

TransExo™ Serum/Plasma Exosome Total RNA Extraction Kit

Cat. No. FE201

Storage: EPS should be stored at 2-8°C for one year, ELB2 should be stored at 2-8°C in the dark for one year, and other reagents in this kit should be stored dry at room temperature (15-25°C) for one year.

Description

TransExo™ Serum/Plasma Exosome Total RNA Extraction Kit is designed to extract total RNA from serum/plasma exosomes with easy and fast column-based purification procedure, featuring high yield. The whole procedure could be completed within two hours.

- Easy, efficient and fast procedure.
- No ultracentrifugation is required.
- Exosome total RNA is extracted with high yield and purity.

Kit Contents

Component	FE201-01 (25 rxns)	FE201-02 (50 rxns)
Exosome Precipitation Solution (EPS)	2×1.6 ml	4×1.6ml
Exosome Lysis Buffer (ELB1)	5 ml	10 ml
Exosome Lysis Buffer (ELB2)	15 ml	30 ml
Exosome Wash Buffer (EWB1)	3 ml	2×3 ml
RNase-free Water	1 ml	2×1 ml
RNase-free Tubes (1.5 ml)	25 tubes	50 tubes
ExoRNA Spin Columns with Collection Tubes	25 tubes	50 tubes

Procedures

Please adjust refrigerated centrifuge to 2-8°C in advance, and add 12 ml of anhydrous ethanol to EWB1 prior to use.

Additional required materials: chloroform, anhydrous ethanol and isopropanol.

1. Centrifuge the serum/plasma at 3,000×g for 15 minutes at 2-8°C to remove cell debris.
2. Add 125 µl of EPS solution to 500 µl of serum/plasma sample, and mix by inverting or flicking the tube.
3. Incubate the mixture at 2-8°C for 30 minutes, and centrifuge at 10,000×g for 10 minutes at 2-8°C. Discard the supernatant carefully.
4. Centrifuge the pellet again at 10,000×g for 5 minutes at 2-8°C. Pipet the residue supernatant with 200 µl tip carefully.
5. Add 150 µl of ELB1 to the pellet, and gently pipet to dissolve the yellow precipitate completely till it turns to clear yellow liquid.
6. Add 500 µl of ELB2 and vortex for 10 seconds to mix well. Incubate at room temperature for 5 minutes. (It is normal to have a small amount of white flake insoluble substance).
7. Add 100 µl of chloroform and shake the tube vigorously for 30 seconds. Incubate at room temperature for 3 minutes.
8. Centrifuge at 10,000×g for 15 minutes at 2-8°C. The mixture separates into 3 phases: a lower pink organic phase, an interphase, and a colorless upper aqueous phase which contains the RNA. The volume of the upper aqueous phase is about 80%-90% of ELB2 reagent.
9. Transfer the colorless upper aqueous phase containing the RNA to a fresh RNase-free tube (to avoid DNA contamination, a portion of aqueous phase could be left in the tube). Add isopropanol at the ratio of 1.25:1 to the upper aqueous phase (e.g. 500 µl isopropanol: 400µl upper aqueous phase). Mix gently by inverting the tube.

All following centrifugation steps are carried out at room temperature.

10. Add the obtained solution into the ExoRNA spin column, and centrifuge at 12,000×g for 1 minute at room temperature. Discard the flow-through. If the solution volume is larger than the maximum volume of the ExoRNA spin column, repeat this step until the entire solution flows through the column.

11. Add 500 μ l of EWB1 (make sure that the anhydrous ethanol has been added) into the spin column. Centrifuge at 12,000 \times g for 1 minute at room temperature. Discard the flow-through.
12. Centrifuge the column at 12,000 \times g for 2 minutes at room temperature to remove ethanol residue completely. No extra air-dry step is needed.
13. Place the ExoRNA spin column into a clean 1.5 ml RNase-free tube. Add 30 μ l of RNase-free Water into the center of spin column matrix and incubate at room temperature for 1 minute.
14. Centrifuge at 12,000 \times g for 1 minute to elute RNA at room temperature.
15. Store the isolated RNA aliquotes at -80°C.

Notes

- Plasma containing heparin anticoagulant is not suitable for this product.
- Serum/plasma is suggested to be aliquoted into small volumes and kept in -80 °C to avoid repeated freeze-thawing.
- It is important to mix well after adding chloroform.
- All the reagents, tubes and tips should be RNase-free.
- Isolated RNAs cannot be quantified with spectrophotometer.
- The quantities of exosome RNAs in serum/plasma vary from different species. Setting parallel groups is suggested in the experiment to collect reliable data.
- No extra air-dry step is needed before elution with RNase-free Water as overdried ExoRNA Spin Column may decrease elution efficiency.
- ELB1 should be added immediately to exosome precipitate for lysis after centrifugation of Step 3 as long incubation on ice may affect yield.

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