RNA Isolation from Urine Pellet - CTOT

Supplies

Life Technologies 12183-018A PureLink RNA mini kit
Fisher 14-826AA 25 gauge needles
Fisher 14-823-2F 1mL syringes
RPI 163236 5 ml sample prep tubes
100% ethanol
ß-mercaptoethanol

Before starting

- If using kit for the first time, prepare Wash Buffer II by adding 10 ml of concentrated solution to 40 ml of 100% ethanol in a 50 ml Falcon tube.
- Add 39ul of ß-mercaptoethanol (in the fume hood) to 3.9 ml of RNA Lysis Solution in a 5 ml sample prep tube.

RNA isolation

1. Add 300 ul of freshly prepared RNA Lysis Solution containing ß-mercaptoethanol to urine pellet and homogenize thoroughly with a syringe **until no visible pellet can be seen. ***

2. Add 300ul of 100% ethanol and disperse the precipitate completely by pipetting up and down >5 times.

3. Transfer the sample to the RNA Spin Cartridge. Centrifuge at 12,000 x g for 30s at 25^{o}C. Discard the flow-through.

4. Add 650ul of Wash Buffer I to the cartridge. Centrifuge at 12,000 x g for 15s at 25^{o}C. Discard the flow-through.

5. Repeat step 4. Add additional washes if necessary, especially for bloody urine. The flow-through must be completely clear and colorless. More washes yield better RNA. After the final wash, discard the flow-through and the tube. Place the spin cartridge into a clean 2 ml RNA Wash Tube.

6. Add 500ul of Wash Buffer II to the cartridge. Centrifuge at 12,000 x g for 15s at 25^{o}C. Discard the flow-through.

7. Repeat step 6 three more times, for a total of **four washes** with Wash Buffer II.

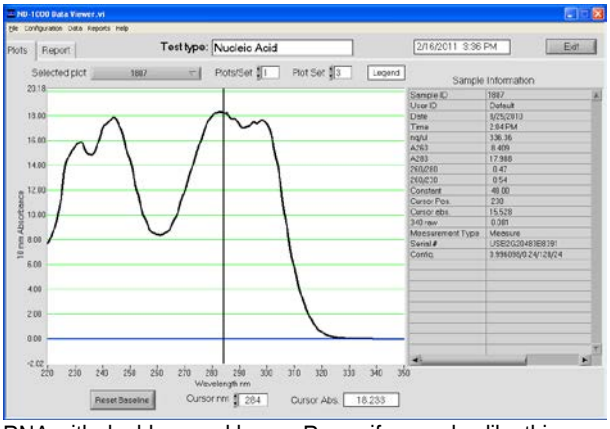
8. Centrifuge the cartridge for 1 min to dry the membrane.

9. Remove the cartridge from the wash tube and place it into an RNA Recovery Tube. Add 30ul of RNase-free Water to the cartridge membrane. Centrifuge at 12,000 x g for 2 min at 25^{o}C. Discard the cartridge.

10. Keep the RNA on ice from this point on! RNA can be frozen at -80° at this point **if absolutely necessary** but it is preferable to quantify and reverse transcribe the RNA immediately.

* **Note:** For difficult pellets, it may be necessary to homogenize with a larger needle (i.e., 18-ga followed by 22-ga) before using the smaller needle. If the pellet still won't homogenize, transfer it to a Qiashredder (Qiagen 79656) and spin for 2 minutes.

If the RNA has a high absorbance at 220-230 nm, as shown, it will need additional purification. Add 120 ul of Rnase-free water to the sample, freeze at -80°C and include it with the next set of samples.



RNA with double camel hump. Re-purify samples like this.