Exosomal RNA and Protein Extraction kit

Cat. #: P200

Storage: keep all bottles upright. Store Exosomal Protein Lysis buffer in -20°C and other bottles at room temperature in dark place.

Shelf Life: 6 months

Application: our "2 in 1" kit is for extracting both **exosomal RNA** and **exosomal protein** from pure exosome isolated by our Exosome Isolation Kits (Cat. #: P100, P101, P120, P121). This product is for research use only.

Product Size: 20 extractions

Product Content

Component	Amount	Storage
Exosomal Protein Lysis buffer *	2 mL	-20°C
N1	5 mL	room temperature
N2	1 mL	room temperature
N3	2.5 mL	room temperature
N4	10 mL	room temperature
RNA Elution buffer	0.5 mL	room temperature

* *Store at* 4° C for 7 days or aliquot and store at -20°C for up to 3 months.

Important: RNA is sensitive to RNase. Before starting RNA extraction, prepare clean lab bench and wipe working surface and pipettors with RNase decontamination solution, such as Ambion® RNaseZap®. Always wear clean laboratory gloves during manipulation.

Protocol

Sample prepare

1. Transfer the isolated exosomes (by our PureExo® Exosome Isolation kits) to an RNase free tube. Add **1× PBS** buffer to the exosomes to a final total volume of **100µl**. Concentrated exosome will precipitate. Pipet up and down to mix well before use.
2. Mix well and split the exosomes into two portions: **75 µl for RNA** extraction and **25 µl for protein** extraction, if both RNA and protein extraction are desired.

Exosomal RNA extraction

Homogenization

3. Transfer the **75 µl exosomes** to an RNase free tube and add **250 µl N1**; mix extensively by pipetting up and down and incubate **5 min** at **room temp**.

   * For other volumes of exosome, adjust N1 volume **proportionally**.

Phase Separation

4. Add **50µl N2** to the sample and vortex vigorously for **15 seconds** and incubate at **room temp for 2-3 min**.

5. Centrifuge sample at **12,000x g** for **15 min.** at **4ºC**.

6. Without disturbing interphase, transfer the upper aqueous phase to a **new RNase-free tube**.

Precipitation

7. In the new RNase free tube, add **125 µl N3** to precipitate exosomal RNA;

8. Incubate for **15 min.** at **room temperature.** Centrifuge at **12,000x g** for **10 min.** at **4ºC**;

9. The **exosomal RNA** precipitates as gel like pellet on the bottom / side of the tube. Carefully remove / discard the supernatant.

Wash

10. Wash RNA pellet with **250 µl N4**, mix and centrifuge at **7,500x g** for **5 min.** at **4ºC**. Remove supernatant without disturbing RNA pellet;

11. Repeat step 10 once;

Elution

12. **Air dry** the exosomal RNA pellet for 10 min. at room temp;

13. Dissolve the exosomal RNA pellet in **10-15 µl RNA Elution buffer.** Use this extracted exosomal RNA for downstream assay or store it at -80ºC for future use.

Exosomal protein extraction

14. Thaw exosomal protein lysis buffer aliquot at **room temperature** and keep it on ice.

15. Transfer the **25 µl** exosome sample to a clean tube and add exosomal protein lysis buffer **50-100 µl**; mix the sample well by pipetting up and down.

16. Incubate **15 min.** at **4ºC** and centrifuge the sample at **14,000x g** for **10 min.** at **4ºC**.

17. The supernatant is the extracted exosomal protein. Transfer the supernatant to a clean tube and keep on ice. Measure the protein concentration. Use it for downstream assay or store the samples at -80ºC for up to 3 months.

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