Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit

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

Kit Specifications			
Minimum Plasma/Serum Input	2 mL	Maximum Plasma/Serum Input	5 mL
Time to Complete Purification	35-40 minutes	Size of RNA Purified	All sizes, including miRNA and small RNA (< 200 nt)
Elution Volume	50-100 µL	Size of DNA Purified	≥ 50 bp

Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit Benefits	
No phenol:chloroform extractions	Circulating DNA/RNA and Exosomal RNA are isolated without the use of harmful chemicals such as phenol or chloroform.
Isolate all sizes of circulating RNA and exosomal 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 35-40 minutes.
Input volume	Isolate circulating nucleic acid from 2 mL to 5 mL of plasma/serum.
Isolate inhibitor-free NA	Purified NA can be used in a number of sensitive downstream applications including reverse transcription qPCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

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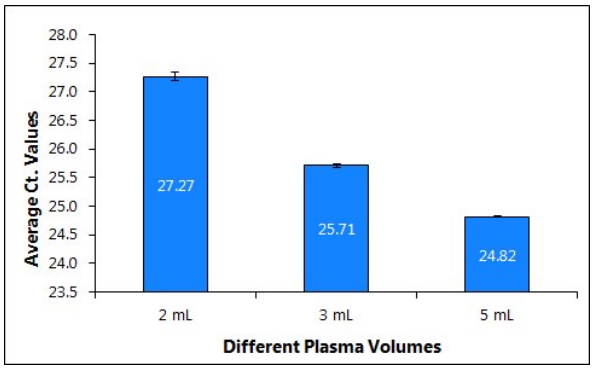

Figure 1. Purification of cell-free circulating DNA from different plasma volumes. Norgen's Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit (Cat# 56500) was used to purify circulating DNA from 2 mL, 3 mL and 5 mL plasma prepared from blood collected on citrate as an anticoagulant. Two microlitres of the purified NA was then used as the template in qPCR reactions to assess the relative amount of the purified the housekeeping 5S rRNA gene. The Ct. values for the 5S rRNA gene is linearly decreasing with increasing the sample input volume.

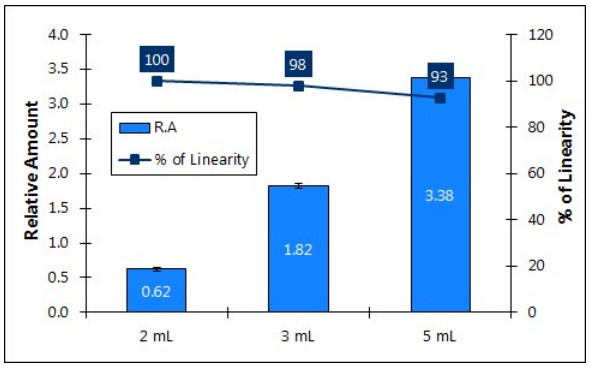

Figure 2. Linearity of DNA purified from increasing plasma volumes. Norgen's Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit (Cat# 56500) was used to purify circulating NA from 2 mL, 3 mL and 5 mL plasma prepared from blood collected on citrate as an anticoagulant. Two microlitres of the purified NA was then used as the template in qPCR reactions to assess the linearity of the purified the housekeeping 5S rRNA gene from the different plasma volumes. Norgen's Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit was able to recover 98% of the 5S rRNA gene from 3 mL plasma relative to the amount that is present in 2 mL plasma. Moreover, 93% of the 5S rRNA gene was recovered from 5 mL plasma relative to the amount that is present in 3 mL plasma.

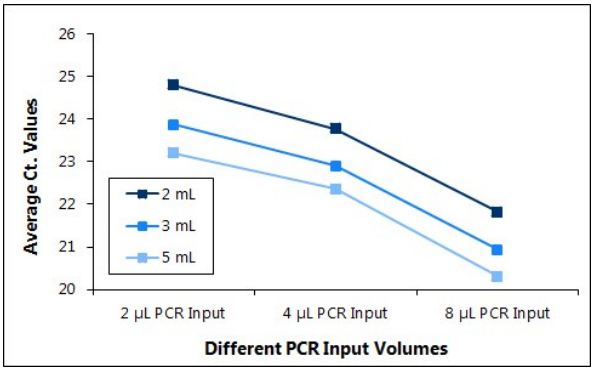

Figure 3. Determination of the amount of inhibition present in plasma cell-free circulating DNA samples when detecting the human 5S gene. DNA was isolated from 2 mL, 3 mL and 5 mL plasma using Norgen's Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit (Cat# 56500). Increasing volumes of the elution (2, 4 and 8 µL) were used in a 20 µL qPCR reaction to observe any decrease in Ct value. An increase in Ct values with increasing amount of template would be a clear indication of PCR inhibitors present in the sample. An increase in elution volume used as a template in the qPCR did not affect the Ct value generated from qPCR and in fact the Ct. values tend to decrease with increasing the PCR input volume indicating that DNA purified from plasma using Norgen's kit is free of the common inhibitors usually present in plasma.

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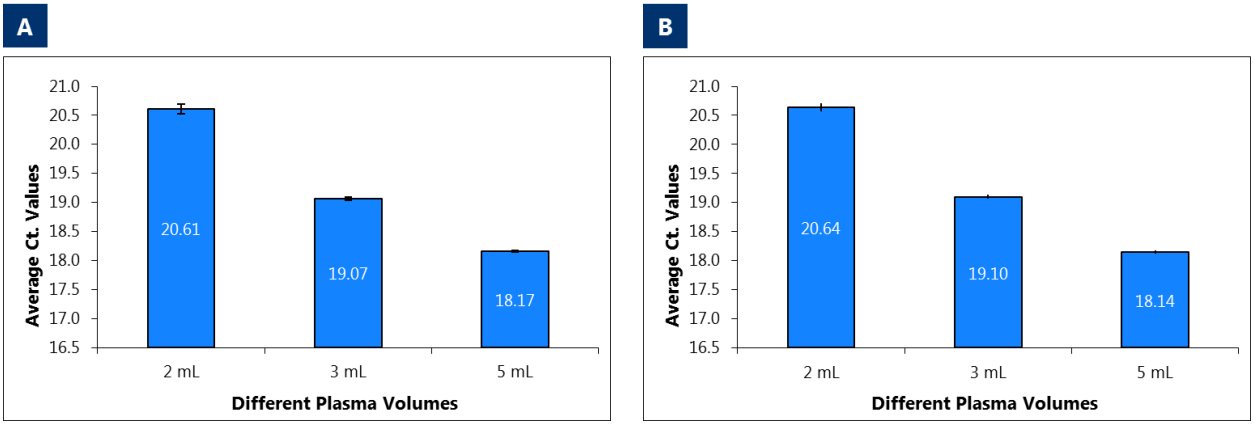

Figure 4. Purification of cell-free Circulating RNA and Exosomal RNA from different plasma volumes. Norgen's Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit (Cat# 56500) was used to purify cell-free circulating and Exosomal RNA from 2 mL, 3 mL and 5 mL plasma prepared from blood collected on citrate as an anticoagulant in comparison. Two microlitres of the purified RNA was then used as the template in RT-qPCR reactions to assess the amplification of the purified the (A) 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.

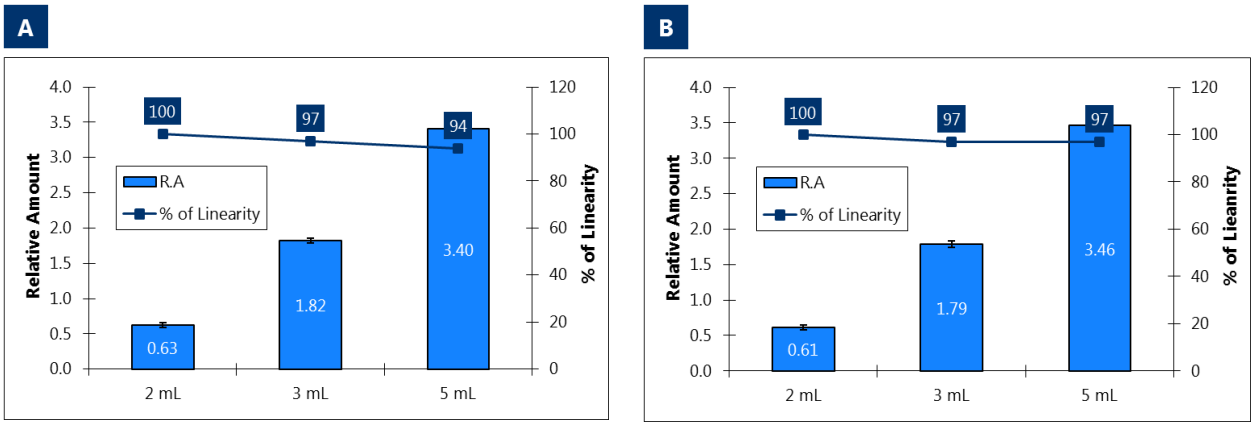

Figure 5. Linearity of RNA purified from increasing plasma volumes. Norgen's Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit (Cat# 56500) was used to purify RNA from 2 mL, 3 mL and 5 mL plasma prepared from blood collected on citrate as an anticoagulant. Two microlitres of the purified RNA was then used as the template in RT-qPCR reactions to assess the linearity of the purified the housekeeping (A) 5S rRNA transcript and (B) miR-21 from the different plasma volumes. Norgen's Plasma/Serum Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit was able to recover 97% of both the 5S rRNA transcript and miR-21 from 3 mL plasma relative to the amount that is present in 2 mL plasma. Moreover, 94% of the 5S rRNA transcript and 97% of the miR-21 was recovered from 5 mL plasma relative to the amount that is present in 3 mL plasma.

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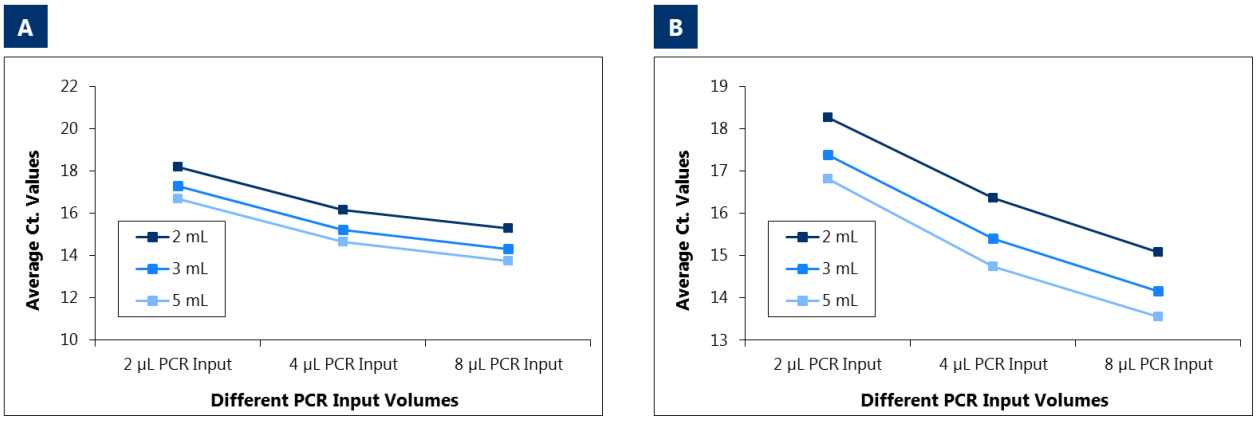

Figure 6. Determination of the amount of inhibition present in plasma RNA samples when detecting the human 5S transcript and mir-21. RNA was isolated from 2 mL, 3 mL and 5 mL plasma using Norgen's Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit (Cat# 56500). Increasing volumes of the elution (2, 4 and 8 µL) were used in a 20 µL reverse transcription reaction followed by qPCR amplification reaction to observe any decrease in Ct value. An increase in Ct values with increasing amount of template would be a clear indication of PCR inhibitors present in the sample. An increase in the PCR input volume used as a template in the reverse transcription reaction did not affect the Ct value generated from the qPCR amplification for both (A) 5S rRNA transcript and (B) miR-21. In fact the Ct. values tend to decrease with increasing the PCR input volume indicating that RNA purified from plasma using Norgen's kit is free of the common inhibitors usually present in plasma.

Kit Contents:

1. Lysis Buffer A
2. Wash Solution A
3. Elution Buffer F
4. Mini Spin Columns
5. Maxi Spin Columns
6. Collection Tubes
7. Elution tubes (1.7 mL)
8. Product Insert

Storage Conditions

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

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Micropipettors
- 96 – 100% ethanol
- β - Mercaptoethanol

Shipping Conditions

The Plasma/Serum Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit is shipped at room temperature.

Cat #	Description	Quantity
56500	Plasma / Serum Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit	10 preps

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