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Urine Extra-cellular RNA Isolation Kit

Component	Contents	Storage
Treatment Buffer	10 mL	4C
Conditioning Buffer	4 mL	Room Temp
Precipitation Buffer	4 x 1 mL	-20C
Lysis Buffer	37.5 mL	-20C
Wash Buffer	15 mL	Room Temp
Elution Buffer	5 mL	Room Temp
Collection Tubes/Filter Columns	50	Room Temp
Elution Tubes	50	Room Temp

Storage Conditions and Product Stability:
All solutions should be kept tightly sealed and stored at the indicated temperature. If any precipitation occurs in the **Precipitation Buffer** then heat to 95C for 5 minutes and invert. Repeat until the precipitate is mostly dissolved. Small residual precipitation will not affect performance. These reagents should remain stable for at least 1 year in their unopened containers.

Precautions and Disclaimers:
This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves, and protective goggles are worn when working with chemicals. **Lysis Buffer** contains guanidine thiocyanate and should be handled with care. Guanidine thiocyanate forms highly reactive compounds when combined with bleach, thus, care must be taken to properly dispose of any of this solution. If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Protocol:

1. Add 0.1 volume of Treatment Buffer to Whole Urine

   Example: for 1mL of urine add 0.1ml of Treatment Buffer; for 3ml of urine add 0.3ml Treatment Buffer

2. Centrifuge at 1,000x*g* for 5min at RT. Transfer the supernatant to a new tube.

3. Add 0.04 volume of Precipitation Reagent. Mix by inverting the tube 3 times. *Example: for 1mL of urine add 0.04ml of Precipitation Reagent; for 3ml of urine add 0.12ml Precipitation Reagent*

4. Add 0.04 volume of Conditioning Buffer. Mix by inverting the tube 3 times.

   Example: for 1mL of urine add 0.04ml of Conditioning Buffer; for 3ml of urine add 0.12ml Conditioning Buffer

5. Incubate on ice for 10min.

6. Centrifuge at 3,000xg for 3min at RT. Discard the supernatant.

7. Add 0.7ml of Lysis Buffer to the precipitate and pipette up and down until completely dissolved.

8. Add 0.3ml of 100% isopropanol. Mix by inverting the tube 3 times.

9. Place the Filter Column in the Collection Tube and add 0.5ml of the sample. Centrifuge at 500x*g* for 1min at RT. Discard the flow through. Repeat with remaining 0.5ml of the sample.

10. Add 0.5ml of Wash Buffer. Centrifuge at 10,000x*g* for 15sec. Discard the flow through. Repeat 2 times (a total of 3 spins).

11. Centrifuge at 10,000x*g* for 2min at RT.

12. Place the Filter Column in a new Collection tube.

Add 0.05ml of Elution Buffer to the Filter Column. Centrifuge 200x*g* for 2min at RT then 10,000x*g* for 2min.