Exosomal RNA and Protein Extraction kit (Cat.#: P200)

Exosomal RNA Extraction kit (Cat. #: P200R)

Exosomal Protein Lysis Buffer (Cat. #: P200P) (store at −20°C)

Storage: keep all bottles upright. Store Exosomal Protein Lysis buffer (P200P) at −20°C and other bottles at room temperature in dark place.

Shelf Life: 6 months

Application: This kit is for extraction of **exosomal RNA (including microRNA)** and/or **exosomal protein** from pure exosome isolated by our PureExo® Exosome Isolation Kits (Cat. #: P100, P101, P120, P121). This product is for research use only.

Product Size: 20 extractions

Product Components

Cat. #		Components	Amount	Storage
P200	P200R	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
	P200P	Exosomal Protein Lysis Buffer *	2 ml	−20°C

* *Store at* 4° C for 7 days or aliquot and store at −20°C for up to 6 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 experiment.

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 **100 µl**. Concentrated exosome will precipitate. Pipet up and down to mix well before use.
2. Mix well and split the exosome sample 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 (using P200R)

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 minutes** at **RT** (room temperature).

* For other volumes of exosome, adjust all buffer volumes **proportionally**.

Phase Separation

4. Add **50 µl N2** to the sample and vortex vigorously for **15 seconds.** Incubate at **RT for 2 ~ 3 minutes**.

5. Centrifuge sample at **12,000x g** for **15 minutes** 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 **RT.** Centrifuge at **12,000x g** for **10 minutes** at **4ºC**;

9. The **exosomal RNA** precipitates as gel like pellet at 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 minutes** 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 RT. Do not over dry.

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 -80°C for up to 6 months.

Exosomal protein extraction (using P200P)

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

15. Transfer the **25 µl** exosome sample to a clean tube and add **50 ~ 100 µl** Exosomal Protein Lysis Buffer and mix well by pipetting up and down.

16. Incubate **15 minutes** at **4ºC** and centrifuge the sample at **14,000x g** for **10 minutes** 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 -80°C for up to 3 months.

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