

**Total RNA Purification Micro Kit - Supplementary Protocol for Exosomal
RNA Purification from Exosomes Already Purified via Ultracentrifugation,
Exoquick, Filtration or any other Precipitation Method
Product # 35300**

Customer-Supplied Reagents

- 0.2 μ 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 μ L 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 μ L.

2. Lysate Preparation

- a. Add 600 μ L of **Buffer RL** to your exosome sample prepared in section 1. Mix by vortexing for 10 seconds.
- b. Add 800 μ L of **96-100% ethanol** (provided by the user). Mix by vortexing for 10 seconds.

3. Binding RNA to Column

- a. Assemble a Micro Spin Column with one of the provided collection tubes.
- b. Apply up to 600 μ L of the lysate with the ethanol (from **Step 2b**) onto the column and centrifuge for 1 minute at **$\geq 3,500 \times g$ (~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 **$14,000 \times g$ (~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.

4. Column Wash

- a. Apply 400 μ L 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 μ L 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 20 μ L up to 50 μ L of **Elution Solution A** to the column and let stand at room temperature for 2 minutes.
- 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 20 μ L - 50 μ L 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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