to both EDTA tubes (1 day) and the competitor (4 days).

### **Maximum Plasma Volume Recovery After Shipping**



**No plasma volume loss after shipping/transportation.** Blood was drawn from 6 different donors into Norgen, Competitor, and EDTA tubes. One set was kept in the lab at room temperature and the other was packed in an insulated box and shipped from Thorold, ON via overnight air freight to Winnipeg, MB and then back to Thorold, ON (elapsed time 72 h). The plasma volume recovered from Norgen's cf-DNA/cf-RNA Preservative Tubes did not change before shipping or after shipping (6-7 mL recovered plasma) whereas for both Competitor tubes and EDTA Tubes the plasma volume recovered before shipping was ~ 4mL and after shipping was ~ 2.5mL.

### **Ordering Information**

Description	Size	Cat. Number
cf-DNA/cf-RNA Preservative Tubes	50 Tubes	63950

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

Norgen's preservation technology is patent pending.



### **Recent Publication**

### Endorsed by a Leading Research Group



#### Communication



### **Evaluation of Storage Tubes for Combined Analysis of Circulating Nucleic Acids in Liquid Biopsies**

Aoife Ward Gahlawat <sup>1,†</sup><sup>(D)</sup>, Judith Lenhardt <sup>1,†</sup>, Tania Witte <sup>1</sup><sup>(D)</sup>, Denise Keitel <sup>1</sup>, Anna Kaufhold <sup>2</sup>, Kendra K Maass <sup>2</sup>, Kristian W Pajtler <sup>2,3</sup>, Christof Sohn <sup>1</sup> and Sarah Schott <sup>1,\*</sup><sup>(D)</sup>

### **Key Findings**

- Norgen BCTs are advantageous as they do not use formaldehyde fixation, which is a harsh treatment.
- Norgen BCTs are compatible for the extraction of both cf-DNA and cf-RNA, whereas the other manufacturers supply separate tubes for RNA analysis, making it costlier, not only financially but also in terms of the sample amount available.
- Therefore, for combinatorial marker studies, Norgen tubes are desirable.



Ward Gahlawat, A.; Lenhardt, J.; Witte, T.; Keitel, D.; Kaufhold, A.; Maass, K.K.; Pajtler, K.W.; Sohn, C.; Schott, S. Evaluation of Storage Tubes for Combined Analysis of Circulating Nucleic Acids in Liquid Biopsies. Int. J. Mol. Sci. 2019, 20, 704.

### App Note 86DNA Sample Preparation

### Norgen's cf-DNA/cf-RNA Preservative Tube Preserves & Prevents the Contamination of Circulating Cell-Free DNA by Cellular Genomic DNA during Blood Collection, Shipping and Storage

T.A. Haj-Ahmad, PhD, Y. Haj-Ahmad, PhD Norgen Biotek Corporation, Thorold, Ontario, Canada

#### INTRODUCTION

It is well known that cell-free DNA (cf-DNA) can be found freely circulating in the bloodstream. Cell-free DNA of fetal origin is also present in maternal blood and is now used for non-invasive prenatal diagnosis. Cell-free DNA is also typically elevated in cancer patients. A challenge with the use of cf-DNA in diagnostic applications is that fetal or cancer derived cf-DNAs typically represent less than 10% of the total circulating cf-DNA. Accurate and consistent detection of those less abundant alleles therefore requires that blood is processed as soon as possible. This is done to separate the plasma from potential contamination with genomic DNA (gDNA) released by peripheral blood leukocytes as they degrade. Because collection is often done in a resourcelimited setting and the samples shipped to a secondary analysis centre, it is not usually possible to process blood immediately. Therefore preserving the integrity of cf-DNAs and preventing their contamination with gDNA should be a primary concern for cf-DNA based studies involving blood sample collection, transportation and/or storage. The present study highlights the efficacy of Norgen's cf-DNA/cf-RNA Preservative Tube in preserving and preventing the contamination of cf-DNA with gDNA during blood collection, shipping and storage.

#### MATERIALS AND METHODS

#### **Sample Collection**

Blood samples were collected from six different volunteers recruited by Norgen Biotek Corp. in Thorold, ON Canada. Volunteers were from both sexes and presumed to be healthy. Blood samples from all six donors were drawn onto three different blood collection tubes: K2EDTA tubes (BD Vacutainer®, Becton Dickinson, Franklin Lakes, NJ), CellFree DNA<sup>™</sup> BCT tubes (BCT) (Streck Inc., Omaha, NE) and cf-DNA/cf-RNA Preservative Tubes (Norgen Biotek Corp., Thorold, ON Canada). Blood samples were gently mixed immediately after blood collection by inverting 10 times.

#### Sample Shipping and Storage

Blood was drawn into two of each type of blood collection device from each of the 6 donors (36 tubes total). One Norgen cf-DNA/ cf-RNA Preservative tube, one Streck tube and one K2EDTA tube from each donor was packed in an insulated box and shipped from Thorold, ON via overnight air freight to Winnipeg, MB and then back to Thorold, ON (elapsed time 72 hrs). Upon return an aliquot was collected and the remainder of the preserved sample was stored at room temperature for 7 days before final processing (10 days total since collection). The 18 tubes that were not shipped were left at room temperature for 10 days. An aliquot from each unshipped sample was separated at time 0 and used as a control, and then again after 3 days and 10 days. DNA was subsequently isolated from each aliquot and gene targets assessed by qPCR.

#### **Plasma Preparation**

For blood samples collected in K2EDTA and BCT tubes plasma was separated from each aliquot by centrifuging for 20 minutes at room temperature at 1600 × g. For blood samples collected in Norgen's cf-DNA/cf-RNA Preservative Tubes plasma was separated from each aliquot by centrifuging for 20 minutes at room temperature at 425 × g.

#### **Cell-free DNA Isolation from Plasma**

The Plasma/Serum Cell-Free Circulating DNA Purification Mini Kit (Cat# 55100) (Norgen Biotek Corp., Thorold, ON Canada) was used to isolate the DNA present in each plasma fraction. Isolation was performed according to the manufacturer's instructions. Samples were eluted in 50  $\mu$ L and stored at -20°C until analysis by quantitative PCR.

#### **Quantitative PCR**

The level of total plasma DNA was assessed by amplifying a short DNA fragment (136 bp) from the human  $\beta$ -actin gene (forward primer 5'-GCG CCG TTC CGA AAG TT-3'; reverse primer 5'-CGG CGG ATC GGC AAA-3'). The level of contaminating genomic DNA was assessed by amplifying a longer  $\beta$ -actin fragment (420 bp)

### Liquid Biopsies

(forward primer 5'-CCG CTA CCT CTT CTG GTG-3'; reverse primer 5'-GAT GCA CCA TGT CAC ACT G-3'). The DNA template input volume was 3  $\mu$ L amplified in a final volume of 20  $\mu$ L using 2X Real-Time PCR Master Mix (Norgen Biotek Corp., Thorold, ON Canada). Data analysis was done on GraphPad Prism, version 6.01.

#### **Quality of Plasma and Purified cf-DNA**

Hemolysis was determined by measuring the absorbance of free hemoglobin at 414 nm in the plasma of 6 subjects using a Nanodrop 2000/2000c. The size distribution of the purified plasma cf-DNA was also assessed using an Agilent Bioanalyzer 2100 High Sensitivity DNA Chip.

#### **RESULTS AND DISCUSSION**

#### **Measurement of Hemolysis**

Hemolysis was determined by measuring the absorbance at 414 nm of plasma collected from 6 individuals in each tube type. Data collected include initial absorbance at day 0, and absorbance 10 days later for shipped and unshipped samples. Mean absorbance and standard deviation are shown (Fig. 1). A paired, two-tailed Student's t-test showed that there was no statistically significant difference in the initial mean absorbance at 414 nm and after 10 days (either shipped or unshipped) for blood collected on Norgen's and Streck's preservatives. Blood collected on K2EDTA however showed a significant difference between mean initial absorbance and mean absorbance after 10 days for shipped samples. A difference was only considered significant if it occurred at p < 0.05.



Figure 1. Norgen's cf-DNA/cf-RNA Preservative Tube helps prevent hemolysis of whole blood during shipping and storage (Day 0 and Day 10 data shown).

#### Size distribution of plasma cf-DNA

Total DNA was isolated from preserved whole blood that was first shipped (elapsed time 72 h) and then stored at room temperature for 7 days (total elapsed time 10 days). Eluate (1  $\mu$ L) was analyzed on an Agilent Bioanalyzer 2100 using a High Sensitivity DNA chip. Plasma from blood collected on either K2EDTA or Streck preservative showed an increased amount of high molecular weight DNA (Figure 2a and Figure 2b).



Figure 2. Norgen cf-DNA/cf-RNA Preservative Tube helpsprevent the release of high molecular weight gDNA into plasma (Fig. 2A) while also minimizing the accumulation of contaminating apoptotic ladder from dying peripheral blood leukocytes (Fig. 2B)

### Effect of shipping on level of high molecular weight DNA and total level of DNA in plasma

Relative levels of total DNA (Fig.3A) and relative levels of high molecular weight DNA (Fig.3B) were determined by quantitative PCR on different sized amplicons of the  $\beta$ -actin gene. Initial levels were compared to unshipped levels of the same samples 3 days later or against the shipped levels of the same samples after 3 days. In general, shipping caused an increase in DNA levels in all tubes except for Norgen's tubes. This was only significant (p < 0.05) between those blood samples initially collected on K2EDTA and those same samples after 3 days that were shipped.



Figure 3. Effect of shipping on (A) total DNA level and (B) level of high molecular weight DNA (>420 bp) (Day 0 and Day 3 Data Shown).

#### CONCLUSIONS

- Norgen's cf-DNA/cf-RNA Preservative Tubes help prevent hemolysis and preserve cf-DNA during shipping as measured by free hemoglobin in plasma and Quantitative PCR of short fragment β-actin.
- 2. Norgen's cf-DNA/cf-RNA Preservative Tubes help prevent the release and subsequent fragmentation of gDNA in whole blood.

## For information on cf-DNA/cf-RNA preservation technologies

check out our webinar, **"Recent Advances in the Preservation and Purification of Plasma cf-DNA and cf-RNA"** from Dr. Moemen Abdalla

norgenbiotek.com/webinars

### Liquid Biopsies



exosomal RNA from plasma/serum



RNA isolated using Norgen's kit with various amounts of plasma collected on EDTA tubes was used as a template in qRT-PCR directed to the housekeeping 5S rRNA to assess relative amount of RNA, linearity and level of inhibition at various volumes.

### **Ordering Information**

Description	Size	Cat. Number
Plasma/Serum RNA Purification Mini Kit	50 preps	55000
Plasma/Serum RNA Purification Midi Kit	20 preps	56100
Plasma/Serum RNA Purification Maxi Kit	10 preps	56200







# Rapid and simple purification of cell-free circulating

### DNA from plasma/serum

#### **Excellent Linearity**



#### Robust Plasma Volumes

300 µL

Different Plasma Vo

400 µL

Norgen

200 µL

Competitor Q

### Inhibitor-Free DNA



### **Ordering Information**

Description	Size	Cat. Number
Plasma/Serum Cell-Free Circulating DNA Purification Micro Kit	50 preps	55500
Plasma/Serum Cell-Free Circulating DNA Purification Mini Kit	50 preps	55100
Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit	20 preps	55600
Plasma/Serum Cell-Free Circulating DNA Purification Maxi Kit	10 preps	55800

Relative Amount of the 5S rRNA Gene

2

2

1

ſ



### **Recent Publication**



# **Evaluation of commercial kits for purification of circulating free DNA**

Russell J. Diefenbach, Jenny H. Lee, Richard F. Kefford, Helen Rizos



### Key Points

- Highest recovery of low MW DNA from plasma (red arrows)
- Competitor demonstrates clear decrease in recovery of DNA below 150 bp (blue line)
- Competitor recovers more gDNA contaminants at the expense of recovering more cf-DNA/ct-DNA sizes of interest for biomarker discovery
- Norgen has highest recovery of small MW DNA containing potential targets of interest

"Evaluation of commercial kits for purification of circulating free DNA", Russell J.Diefenbach et al.Cancer Genetics, Volumes 228–229, December 2018, Pages 21-27

### **Urine Collection and Preservation**

Cat No. 18118, 18122, 18113, 18129, 18126





### **Ordering Information**

Description	Size	Cat. Number
Urine Collection and Preservation Tubes - 5 cc	50 tubes	18118
Urine Collection and Preservation Tubes - 15 cc	50 tubes	18122
Urine Collection and Preservation Tubes - 50 cc	50 tubes	18113
Urine Collection and Preservation Cup - 120 cc	1 Cup	18129
Urine Preservative Single Dose	50 units	18126

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.

Single unit sizes are available for all of Norgen's Urine Collection and Preservation Products



### Liquid Biopsies



### **Urine Cell-Free Circulating RNA Purification Kits**

Cat No. 56900, 57000, 57100



### Isolate all sizes of circulating and exosomal RNA, including microRNA



**Figure 1. Purification of cell-free circulating RNA and exosomal RNA from different urine volumes.** Norgen's Urine Cell-Free Circulating RNA Purification Mini Kit (Cat# 56900) was used to purify cell-free circulating and exosomal RNA from 0.5 mL, 1 mL and 2 mL urine samples. Two microlilitres of the purified RNA was then used as the template in RT-qPCR reactions to assess the amplification of (A) the housekeeping 5S rRNA transcript and (B) miR-21. The average Ct value for both (A) 5S rRNA transcript and (B) miR-21 is linearly decreasing with increasing the sample input volume.



**Figure 2. Linearity of RNA purified from increasing urine volumes.** Norgen's Urine Cell-Free Circulating RNA Purification Mini Kit (Cat# 56900) was used to purify RNA from 0.5 mL, 1 mL and 2 mL urine samples. Two microlitres of the purified RNA was then used as the template in RT-qPCR reactions to assess the linearity of the (A) 5S rRNA transcript and (B) miR-21 from the different urine volumes. Norgen's Urine Cell-Free Circulating RNA Purification Mini Kit was able to recover 96% of the 5S rRNA transcript from 1 mL urine relative to the amount that is present in 0.5 mL plasma. Moreover, 96% of the 5S rRNA transcript was recovered from 2 mL urine relative to the amount that is present in 1 mL urine. As for miR-21, Norgen's Urine Cell-Free Circulating RNA Purification Mini Kit was able to recover 92% of miR-21 from 1 mL urine relative to the amount that is present in 0.5 mL 00% of miR-21 was recovered from 2 mL urine relative to the amount that is present in 1 mL urine. Furthermore, 100% of miR-21 was recovered from 2 mL urine relative to the amount that is present in 1 mL urine.

### **Ordering Information**

Description	Size	Cat. Number
Urine Cell-Free Circulating RNA Purification Mini Kit	50 preps	56900
Urine Cell-Free Circulating RNA Purification Midi Kit	20 preps	57000
Urine Cell-Free Circulating RNA Purification Maxi Kit	10 preps	57100





Isolate all sizes of circulating DNA from fresh, preserved or

### frozen urine samples



Linearity of DNA purified from increasing urine volumes



Detection of the human 5S gene

commercially available urine

preservatives



### **Ordering Information**

Description	Size	Cat. Number
Urine Cell-Free Circulating DNA Purification Mini Kit	50 preps	56600
Urine Cell-Free Circulating DNA Purification Midi Kit	20 preps	56700
Urine Cell-Free Circulating DNA Purification Maxi Kit	10 preps	56800

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.





### **RNA Clean-Up and Concentration Micro-Elute Kit** Cat No. 61000



✓	Concentration of small amounts of RNA into 8 $\mu\text{L}$
	Ideal for concentrating RNA samples prior to NGS library preparation
	Concentrate from larger elution volumes to more manageable elution volumes
	Ideal for concentrating RNA purified from exosomes, plasma, serum, urine, and other bodily fluids, and any RNA samples initially purified in large volumes
	Efficient RNA cleanup from enzymatic reactions – labeling, DNase treatment and in vitro transcription
	Cleanup of RNA isolated using different methods, including phenol/chloroform extractions
	Fast and easy processing using rapid spin-column format in 15 minutes
	Suitable for all sizes of RNA, including microRNA (miRNA) without bias

For rapid and efficient clean-up and concentration of Total RNA, including microRNA, without phenol from small inputs



**Figure 1. Excellent Quality of Concentrated RNA.** Total RNA isolated from HeLa cells (2 µg) was concentrated to 8 µL using the RNA Clean-Up and Concentration Micro-Elute Kit. The excellent quality is indicated by the electropherogram generated using the Agilent 2100 Bioanalyzer (RIN > 9). The concentrated RNA is a true 'total RNA' as can be observed by the presence of small RNA species.



Figure 2. Concentration of RNA prior to Next Generation Sequencing (NGS) applications. Total RNA was purified from 200 µL of plasma collected on EDTA blood tubes using Norgen's Total RNA Purification Kit (Cat # 17200) and eluted in 50 µL of elution solution. The same RNA was also concentrated two-fold using the Micro-Elute RNA Column by eluting in 25 µL of elution solution. Five microliters of both the RNA without additional concentration and the 2X concentrated RNA were used as inputs to generate RNA libraries (using the NEBNext® Small RNA Library Prep Set for Illumina® and following manufacturer's instructions) for small RNA NGS on the MiSeq (Illumina) platform. A) The prepared small RNA libraries were visualized on a 6% TBE polyacrylamide gel, where the library prepared with 2X concentrated RNA contained more ligated/indexed miRNA cDNA (147-160 bp) products than the library prepared using the RNA without concentration. B) The cDNA was extracted from excised gel bands and interrogated using the Agilent 2100 Bioanalyzer (High Sensitivity DNA Assay). As would be expected based on input, the small RNA library prepared with the 2X concentrated RNA sample was approximately two times more concentrated than the library prepared with RNA without prior concentration (39.8 vs 21.2 nM, respectively).

### **Ordering Information**

Description	Size	Cat. No
RNA Clean-Up and Concentration Micro-Elute Kit	50 preps	61000







# For rapid and efficient clean-up and concentration of DNA, without phenol from small input volumes



Figure 1. Efficient Concentration of DNA from Various Sources. Approximately 25  $\mu$ g of plasmid DNA (panel A), HEK 293 cell line DNA (panel B), or PCR product (panel C) was concentrated using the DNA Clean-Up and Concentration Micro-Elute Kit and eluted into a final volume of 15  $\mu$ L. For each sample, 1  $\mu$ L of unconcentrated (U) and concentrated DNA (C) was then run on an 1.3% agarose gel at 170 V for 25 min. In each case the DNA Clean-Up & Concentration Micro-Elute Kit demonstrates a significantly higher final concentration of DNA. M = Norgen HighRanger DNA Ladder (Cat#11900).

### **Ordering Information**

Description	Size	Cat. Number
DNA Clean-Up and Concentration Micro-Elute Kit	50 preps	67200





### Low Abundance RNA Quantification Kit

Cat No. 58900





RNA is accurately quantified using a standard curve constructed from the provided RNA standard

#### Sensitivity of Norgen's Kit Compared to Other Methods



Figure 1. Sensitivity of RNA Quantification in the Picogram Range using the Low Abundance RNA Quantification Kit. A representative qPCR Baseline Graph showing the amplification of an RNA standard dilution series. The Low Abundance RNA Quantification Kit can quantify purified RNA from low abundance samples such as liquid biopsies (plasma or urine). As little as 500 fg of RNA can be quantified using Norgen's kit.

#### Sensitive RNA Quantification from Small Volumes of Plasma



Figure 2. Sensitivity of RNA Quantification from Small Volumes of Human Plasma using the Low Abundance RNA Quantification Kit. A representative qPCR Baseline Graph showing the amplification of total plasma RNA isolated from either 50 or 200  $\mu$ L of human plasma using Norgen's Plasma/Serum RNA Purification Mini Kit (Cat. 55000). The Low Abundance RNA Quantification Kit could quantify purified RNA from such low abundance samples (and others such as urine, exosomes etc) with purified RNA concentrations that are 100 pg per  $\mu$ L or less.

### **Ordering Information**

Description	Size	Cat. Number
Low Abundance RNA Quantification Kit	48 rxns	58900









Compatible with any Real-Time PCR system

DNA is accurately quantified using a standard curve constructed from the provided DNA standard

### Sensitivity of Norgen's Kit Compared to Other Methods



**Dynamic Range of DNA Quantification Methods** 

Figure 1. Sensitivity of DNA Quantification of the Low Abundance DNA Quantification Kit Compared to Other Methods. A diagram representing the dynamic range of different DNA quantification methods is presented here. The Low Abundance DNA Quantification Kit can quantify purified DNA from low abundance samples in the pg and sub-pg range.

### **Ordering Information**

Description	Size	Cat. Number
Low Abundanc <mark>e DN</mark> A Quantification Kit	48 rxns	57200



Liquid Biopsies



**Synovial Fluid Bacterial Genomic DNA Isolation Kit** Cat No. 67900



# Isolate genomic DNA from all types of bacteria (both Gram-positive and Gram-negative)



Figure 1. Isolation and Detection of the Gram +Ve S. Aureus Bacterial Genomic DNA from 1 mL of spike-in Synovial fluid. Genomic DNA was isolated from 1 mL synovial fluid spiked with a serially diluted Gram +Ve S. Aureus using Norgen's Synovial Fluid Bacterial Genomic DNA Purification Kit. The efficiency of the purified bacterial gDNA from the spiked-in synovial fluid was evaluated against the gDNA isolation from pure culture containing the same amount of S. Aureus spiked in 1mL synovial fluid. The purified gDNA was subsequently detected using quantitative PCR. All serially diluted spiked-in Gram +Ve S. Aureus was purified with high efficiency from 1mL synovial fluid as compared to the amplification of S. Aureus from pure culture. The limit of detection for the Gram +Ve S. Aureus was down to 5 CFU/mL.



Figure 2. Isolation and Detection of the Gram -Ve *E.coli* Bacterial Genomic DNA from 1 mL of spike-in Synovial fluid. Genomic DNA was isolated from 1 mL synovial fluid spiked with a serially diluted Gram -Ve *E.coli* using Norgen's Synovial Fluid Bacterial Genomic DNA Purification Kit. The efficiency of the purified bacterial gDNA from the spiked-in synovial fluid was evaluated against the gDNA isolation from pure culture containing the same amount of *E.coli* spiked in 1mL synovial fluid. The purified gDNA was subsequently detected using quantitative PCR. All serially diluted spiked-in Gram -Ve *E.coli* was purified with high efficiency from 1mL synovial fluid as compared to the amplification of *E.coli* from pure culture. The limit of detection for the Gram -Ve *E.coli* was down to 5 CFU/mL.

### **Ordering Information**

Description	Size	Cat. Number
Synovial Fluid Bacterial Genomic DNA Isolation Kit	50 preps	67900



### Small RNA Library Prep Kit for Illumina

Cat No. 63600, 63610, 63620



- Complete kits with reagents for all steps from 3' ligation through size selection
- Includes all clean-up columns and reagents in one convenient kit no need to purchase several kits
- Tremendous time savings with size selection without gels and lengthy elution steps
- Enhancement of useful small RNA detection (3-4x increase) in human plasma/serum by depleting an abundant sequence that may occupy up to 50% of NGS reads

## Optimized for ultra-low RNA inputs

Our workflow, using Norgen's Small Library Prep Kit for Illumina, will yield higher numbers of microRNA detected when compared to competitor's workflows. Norgen's Small RNA Library Kit exhibits outstanding performance, specifically when working with ultra-low input samples..

- Dilution series of purified plasma RNA used for different vendor's library prep kits
- Sequenced at equal molarity at ~ 1.5-2M Total Raw Reads per sample
- Norgen workflow showed much higher number of miRNA detected at ≥ 5 raw reads across all concentration tested



### Workflow Comparisons



### **Related Kit Data**



**Figure 1.** Norgen's Abundant Sequence Depletion Kit (For KNA Publied from Human Plasma/Serum) specifically removes an abundant RNA in human plasma/serum that usually takes up > 50% reads of a standard Small RNA-Seq run (see green bars, Panel A) resulting in microRNA-related reads in < 10% of the reads. With the depletion (blue bars, Panel A), the reads dedicated to microRNA significantly increase to > 40% (Panel B), resulting in more microRNA detected (Panel C).



**Figure 2.** A representative qPCR baseline graph showing the successful amplification of Quantified NGS Library Standards (Green) with a range from 20 pM to 2 fM, using Norgen's NGS Library Quantification Kit (for Small RNA-Seq) (Panel A.) Duplicate amplification of a sample Small RNA-Seq library (at 1:10,000 dilution) was performed (Orange). The derived library concentration was 9.41 nM. Norgen's NGS Library Quantification Kit (for Small RNA-Seq) is of good quality as shown with the high PCR efficiency and correlation in the standard curve (Panel B) with low background singals (No Template Control - NTC as Yellow in Panel A)

### **Ordering Information**

Description	Size	Cat. No
Small RNA Library Prep Kit for Illumina	6 rxns	63610
Small RNA Library Prep Kit for Illumina (Indexes 1-24)	24 rxns	63600
Small RNA Library Prep Kit for Illumina (Indexes 25-48)	24 rxns	63620

For more detailed information on these products please scan the **QR code** with your mobile device or visit us at **www.norgenbiotek.com**.



Donor 1

50% Increase in microRNA Detected.

Donor 2

Applications

## Next Generation Sequencing Services

for Small RNA and microRNA





**Norgen Biotek** offers comprehensive services for Next-Generation Sequencing (NGS) in an accredited state-of-the-art laboratory from sample isolation to sequencing and bioinformatics analysis. We have extensive expertise in sample preparation, sequencing and analysis of all types of samples, specializing in ultra-low input samples including liquid biopsies (plasma/serum, urine and exosomes).

for Liquid Biopsies (Plasma/Serum, Urine and Exosomes), FFPE, Cells and Tissues





Figure 1. Norgen's Patented RNA Sample Preparation Technology Allows for Better Diversity of miRNA Detected from HeLa Cells using Illumina Small RNA Next Gen Sequencing. Example shown here with Total RNA Purification Kit, (Cat # 17200) and a leading competitor kit. Panel A shows a higher number of microRNA detected from Norgen's isolation. Panel B is the relative expression levels between the two kits and indicates the number of miRNAs with higher recovery. Panel C shows the number of miRNAs with 3-fold improvement in recovery. Norgen's technology showed higher efficiency with larger number of identified miRNAs



Figure 2. Small RNA Sequencing from as little as 50 µL of Plasma. Norgen Biotek has developed an effective pipeline of small RNA sequencing from small volumes of plasma or serum. RNA could be effectively and consistently recovered from as little as 50 µL of plasma using Norgen's patented sample preparation technology (example shown here with Plasma/Serum RNA Purification Mini Kit, [Cat # 55000]). Panel A shows that the number of microRNA detected from 50 or 200 µL of Plasma was almost identical to that of 4 mL. Panel B is a Venn diagram showing that of the microRNAs identified, the majority are detected in all volumes of plasma input from 50 µL to 4 mL. In fact, the scatter plots in Panel C show the relative expression level of each microRNA detected was highly correlated between 50 or 200 µL of Plasma and 4 mL plasma.



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Figure 3. Norgen's Data Analysis provides statistically significant differential expression information between conditions and displays the data in various

illustrations. Panel A shows the volcano plot that illustrates the relation between -log10 FDR and log2 fold change between the control and indicates upregulated and downregulated miRNAs. Panel B is the principal component analysis (PCA) plot that illustrates the distance between samples based on miRNA profile. Samples are clustered in the PCA plot based on their biology unless other factors, such as sample purity, are causing a more pronounced source of variation. Panel C is a Heat map with hierarchal clustering of miRNAs and samples. The color scale indicates the relative expression level of a miRNA to the mean, where green and red indicate higher or lower expression, respectively.

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**Contact Us for a FREE Consultation** services@norgenbiotek.com

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### Illumina **Propel Certified**<sup>®</sup>

2018-19 NextSeq®

 $R^2 = 0.9905$ 

1000

100

10000

·2/FoldChr nge

Not Deregulate

Α



### Small RNA (and microRNA) Sequencing for Ultra Low Input Samples - including Exosomes, Liquid

Biopsies, FFPE, & More

- Comprehensive services from RNA isolation to bioinformatic analysis
- Expertise in deriving meaningful data from low concentration samples
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### **Ordering Information**



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