MANUAL

RayBio® Urine Exosome Nucleic Acid Extraction Kit (Magnetic Bead Method)

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ISO 13485:2016

Catalog #: NAE-UENA-MAG

Purpose

This kit is used for the extraction, enrichment, and purification of exosome RNA from urine.

Kit Components (50 sample kit)

Store kit in a dry environment at 2 – 30ºC for up to 24 months. Kit must be used within the validity period to ensure correct performance.

Components	Catalog #	Size	Number
Exosome Separation Solution	NAE-EXO	40 mL/bottle	1 bottle
Lysis Buffer A	NAE-LB-A	16 mL/bottle	1 bottle
Magnetic Bead Solution C	NAE-MAG-C	0.6 mL/vial	1 vial
DNA Digestive Enzyme	NAE-DIG	1.5 mL/vial	2 vials
Wash Buffer A	NAE-WB-A	30 mL/bottle	1 bottle
Wash Buffer B	NAE-WB-B	30 mL/bottle	2 bottles
Wash Buffer C	NAE-WB-C	30 mL/bottle	1 bottle
Elution Buffer A	NAE-EB-A	1.5 mL/vial	2 vials

Note: Do not mix reagents from different lots.

Required Materials (NOT INCLUDED)

1. Magnetic frame
2. Centrifuges (>12,000 x g)
3. Tabletop centrifuge
4. Vortex Mixer
5. Pipettes and sterile nuclease free pipette tips (barrier tips recommended)
6. Nuclease free microfuge and conical tubes
7. 100% ethanol

General Considerations

1. Please read this manual carefully before the experiment.
2. All samples should be regarded as potentially infectious and handled with extreme caution. Measures should be taken to minimize the risk of laboratory transmission based on risk assessment when testing any samples. These precautions, at a minimum, should include proficiency and competency testing, appropriate PPE, hazardous waste disposal, avoiding aerosols, and using effective disinfectants (quaternary ammonium compounds and 0.5% bleach). It is also recommended that extractions be performed in an appropriate biosafety cabinet.
3. Please use calibrated pipettes and nuclease free reaction tubes centrifuge tubes, conical tubes, and pipette tips.
4. Laboratory management should strictly follow the standards for PCR gene amplification.

Sample Requirements

1. Sample type: Human urine.
2. Sample collection: Collect 10 mL of human urine in a 15 mL collection tube according to the manufacturer's recommendations. Store at 4ºC until ready to use (up to 5 hours).

Protocol

1. **Reagent preparation**: Ensure that Lysis Buffer A is completely thawed and free from crystallization.

   Note: *If crystals are apparent, warm Lysis Buffer A to 30ºC and gently vortex until crystals dissipate.*
2. **Sample preparation**:
   - 2.1. Centrifuge at 2200 RCF for 15 minutes. Collect supernatant and aliquot into individual 1.5 mL microcentrifuge tubes (1 mL/vial).
   - 2.2. Proceed to step 3 immediately or store at -20ºC. Store unused aliquots at -20ºC for later use. If not
3. **Exosome separation**
   - 3.1. Retrieve two sample vials from step 2 (2 mL total sample volume). If using frozen samples, thaw at room temperature and then place on ice.
   - 3.2. Add 400 µL Exosome Separation Solution to each tube. Vortex for 10 seconds.

3.3. Centrifuge at 14000 RCF for 10 minutes. Discard the supernatant.

Note: *Remove as much excess liquid as possible.*

4. **Exosome nucleic acid cleavage**

4.1. Add 155 µL Lysis Buffer A to each microcentrifuge tube from step 3. Vortex for 10 seconds and then centrifuge at low speed for 5 minutes at room temperature.

4.2. Combine the contents of the two vials into a new labeled tube (155 µL from each vial).

4.3. Add 270 µL 100% ethanol and 10 µL Magnetic Bead Solution C. Vortex for 10 seconds. Incubate at room temperature for 10 minutes. Invert 5 times every 2 minutes.

4.4. Centrifuge at low speed for 2 minutes and place on a magnetic stand for 2 minutes. Discard the clarified liquid waste.

5. **Nucleic acid washing**

5.1. Add 600 µL Wash Buffer A and vortex for 10 seconds. Centrifuge briefly at low speed and place on magnetic stand for 2 minutes. Discard the clarified liquid waste.

5.2. Repeat previous wash step for a second wash (still using Wash Buffer A).

6. **Exosome DNA digestion**

6.1. Add 60 µL DNA Digestive Enzyme to the centrifuge tube and mix by vortexing for 10 seconds. Incubate at room temperature for 15 minutes.

7. **Nucleic acid RNA washing**

7.1. Add 600 µL Wash Buffer B and vortex for 10 seconds. Centrifuge briefly at low speed and place on magnetic stand for 2 minutes. Discard the clarified liquid waste.

7.2. Add 600 µL Wash Buffer C and vortex for 10 seconds. Centrifuge briefly at low speed and place on magnetic stand for 2 minutes. Discard the clarified liquid waste.

7.3. Open tube in clean environment and dry at room temperature for 2 minutes.

Note*: We recommend doing this step in a clean, sterilized PCR hood.*

8. **Nucleic acid RNA elution**

8.1. Add 30-60 µL Elution Buffer A and vortex for 10 seconds. Incubate at room temperature for 3 minutes. Vortex for 5 seconds every minute.

8.2. Centrifuge briefly at low speed and place on magnetic stand for 2 minutes. Transfer clarified liquid to a new 1.5 mL centrifuge tube.

***Note**: The clarified liquid contains the extracted nucleic acid. Do not discard!*

8.3. If the extracted nucleic acid is not immediately used, it can be stored below -20 °C.

Product Performance

Repeatability: Precision testing showed that the coefficient of variation (CV) of the precision Cq values within lots is less than 5%.

References

1. Zhang *et al*. Exosomes in cancer: small particle, big player. Journal of Hematology & Oncology, 2015, 8:83.
2. Richard J. Lobb *et al*. Optimized exosome isolation protocol for cell culture supernatant and human plasma. Journal of Extracellular Vesicles 2015, 4: 27031.
3. Taixue An *et al*. Exosomes serve as tumor markers for personalized diagnostics owing to their important role in cancer metastasis. Journal of Extracellular Vesicles 2015, 4: 27522.