The Range of RNA Yield and Purity with Norgen’s Urine RNA Concentration, Preservation and Isolation Kit

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INTRODUCTION
In recent years attention has been turning to the use of non-invasive samples for genetic and diagnostic analysis, including the use of urine. Norgen Biotek Corp. has developed a simple method for the concentration, preservation, storage and isolation of RNA from urine using Individual Urine RNA Concentration and Preservation Devices. Donors simply collect their urine and transfer it directly into the Urine Concentration Tube, and then mix the urine with Norgen’s resin that is inside the tube. All the macromolecules present within the urine will bind to the resin in the presence of the buffer located within the Urine Concentration Tube. The resin is then allowed to precipitate by gravity for 10 minutes, then the urine supernatant is discarded and Norgen’s Urine Preservative is added. The preservative is an aqueous storage buffer designed for rapid preservation of urine RNA from fresh, concentrated urine specimens.

Norgen’s Urine Preservative stabilizes the RNA for long-term storage at ambient temperature. Since the buffer prevents the growth of microorganisms and inactivates viruses it also allows the samples to be handled and shipped safely. The RNA subsequently isolated from the preserved samples is of a high quality and can be used directly in sensitive downstream diagnostic assays such as real-time PCR. This application note reports on the amount of RNA obtained from urine using Norgen’s Urine RNA Concentration, Preservation and Isolation kit.

MATERIALS AND METHODS

RNA Isolation
Twenty different urine samples were collected using Norgen’s Urine RNA Concentration, Preservation and Isolation Kit and stored for 1 day at room temperature. RNA was purified from 30 mL of the concentrated urine/preservative mix according to the supplied protocol using a 150 µL elution volume.

Determination of RNA Yield
RNA yield and purity (OD260/280) was determined using a NanoVue Plus™ (GE Healthcare) according to the manufactures instructions.

RESULTS AND DISCUSSION
The amount of RNA recovered from urine samples can vary greatly depending on the collection method, preservation method, isolation method and the donor. A high yield of RNA is often required for certain downstream applications. Here, RNA was collected and isolated from 20 urine samples using Norgen’s Urine RNA Concentration, Preservation and Isolation Kit, and the yield from each sample determined using a NanoVue Plus™.

The total average urine RNA concentration from the 20 urine samples processed using Norgen’s kit was 22 ng/µL. Figure 1 shows the urine RNA concentration from 30 mL of each of the 20 samples when eluted into a 150 µL elution volume. The RNA concentration from the 30 mL concentrated urine/preservative samples stored for 1 day at room temperature is between 12.5 ng/µL to 48 ng/µL, with an average RNA concentration of 22 ng/µL.

Figure 1. RNA Concentration from 30 mL Concentrated Urine/Preservative Samples. RNA was isolated from 30 mL concentrated urine/preservative samples using Norgen’s Urine RNA Concentration, Preservation and Isolation Kit. Based on these results the RNA concentration from 30 mL of saliva/preservative samples were calculated and graphed.
The value of OD260/280 was calculated to measure the purity of the RNA isolated from the 30 mL concentrated urine/preservative samples (OD260 is corrected automatically by subtracting the value of OD320). The purity data from the 20 samples is shown in Figure 2, with an average purity of 1.74 ± 0.1.

**Figure 2. RNA Purity from 30 mL Concentrated Urine/Preservative Samples.** Histogram of RNA purity from 20 donors isolated using Norgen’s Urine RNA Concentration, Preservation and Isolation kit.

**CONCLUSION**

The results demonstrate that the urine RNA isolated using Norgen’s Urine RNA Concentration, Preservation and Isolation kit is of a high yield and purity. Norgen’s Urine RNA Concentration, Preservation and Isolation kit offers a non-invasive method for the concentration and the preservation of urine samples for use in diagnostic studies. The average RNA yield was 22 ng/µL with an average OD260/280 of 1.74.