TransExo™ Serum/Plasma Exosome miRNA Extraction Kit

Cat. No. FE301

Storage: EPS at 2-8°C for one year, ELB at 2-8°C away from light for one year, others at room temperature (15-25°C) for one year.

Description

TransExo™ Serum/Plasma Exosome miRNA Extraction Kit is designed to extract miRNA from serum/plasma exosomes with easy column-based purification procedure.

- Easy, efficient and fast procedures. No ultracentrifugation required.
- High yield and purity. Suitable for varieties of applications, such as qPCR.

Kit Contents

Component	FE301-01 (25 rxns)	FE301-02 (50 rxns)
Exosome Precipitation Solution (EPS)	1.25 ml	2×1.25 ml
Exosome Lysis Buffer (ELB)	15 ml	30 ml
Exosome Wash Buffer (EWB)	6 ml	2×6 ml
RNase-free Water	5 ml	10 ml
RNase-free Tubes (1.5 ml)	25 tubes	50 tubes
RNA Spin Columns with Collection Tubes	25 tubes	50 tubes
miRNA Spin Columns with Collection Tubes	25 tubes	25 tubes

Procedures

Please adjust refrigerated centrifuge to 4°C in advance, and add 24 ml of 96-100% ethanol to EWB prior to use.

Materials required but not included: chloroform (or 4-Bromoanisole), 96-100% ethanol.

1. Centrifuge the serum/plasma at 3,000×g for 15 minutes at 2-8°C to remove cell debris. Collect the supernatant.
2. Add 50µl of EPS solution to 200µl of serum/plasma sample, 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 at 10,000×g for 5 minutes, or at 3,000×g for 30 minutes at 2-8°C. Discard the supernatant carefully.
5. Add 500 µl of ELB to the precipitation, gently pipette to dissolve (A little faint yellow phospholipid may not be dissolved, but will not affect the results).
6. Add 100 µl of chloroform to each 500 µl of ELB. Vortex the tube vigorously for 30 seconds. Incubate at room temperature for 3 minutes.
7. Centrifuge at 10,000×g for 15 minutes at 2-8°C. The mixture will be separated into a lower pink organic phase, an interphase, and a colorless upper aqueous phase which contains the RNA. The volume of the aqueous upper phase is about 50-60% volume of ELB reagent.
8. Transfer the colorless, upper phase containing the RNA to a fresh Nuclease-free tube (to avoid DNA contamination, a portion of aqueous phase could be left in the tube). Add 96-100% of ethanol (equal to 1/3 volume of the transferred solution) to the transferred solution (e.g. add 200 µl of 96-100% of ethanol to 600 µl of transferred solution. Some precipitates may form at this moment). Mix gently by inverting tube.

   All following centrifugation steps are carried out at room temperature.
9. Add the entire lysate into the RNA spin column, centrifuge at 12,000×g for 30 seconds, collect the flow-through.
10. Measure the volume of flow-through and transfer to a clean 1.5 ml or 2 ml Nuclease-free tube. Add 96-100% ethanol (equals to 1.25 volume of flow-through) to the tube (e.g. add 812.5 µl of 96-100% ethanol to 650 µl of flow-through. Some precipitates may form at this moment). Mix gently by inverting tube.

11. Add the entire lysate into the miRNA spin column, centrifuge at 12,000×g for 30 seconds, discard the flow-through (if the volume of the lysate is more than the loading volume, repeat this step until all the lysate is applied).
12. Add 500 µl of EWB (make sure that the ethanol has been added) into the spin column. Centrifuge at 12,000×g for 30 seconds. Discard the flow-through.
13. (Optional) Repeat step 13 once.
14. Centrifuge the column at 12,000×g for 2 minutes to remove ethanol residue.
15. Place the miRNA spin column into a clean 1.5 ml Nuclease-free tube. Add 20-30 µl of Nuclease-free Water into the spin column matrix and incubate for 1 minute at room temperature.
16. Centrifuge at 12,000×g for 1 minute to elute miRNA.
17. Store the isolated miRNA at -80°C.

Notes

- Plasma containing heparin anticoagulant is not suitable for this product.
- Serum/plasma is suggested to be aliquoted into single-use aliquots and kept in -80°C, avoid repeated freeze-thaw cycles.
- It is important to vortex vigorously after adding chloroform.
- All of the reagents, tubes and tips should be Nuclease-free..
- Isolated miRNAs cannot be quantified with spectrophotometer, because of the low amount of single extraction.
- The characteristics and quantities of exosome miRNAs in serum/plasma vary from different samples of human or animals. Setting parallel groups is suggested in the experiment to collect enough information.

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