Urine Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit

This kit provides a fast, reliable and convenient method to purify and concentrate high quality, high purity and inhibitor-free cell-free circulating DNA, circulating including exosomal RNA as well as viral DNA/RNA from fresh, frozen or preserved urine samples from volumes ranging from 10 mL to 30 mL.

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 Urine Cell-Free Circulating and Viral Nucleic Acid Purification Kits provides a clear advantage over other available kits in that they do not require phenol/chloroform or any protease treatments. Moreover, the kit allows the user to elute into a flexible elution volume ranging from 50 µL to 100 µL. The purified nucleic Acid is of the highest integrity, and can be used in a any downstream applications including PCR, qPCR, methylation-sensitive reverse transcription qPCR, reverse transcription PCR, methylation-sensitive PCR and Southern Blot analysis, Northern blotting, RNase protection and primer extension, expression array assays, and NGS.

Kit Specifications			
Minimum Urine Input	10 mL	Elution Volume	50-100 µL
Maximum Urine Input	30 mL	Time to Complete 10 Purifications	40-45 minutes
Size of Nucleic Acid Purified	All sizes, including miRNA and small RNA (< 200 nt)	Average Yields	Variable depending on specimen

Features and Benefits	
Diverse	Isolate all sizes of circulating DNA, circulating and exosomal RNA, including microRNA, viral DNA/RNA in one elution
Versatile	Versatile urine input ranging from 10 mL - 30 mL
Phenol-Free	No phenol extractions nor carrier RNA
Flexible	Concentrate circulating DNA, circulating RNA and exosomal RNA, viral DNA, viral RNA into a flexible elution volume ranging from 50 µL - 100 µL
Fast and Accurate	Purify high-quality RNA/DNA in 40 - 45 minutes

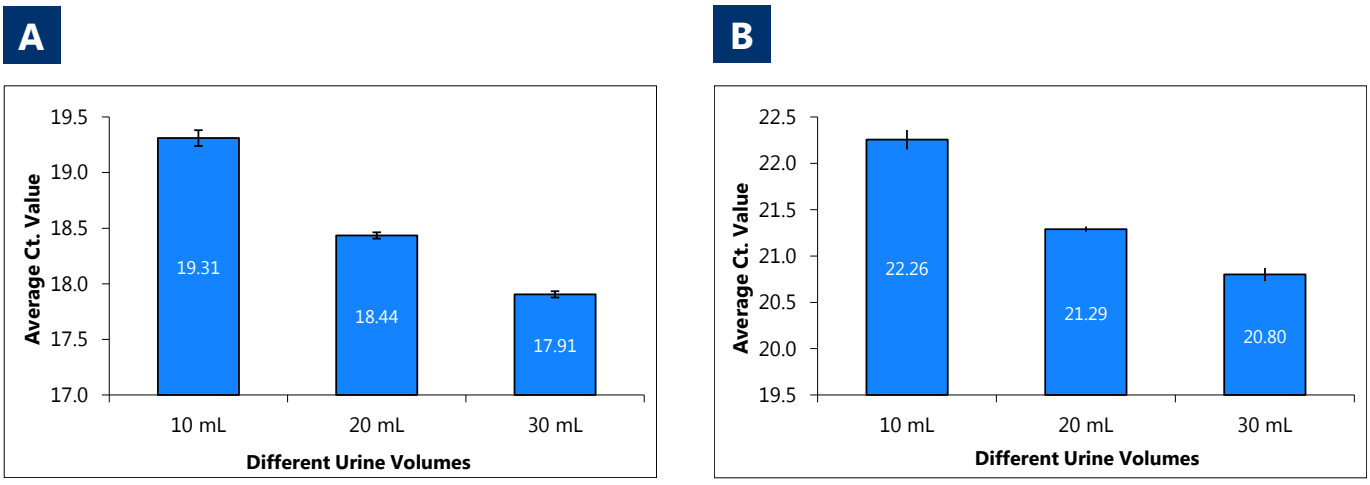

Figure 1. Purification of cell-free circulating RNA and exosomal RNA from different urine volumes. Norgen's Urine Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit (Cat# 60100) was used to purify cell-free circulating and exosomal RNA from 10 mL, 20 mL and 30 mL urine. Two microlitres of the purified RNA was then used as the template in RT-qPCR reactions to assess the amplification of the purified (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.

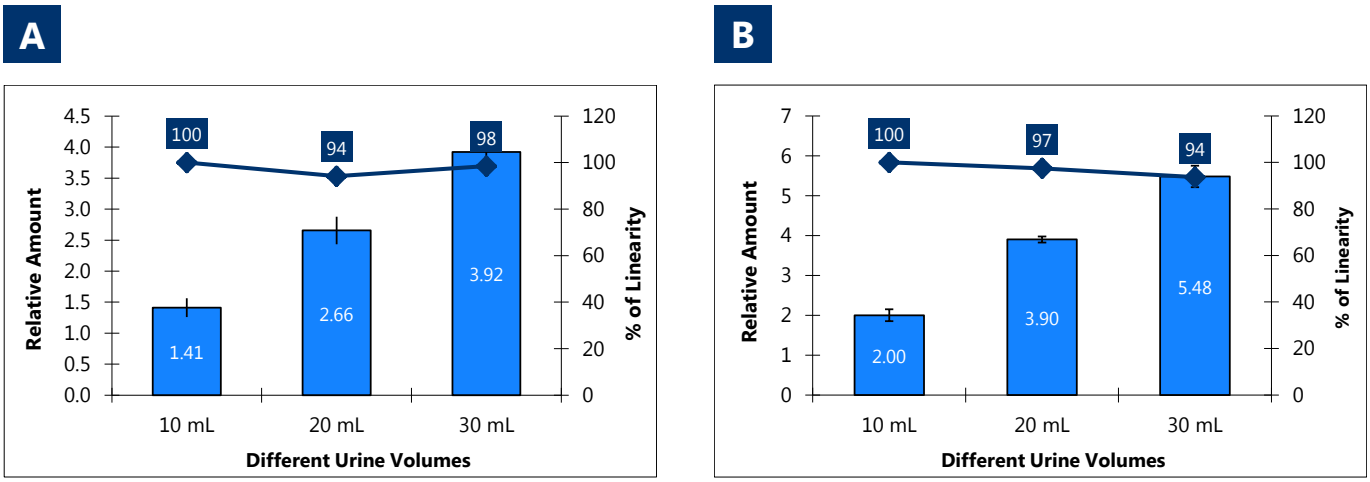

Figure 2. Linearity of RNA purified from increasing urine volumes. Norgen's Urine Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit (Cat# 60100) was used to purify RNA from 10 mL, 20 mL and 30 mL urine. Two microlitres of the purified RNA was then used as the template in RT-qPCR reactions to assess the linearity of the purified (A) housekeeping 5S rRNA transcript and (B) miR-21 from the different urine volumes. Norgen's Urine Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit was able to recover 94% of the 5S rRNA transcript and 97% of miR-21 from 20 mL urine relative to the amount that is present in 10 mL urine. Moreover, 98% of the 5S rRNA transcript and 94% of the miR-21 was recovered from 30 mL urine relative to the amount that is present in 20 mL urine.

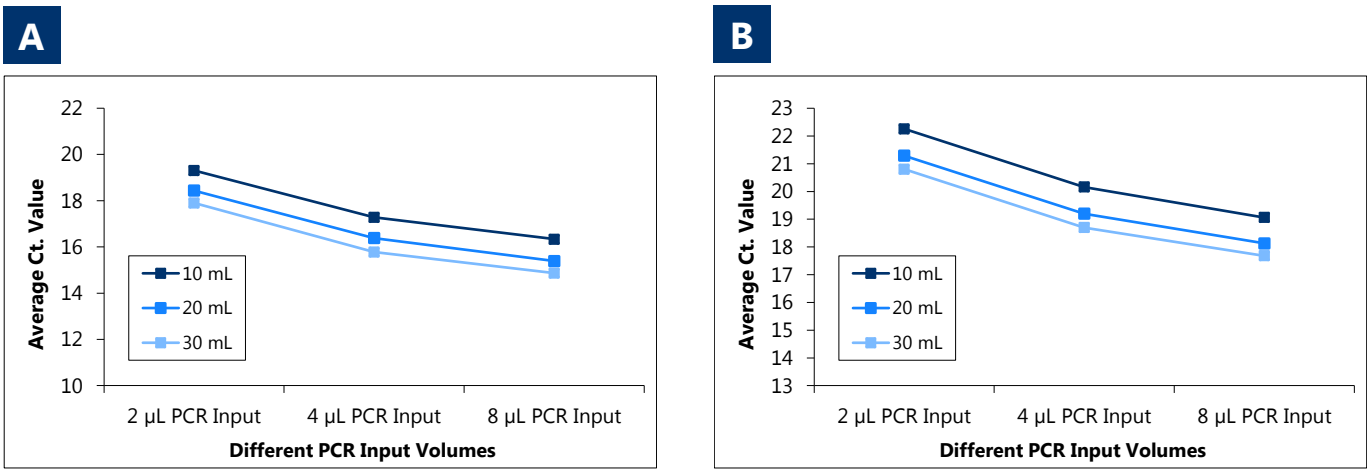

Figure 3. Determination of the Amount of Inhibition Present in Urine RNA Samples when Detecting the Human 5S transcript and miR-21. RNA was isolated from 10 mL, 20 mL and 30 mL urine using Norgen's Urine Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit (Cat# 60100). Increasing volumes 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,

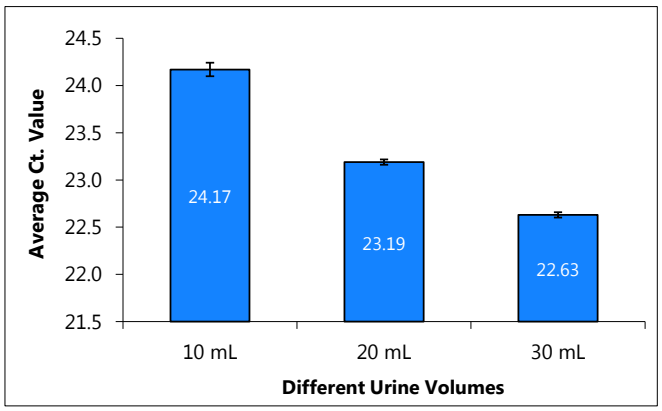

Figure 4. Purification of cell-free circulating DNA from different urine volumes. Norgen's Urine Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit (Cat# 60100) was used to purify circulating DNA from 10 mL, 20 mL and 30 mL Urine. Two microlitres of the purified NA was then used as the template in qPCR reactions to assess the relative amount of the purified housekeeping 5S rRNA gene. The Ct values for the 5S rRNA gene is linearly decreasing with increasing the sample input volume.

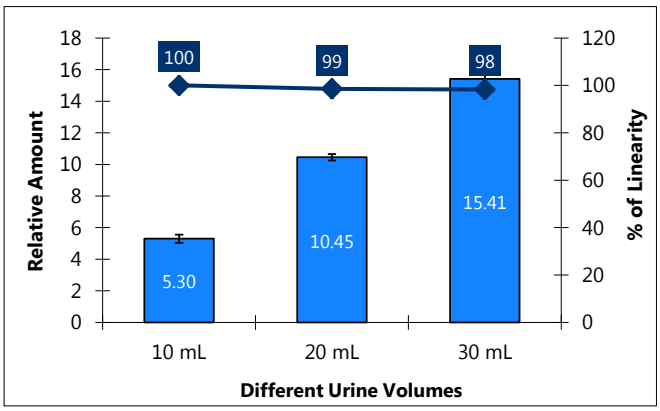

Figure 5. Linearity of DNA purified from increasing urine volumes. Norgen's Urine Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit (Cat# 60100) was used to purify circulating NA from 10 mL, 20 mL and 30 mL Urine. Two microlitres of the purified NA was then used as the template in qPCR reactions to assess the linearity of the purified housekeeping 5S rRNA gene from the different urine volumes. Norgen's Urine Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit was able to recover 99% of the 5S rRNA gene from 20 mL urine relative to the amount that is present in 10 mL urine. Moreover, 98% of the 5S rRNA gene was recovered from 30 mL urine relative to the amount that is present in 20 mL urine.

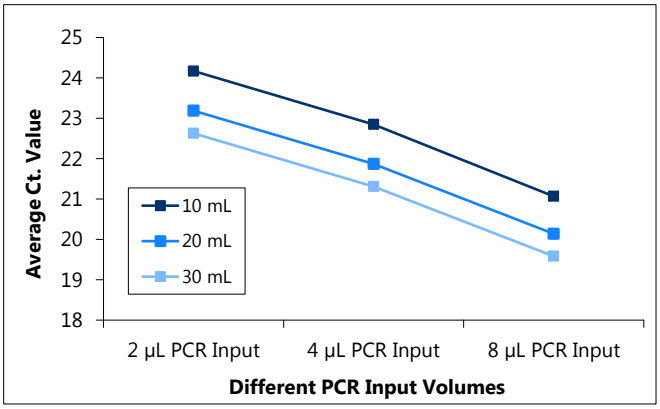

Figure 6. Determination of the amount of inhibition present in urine cell-free circulating DNA samples when detecting the human 5S gene. DNA was isolated from 10 mL, 20 mL and 30 mL urine using Norgen's Urine Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit (Cat# 60100). Increasing 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. In fact, the Ct values tend to decrease with increasing the PCR input volume indicating that DNA purified from urine using Norgen's kit is free of the common inhibitors usually present in urine.

Kit Components	Cat. 60100
Binding Solution K	25 mL
Lysis Buffer A	30 mL
Wash Solution A	18 mL
Elution Buffer F	6 mL
Mini Spin Columns	10
Maxi Spin Columns	10
Collection Tubes	10
Elution Tubes (1.7 mL)	10
Product Insert	1

Customer-Supplied Reagents and Equipment

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

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.

Cat #	Description	Size
60100	Urine Cell-Free Circulating and Viral Nucleic Acid Purification Maxi Kit	10 preps

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