NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Kits
Product Insert

Product # 56300, 56400, 56500

Cell-free circulating (cfc) Nucleic Acids such as DNA and RNA in plasma or serum, including exosomal RNA, has the potential to provide biomarkers for certain cancers and disease states as well as fetal DNA in maternal blood. Moreover, plasma and serum has been widely used for the detection of viral infections, including HIV, HCV and HBV. Currently, significant advancements are being made in utilizing cfc-DNA as well as free circulating RNA as biomarkers for the early diagnosis, prognosis and monitoring of therapy for several cancer types and autoimmune diseases, as well as for gene expression analysis. Exosomes are 40 - 100 nm membrane vesicles, which are secreted by most cell types. Exosomes can be found in saliva, blood, urine, amniotic fluid and malignant ascitic fluids, among other biological fluids. Evidence has been accumulating recently that these vesicles act as cellular messengers, conveying information to distant cells and tissues within the body. The exosomes contain cell-specific proteins, lipids and RNAs, which are transported to other cells, where they can alter function and/or physiology. These exosomes may play a functional role in mediating adaptive immune responses to infectious agents and tumours, tissue repair, neural communication and transfer of pathogenic proteins. Recent work has demonstrated the presence of distinct subsets of microRNAs within exosomes which depend upon the tumour cell type from which they are secreted. For this reason exosomal RNAs may serve as biomarkers for various diseases including cancer. Cell-free mitochondrial DNA (cfmtDNA) is also under investigation for its clinical significance. In addition, cell-free fetal DNA has been widely used as a non-invasive method for prenatal diagnosis including early identification of fetal sex, genetic studies for families at high risk for inherited genetic disorders, screening for Rhesus factor, screening for aneuploidy and identification of preeclampsia.

Norgen's Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Kits provides a fast, reliable reproducible and simple procedure for isolating total circulating Nucleic Acid (DNA and RNA) from various amounts of plasma/serum inputs ranging from 50 µL up to 5 mL, with various kit formats address different plasma/serum input volumes. Purification is based on spin column chromatography that uses Norgen's proprietary resin separation matrix. The kit is designed to isolate all sizes of cfc-DNA and circulating RNA, including microRNA, as well as all sizes of exosomal RNA. Norgen's Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Kits provide a clear advantage over other available kits in that they do not require phenol/chloroform or any protease treatments. Nucleic Acids can be isolated from either fresh or frozen samples using this kit. Moreover, the kit allows the user to elute into a flexible elution volume ranging from 25 µL to 100 µL. ***This kit is suitable for the isolation of total nucleic acid (RNA and DNA) from fresh or frozen serum or plasma prepared from blood collected on either EDTA or Citrate. Plasma samples prepared from blood collected on heparin should not be used, especially if the primarily interest is purifying RNA, as heparin can significantly interfere with many downstream applications such as RT-PCR. If the main interest is to purify DNA, then heparinised plasma can be used.***

Kit Descriptions and Components			
Component	**Mini Kit Cat.# 56300**	**Midi Kit Cat.# 56400**	**Maxi Kit Cat.# 56500**
Number of Preps	50 preps	20 preps	10 preps
Lysis Buffer A	2 x 20 mL	100 mL	1 x 130 mL 1 x 30mL
Wash Solution A	18 mL*	1 x 38 mL* 1 x 18mL*	38 mL*
Elution Buffer F	6 mL	15 mL	15 mL
Micro Spin Columns	50		
Mini Spin Columns		20	10
Midi Spin Columns		20	
Maxi Spin Columns			10
Collection Tubes	50	20	10
Elution tubes (1.7 mL)	50	20	10
Product Insert	1	1	1

** For the preparation of working solutions, please see Important Notes (Notes prior to use)*

Kits Specifications			
	Mini Kit Cat.# 56300	**Midi Kit Cat.# 56400**	**Maxi Kit Cat.# 56500**
Sample Type	Plasma/Serum	Plasma/Serum	Plasma/Serum
Anti-coagulant (for Plasma) †	EDTA or Citrate	EDTA or Citrate	EDTA or Citrate
Sample volume Range	50 to 200 µL	250 µL to 1.5 mL	2 to 5 mL
Minimum Elution Volume	10 µL	50 µL	50 µL
Maximum Elution Volume	25 µL	100 µL	100 µL
Time to Complete 10 Purifications	15 - 20 minutes	35 - 40 minutes	35 - 40 minutes
Size of RNA Purified	All sizes, including miRNA and small RNA (<200 nt)		
Size of DNA Purified	≥ 50 bp		
Average Yields ¥	Variable depending on specimen		

† ***This kit is suitable for the isolation of total nucleic acid (RNA and DNA) from fresh or frozen serum or plasma prepared from blood collected on either EDTA or Citrate. Plasma samples prepared from blood collected on heparin should not be used, especially if the primarily interest is RNA, as heparin can significantly interfere with many downstream applications such as RT-PCR. if the main interest is DNA then heparinised plasma can be used.***

¥ ***Please check page 7 for Average Plasma/Serum Yields and Common RNA/DNA Quantification Methods***

Customer-Supplied Reagents and Equipments

- Benchtop microcentrifuge
- Swinging bucket centrifuges
- Vortexer
- Micropipettors
- 96 – 100% ethanol
- 100% Isopropanol
- β - Mercaptoethanol

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25^{o}C) for up to 2 year without showing any reduction in performance. It is recommended to warm Lysis Buffer A for 20 minutes at 60^{o}C if any salt precipitation is observed.

Quality Control

In accordance with Norgen's Quality Management System, each lot of Norgen's Plasma/Serum Nucleic Acid Purification Kits are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's Plasma/Serum Nucleic Acid Purification Kits are designed for research purposes only. They are not intended for human or diagnostic use.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

Lysis Buffer A contain guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions. Plasma or serum of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with plasma or serum.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Working with RNA

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defence against these enzymes.

- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA, ensure that they remain on ice during downstream applications

Notes Prior to Use

- All centrifugation steps are performed at room temperature.
- Ensure that centrifuge tubes used are capable of withstanding the centrifugal forces required.
- The provided spin columns are optimized to be used with a benchtop centrifuges and not to be used on a vacuum apparatus
- Most standard benchtop microcentrifuges will accommodate Norgen's Micro and Mini Spin Columns.
- Most standard swinging bucket centrifuges will accommodate Norgen's Midi and Maxi Spin Columns. Do not use a fixed-angle rotor
- Norgen's Midi and Maxi Spin Columns are centrifuged in 15 mL and 50 mL centrifuge tubes, respectively.
- Centrifuging Norgen's Spin columns at a speed higher than recommended may affect RNA yield.
- Centrifuging Norgen's Spin columns at a speed lower than recommended will not affect RNA yield. However, centrifugation at a lower speed may require longer time for the solutions to pass through the spin column
- When placing Norgen's Midi and Maxi Spin Columns into the swinging bucket centrifuge make sure that lids of the tubes are not tightly closed. Tightly closed lids may cause back pressure which may cause column clogging or disintegration**.**
- Ensure that all solutions are at room temperature prior to use.
- It is highly recommended to warm up **Lysis Buffer A** at 60^{o}C for 20 minutes and mix well until the solutions become clear again if precipitates are present.
- Prepare a working concentration of the **Wash Solution A:**
    - Add **42 mL** of 96 - 100% ethanol (provided by the user) to the supplied bottle containing **18 mL** of the concentrated Wash Solution A. This will give a final volume of **60 mL.**
    - Add **90 mL** of 96 - 100% ethanol (provided by the user) to the supplied bottle containing **38 mL** from the concentrated Wash Solution A. This will give a final volume of **128 mL**.
    - The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.
- The use of β-mercaptoethanol in lysis is highly recommended to isolate RNA for sensitive downstream applications. Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of **Lysis Buffer A**.
- Ensure that samples have not undergone more than one freeze-thaw cycle, as this may lead to RNA degradation.
- It is recommended to not work with samples that were hemolyzed as this will affect the nucleic acid profile outcome
- ***This kit is suitable for the isolation of total nucleic acids (RNA and DNA) from fresh or frozen serum or plasma prepared from blood collected on either EDTA or Citrate. Plasma samples prepared from blood collected on heparin should not be used, especially if the primarily interest is RNA, as heparin can significantly interfere with many downstream applications such as RT-PCR. If the main interest is DNA then heparinised plasma can be used.***
- ***Frozen plasma or serum samples should be centrifuged for 2 minutes at 400 x g (~2,000 RPM) before processing. Only clear supernatant should be processed, as column clogging may be encountered if frozen samples are directly processed.***
- ***If any of the solutions do not go through the Spin Columns within the specified centrifugation time, spin for an additional 1-2 minutes until the solution completely passes through the Column. Do NOT exceed the centrifugation speed as this may affect DNA yield.***

Please check the product # and proceed to the appropriate section for your Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification

Purification Mini Kit (Product # 56300)

Note: The procedure outlined below is for 200 µL inputs of Plasma/Serum. If processing a sample volume lower than 200 µL Plasma/Serum, simply bring the volume of your samples up to 200 µL using Nuclease-free water and proceed as outlined below.

1. Place 200 µL of plasma/serum sample in a 2 mL tube (provided by the user) and add 600 µL of **Lysis Buffer A**. Mix well by vortexing for 10 seconds.
2. Add 800 µL of 96-100% ethanol (provided by the user). Mix well by vortexing for 10 seconds.
3. Transfer 650 µL of the mixture from **Step 2** into a Micro Spin Column. Centrifuge for **2 minutes at 3,300 *x g* (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
4. Repeat **Step 3** two more times until all the mixture from **Step 2** has been transferred to the Micro Spin column.

Optional Step: If only RNA is the primary interest, an optional **On-Column DNA Removal Protocol** is provided in **Appendix A** for the maximum removal of DNA that may affect sensitive downstream applications. This step should be performed at this point in the protocol

5. Apply 400 µL of **Wash Solution A** to the column and centrifuge for **30 seconds at 3,300 *x g* (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
6. Repeat **step 5** two more times, for a total of three washes.
7. Spin the column, empty, for **2 minutes at 13,000 *x g* (~14,000 RPM)**. Discard the collection tube.
8. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply from 10 µL up to 25 µL of **Elution Buffer F** to the column and let stand at room temperature for 2 minutes. Centrifuge for **1 minute at 400 x *g* (~2,000 RPM),** followed by **2 minutes at 5,800 x g (~8,000 RPM)**.
9. For maximum recovery, transfer the eluted buffer back to the column and let stand at room temperature for 2 minutes. Centrifuge for **1 minute at 400 x *g* (~2,000 RPM),** followed by **2 minutes at 5,800 x g (~8,000 RPM)**.

   - ***Plasma/Serum nucleic acid is ready for the downstream application of your choice. For an explanation of expected yields and recommendations for quantification of the nucleic acids, please refer to Appendix B***

Section 2: Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Midi Kit (Product # 56400)

Note: The procedure outlined below is for processing 250 µL to 1.5 mL inputs of Plasma/Serum. If the sample volume is lower than 1.5 mL Plasma/Serum, simply bring the volume of your sample up to 1.5 mL using Nuclease-free water and proceed as outlined below.

1. Place 1.5 mL of plasma/serum sample in a 15 mL tube (provided by the user) and add 4.5 mL of **Lysis Buffer A**. Mix well by vortexing for 10 seconds.
2. Add 3 mL of 100% Isopropanol (provided by the user). Mix well by vortexing for 10 seconds.
3. Transfer 3 mL of the mixture from **Step 2** into a Midi Spin Column assembled with one of the provided collection tubes. Centrifuge for **3 minutes at 1,000 *x g* (~2,200 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube. (***Note: Make sure that lid of the tubes is not tightly closed during centrifugation***).
4. Repeat **Step 3** one more time until all the mixture from **Step 2** has been transferred to the Midi Spin Column.
5. Apply 3 mL of **Wash Solution A** to the column and centrifuge for **3 minutes at 1,000 *x g* (~2,200 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube. (***Note: Make sure that lid of the tubes is not tightly closed during centrifugation***).
6. Repeat **Step 5** one more time, for a total of two washes.
7. Spin the column, empty, for **3 minutes at 2,000 *x g* (~3,000 RPM)**. Discard the collection tube.
8. Transfer the Midi Spin Column to a fresh 15 mL tube (not provided). Apply 400 µL of **Elution Buffer F** to the column and let stand at room temperature for 2 minutes. Centrifuge for **2 minutes at 500 x *g* (~1,600 RPM).**
9. Reload the eluted RNA from **Step 8** back to the Midi Spin Column and let stand at room temperature for 2 minutes. Centrifuge for **2 minutes at 500 x *g* (~1,600 RPM).**

10. To the elution from **Step 9**, add 300 µL of **Lysis Buffer A** and mix well by vortexing for 10 seconds.
11. Add 400 µL of 96-100% Ethanol (provided by the user). Mix well by vortexing for 10 seconds.
12. Transfer 700 µL of the mixture from **Step 11** into a Mini Spin Column assembled with one of the provided collection tubes. Centrifuge for **2 minutes at 3,300 *x g* (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
13. Repeat **Step 12** one more time to transfer the remaining mixture into the Mini Spin column.

Optional Step: If only RNA is the primary interest, an optional **On-Column DNA Removal Protocol** is provided in **Appendix A** for the maximum removal of DNA that may affect sensitive downstream applications. This step should be performed at this point in the protocol

14. Apply 400 µL of **Wash Solution A** to the column and centrifuge for **1 minute at 3,300 *x g* (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
15. Repeat **Step 14** two more times, for a total of three washes.
16. Spin the column, empty, for **2 minutes at 13,000 *x g* (~14,000 RPM)**. Discard the collection tube.
17. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 50 µL of **Elution Buffer F** to the column and let stand at room temperature for 2 minutes. Centrifuge for **1 minute at 400 x *g* (~2,000 RPM),** followed by **2 minutes at 5,800 x g (~8,000 RPM)**.
18. For maximum recovery, transfer the eluted buffer back to the column and let stand at room temperature for 2 minutes. Centrifuge for **1 minute at 400 x *g* (~2,000 RPM),** followed by **2 minutes at 5,800 x g (~8,000 RPM)**.

- ***Plasma/Serum nucleic acid is ready for the downstream application of your choice. For an explanation of expected yields and recommendations for quantification of the nucleic acid, please refer to Appendix B***

Section 3: Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit (Product # 56500)

Note: The procedure outlined below is for processing 1.5 mL to 5 mL inputs of Plasma/Serum. If the sample volume is lower than 5 mL Plasma/Serum, simply bring the volume of your sample up to 5 mL using Nuclease-free water and proceed as outlined below.

1. Place 5 mL of plasma/serum sample in a 50 mL tube (provided by the user) and add 15 mL of **Lysis Buffer A**. Mix well by vortexing for 10 seconds.
2. Add 10 mL of 100% Isopropanol (provided by the user). Mix well by vortexing for 10 seconds.
3. Transfer 15 mL of the mixture from **Step 2** into a Maxi Spin Column assembled with one of the provided collection tubes. Centrifuge for **3 minutes at 1,000 *x g* (~2,200 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube. **(*Note: Make sure that lid of the tubes is not tightly closed during centrifugation*)**.
4. Repeat **Step 3** one more time until all the mixture from **Step 2** has been transferred to the Maxi Spin Column.
5. Apply 5 mL of **Wash Solution A** to the column and centrifuge for **3 minutes at 1,000 *x g* (~2,200 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube. **(*Note: Make sure that lid of the tubes is not tightly closed during centrifugation*)**.
6. Repeat **Step 5** one more time, for a total of two washes.
7. Spin the column, empty, for **3 minutes at 2,000 *x g* (~3,000 RPM)**. Discard the collection tube.
8. Transfer the Maxi Spin Column to a fresh 50 mL tube (not provided). Apply 800 µL of **Elution Buffer F** to the column and let stand at room temperature for 2 minutes. Centrifuge for **2 minutes at 500 x *g* (~1,600 RPM).**
9. Reload the eluted RNA from **Step 8** back to the Maxi Spin Column and let stand at room temperature for 2 minutes. Centrifuge for **2 minutes at 500 x *g* (~1,600 RPM).**
10. To the elution from **Step 9**, add 600 µL of **Lysis Buffer A** and mix well by vortexing for 10 seconds.
11. Add 800 µL of 96-100% Ethanol (provided by the user). Mix well by vortexing for 10 seconds.
12. Transfer 750 µL of the mixture from **Step 11** into a Mini Spin column assembled with one of the provided collection tubes. Centrifuge for **2 minutes at 3,300 *x g* (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
13. Repeat **Step 12** two more time to transfer the remaining mixture into the Mini Spin column.

Optional Step: If only RNA is the primary interest, an optional **On-Column DNA Removal Protocol** is provided in **Appendix A** for the maximum removal of DNA that may affect sensitive downstream applications. This step should be performed at this point in the protocol

14. Apply 400 µL of **Wash Solution A** to the column and centrifuge for **1 minute at 3,300 *x g* (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
15. Repeat **Step 14** two more times, for a total of two washes.

16. Spin the column, empty, for **2 minutes at 13,000 *x g* (~14,000 RPM)**. Discard the collection tube.
17. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 50 µL of **Elution Buffer F** to the column and let stand at room temperature for 2 minutes. Centrifuge for **1 minute at 400 x *g* (~2,000 RPM),** followed by **2 minutes at 5,800 x g (~8,000 RPM)**.
18. For maximum recovery, transfer the eluted buffer back to the column and let stand at room temperature for 2 minutes. Centrifuge for **1 minute at 400 x *g* (~2,000 RPM),** followed by **2 minutes at 5,800 x g (~8,000 RPM)**.

- ***Plasma/Serum nucleic acid is ready for the downstream application of your choice. For an explanation of expected yields and recommendations for quantification of the nucleic acid, please refer to Appendix B***

Appendix A

Protocol for Optional On-Column DNA Removal

Norgen's Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Kits isolate total nucleic acid (RNA and DNA) in one elution. However, an optional protocol is provided below for the maximum removal of DNA that may affect sensitive downstream applications if your primary interest is cell-free circulating RNA. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

1. For every on-column reaction to be performed, prepare a mix of 15 µL of **DNase I** and 100 µL of **Enzyme Incubation Buffer** using Norgen's RNase-Free DNase I Kit (Product # 25710). Mix gently by inverting the tube a few times. **DO NOT VORTEX**.
   Note: If using an alternative DNase I, prepare a working stock of 0.25 Kunitz unit/µL RNase-free DNase I solution according to the manufacturer's instructions. A 100 µL aliquot is required for each column to be treated.
2. Perform the procedure up to **Step 4 (Mini Format)** or up to **Step 13 (Midi and Maxi Format).**
3. Apply 400 µL of **Wash Solution A** to the column and centrifuge for **30 seconds at 6,000 RPM**. Discard the flowthrough and reassemble the spin column with its collection tube.
4. Apply 50 µL of the **RNase-free DNase I** solution prepared in **Step 1** to the column and centrifuge at **8,000 x g (~10,000 RPM)** for 1 minute.
   Note: Ensure that the entire DNase I solution passes through the column. If needed, spin at **13,000 x g (~14,000 RPM)** for an additional minute.
5. After the centrifugation in **Step 4**, pipette the flowthrough that is present in the collection tube back onto the top of the column.
   Note: Ensure **Step 5** is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.
6. Incubate the column assembly at 25 - 30^{o}C for 15 minutes.
7. Without any further centrifugation, proceed directly **to the second wash step** in **Step 6 (Mini Format) or to the second wash step** in **Step 15 (Midi and Maxi Format)**.

Appendix B

Cell-Free Circulating Nucleic Acid Yield

Plasma/Serum total Nucleic Acid, like RNA and DNA in other cell-free bodily fluids, is normally found in very low amounts (1 - 100 pg/µL), therefore measuring cell-free RNA or DNA concentration using common quantification methods is very difficult and challenging. Typical yields of plasma/serum Nucleic Acid vary significantly from sample to sample. Variability is also observed between samples collected from the same donor at different times during the day and therefore there is no absolute yield for RNA purified from bodily fluids including plasma or serum. Cell-free circulating RNA yield varies depending on a number of factors including age, sex, diet, exercise and most importantly the health status of the donor.

Below is a list of the most common RNA and DNA quantification methods, as well as the limit of detection for each of these methods. ***Unfortunately, none of these methods can be used reliably for measuring the concentration of the purified nucleic acid from plasma or serum unless large plasma/serum volumes have been processed***. This would only be applicable if plasma/serum contains the maximum amount of nucleic acid that can fit within the specification range of these quantification tools. It should be noted that the specifications outlined below are based on measuring a pure RNA/DNA, which will not be the case for the nucleic acid purified from plasma or serum. Plasma/Serum nucleic acid is short and fragmented, and is usually present in less than 1000 bp. Purified plasma/serum nucleic acid usually contains traces of proteins

which will interfere with most quantification methods, leading to the overestimation of the purified nucleic acid concentration. Therefore purified nucleic acid contaminated with more proteins will be presented at a higher concentration as compared to nucleic acid purified with less protein contaminants, which in this case will depend on the method used for plasma/serum nucleic acid purification. ***The only reliable method that can assess the quality and the relative quantity of the purified plasma/serum nucleic acid is RT-qPCR (RNA) or qPCR (DNA) amplification of a standard RNA or DNA using a small amplicon such as the 5S rRNA housekeeping gene***.

Common Nucleic Acid Quantification Methods

1) Bioanalyzer RNA Quantification Kits

	RNA 6000 Nano Kit		RNA 6000 Pico Kit		Small RNA kit
	Total RNA	**mRNA**	**Total RNA**	**mRNA**	**Total RNA**
Quantitative range	25 - 500 ng/µL	25 - 250 ng/µL	----	----	50-2000 pg/µL
Qualititative range	5 - 500 ng/µL	5 - 250 ng/µL	50 - 5000 pg/µL	250 - 5000 pg/µL	50-2000 pg/µL
Quantitation accuracy	20% CV	20% CV	30% CV	----	------

2) Bioanalyzer DNA Quantification Kits

	DNA 1000 Kit	DNA 7500 Kit	DNA 12000 Kit	High Sensitivity DNA Kit
Size Range	**25–1000 bp**	**100–7500 bp**	**100–12000 bp**	**50-7000 bp**
Quantitation accuracy	**20% CV***	**20% CV***	**25% CV***	**20% CV**
Quantitative range	**0.5-50 ng/µL**	**0.5-50 ng/µL**	**0.5-50 ng/µL**	**5-500 pg/µL**

3) NanoDrop 2000

- Detection Limit: 2 ng/µl (dsDNA)

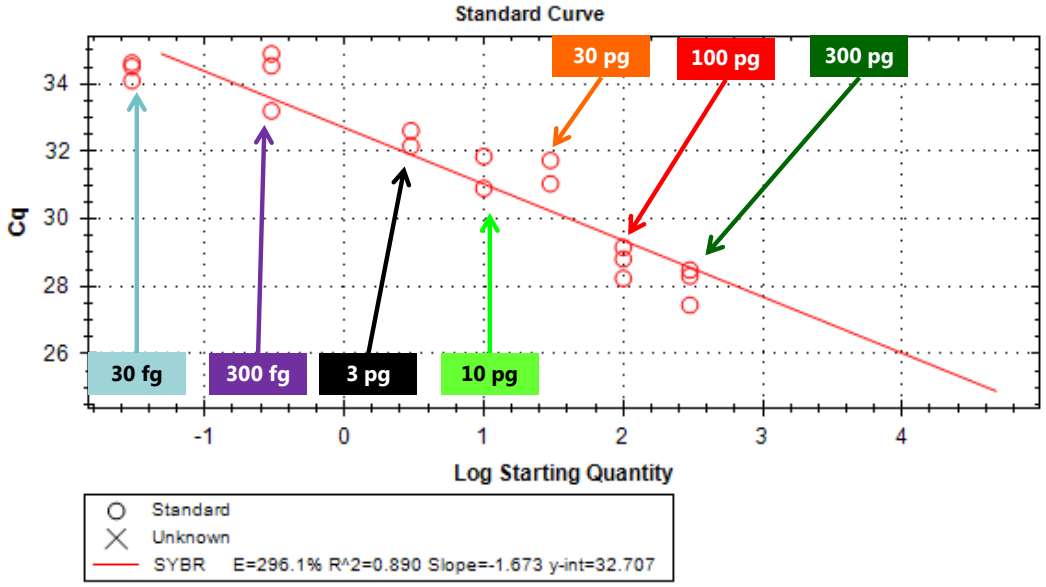
4) Quant-iT™ RiboGreen® RNA Assay Kit

- Quantitation Range: 1-200 ng

5) Quant-iT™ Pico Green® dsDNA Assay Kit

- Detection Limit: 25 pg/mL

6) qPCR Standard Curve (generated using Norgen's Low Abundance DNA Quantification Kit, Cat# 57200)



Frequently Asked Questions

1. **What If a variable speed centrifuge is not available?**
   - A fixed speed centrifuge can be used, however reduced yields may be observed.
2. **At what temperature should I centrifuge my samples?**
   - All centrifugation steps are performed at room temperature. Centrifugation at 4°C will not adversely affect kit performance.
3. **What if I added more or less of the specified reagents' volume?**
   - Adding more or less than the specified volumes may reduce both the quality and the quantity of the purified nucleic acids. Eluting your nucleic acids in high volumes will increase the yield but will lower the concentration. Eluting in small volumes will increase the concentration but will lower the overall yield.
4. **What If I forgot to do a dry spin before my final elution step?**
   - Your purified nucleic acids will be contaminated with the Wash Solution A. This may reduce the quality of your purified nucleic acid and will interfere with your downstream applications.
5. **Can I perform a second elution?**
   - Yes, but it is recommended that the 2nd elution be in a smaller volume (50% of 1st Elution). It is also recommended to perform the 2nd elution into a separate elution tube to avoid diluting the 1st elution.
6. **Why do my samples show low nucleic acid yield?**
   - Plasma/Serum samples contain very little nucleic acid. This varies from individual to individual based on numerous variables. In order to increase the yield, the amount of Plasma/Serum input could be increased.
7. **Why do the A260:280 ratio of the purified nucleic acid is lower than 2.0?**
   - Most of the Free-Circulating Plasma/Serum nucleic acid is short nucleic acid fragments. The A260:280 ratio is normally between 1 – 1.6. This low A260:280 ratio will not affect any downstream application.
8. **Why does my isolated RNA not perform well in downstream applications?**
   - If a different Elution Buffer was used other than the one provided in the kit, the buffer should be checked for any components that may interfere with the application. Common components that are known to interfere are high salts (including EDTA), detergents and other denaturants. Check the compatibility of your elution buffer with the intended use.
9. **Do I need to do a DNase treatment for my nucleic acid Elution?**
   - You may need to do a DNase treatment to your isolated Plasma/Serum miRNA. It is recommended to use Norgen's RNase-Free DNase I Kit (Cat# 25710). Also please refer to the protocol for optional on-column DNA removal outlined in **Page 6**.

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Kits or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362

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