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Total RNA Purification Kit - Supplementary Protocol for Exosomal RNA Purification from Exosomes Already Purified Ultracentrifugation, Exoquick, Filtration or any other Precipitation Method

Product # 17200, 17240

Customer-Supplied Reagents

- 0.2µ filtered 1X PBS - pH 7.4 (RNase-free).
- 96 - 100% ethanol

Notes Prior to Use

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.

1. Exosome Preparation

a. Resuspend your previously purified exosome pellet in 200 µL 1X PBS (pH 7.4) (provided by the user). Mix by vortexing for 10 seconds.

Note: If your previously purified exosomes are already in a different buffer proceed to Step 2 below - Lysate Preparation. The maximum volume of your sample shouldn't exceed 200 µL

2. Lysate Preparation

a. Add 600 µL of **Buffer RL** to your exosome sample prepared in section 1. Mix by vortexing for 10 seconds.
b. Add 800 µL of **96-100% ethanol**. Mix by vortexing for 10 seconds

3. Binding RNA to Column

a. Assemble a Spin Column with one of the provided collection tubes.
b. Apply up to 600 µL of the lysate with the ethanol (from **Step 2b**) onto the column and centrifuge for 1 minute at **≥ 3,500 x g (~6,000 RPM).**

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at **14,000 x g (~14,000 RPM).**

c. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Depending on your lysate volume, repeat Step **3b** and **3c** as necessary.

Optional Step:

Norgen's Total RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided in Appendix A of the full version manual available on our website for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step. This step should be performed at this point in the protocol

4. Column Wash

a. Apply 400 µL of **Wash Solution A** to the column and centrifuge for 1 minute.

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Repeat steps **4a** and **4b** to wash column a second time.
d. Wash column a third time by adding another 400 µL of **Wash Solution A** and centrifuging for 1 minute.
e. Discard the flowthrough and reassemble the spin column with its collection tube.
f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

5. RNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 µL of **Elution Solution A** to the column.
c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 1 minute at **14,000 x *g* (~14,000 RPM)** Note the volume eluted from the column. If the entire 50 µL has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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