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Exosome RNA Extraction Kit Instructions

Composition

Exosome RNA Extraction Kit Cat. No.	5 Preps 5202005	50 Preps 5202050
Spin Columns	5	50
2 ml Collection Tubes	5	50
Buffer TL	7.5 ml	75 ml
Buffer EX	1.2 ml	12 ml
Buffer WA (concentrate)	1.5 ml	15 ml
Buffer WBR (concentrate)	1 ml	10 ml
RNase-free Water	1.5 ml	2 ml×2
Instructions	1	1

Storage

1. Buffer TL should be stored at 2~8℃.
2. If the product is stored at room temperature (0~30℃), it can keep the performance for two years, and if the product is stored at 2~8℃, the validity period of the product can be extended to more than two years.

Technical Support

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Introduction

This kit is suitable for the recovery of RNA from 100~200 μl isolated exosomes. Column purification technology can efficiently recover small RNA fragments, and lipids, proteins and other substances are filtered out. The exosome RNA is finally eluted into a microvolume of 30~50 μl and can be immediately used in various molecular biology experiments related to exosome RNA.

Equipment And Reagents to Be Supplied by Users

1. Absolute ethanol
2. 1.5 ml centrifuge tubes (RNase-free 1.5 ml tubes are necessary)
3. Pipette and tips (to avoid contamination between samples, use pipette tips with filters)
4. Disposable gloves, tissues and protective equipment
5. Microcentrifuge (s) (rotor with 1.5 ml and 2 ml tubes)
6. Vortexer.

Preparation Before Use

1. If the centrifuge has refrigeration, set the temperature to 25℃.
2. Add absolute ethanol to Buffer WA and Buffer WBR according to the instructions on the label the reagent bottle and tick the box on the label to mark "Ethanol Added".

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Protocol

The following steps are to extract exosome RNA from 100 µl exosome solution, if the volume of exosome solution is large, increase the amount of Buffer TL, Buffer EX and absolute ethanol proportionally, and the amount of reagents used for other washing or elution steps (Buffer WA, Buffer WBR, RNase-free Water) remains the same.

1. **Add 700 µl Buffer TL to the exosome solution and vortex to mix well.**

* Add Buffer TL at 7 times the volume of exosome solution, e.g., 1.4 ml Buffer TL for 200 µl exosome solution RNA extraction.
* If the exosome solution is less than 100 µl, add 700 µl Buffer TL directly.
* You can also add 700 µl Buffer TL directly to a column with exosomes adsorbed (e.g., Qiagen product), centrifuge at 5000×g for 5 min, collect the filtered exosome solution, transfer to a clean 1.5 ml centrifuge tube, and follow step 2.

2. **Add 100 µl Buffer EX, close the lid, and shake vigorously for 15 sec to mix well. Centrifuge at 4°C at 12,000 rpm for 15 min.**

* Add Buffer EX as 1× the volume of the exosome solution, e.g. 200 µl Buffer EX for 200 µl exosome solution RNA extraction.
* If the exosome solution is less than 100 µl, add 100 µl Buffer EX directly.

3. **Transfer the supernatant (about 450 µl) to a clean 1.5 ml centrifuge tube, add 2 times the volume of supernatant (about 900 µl) of absolute ethanol, invert the centrifuge tube several times and mix well.**
4. **Transfer 700 µl mixture from step 3 to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12,000 rpm for 30 sec.**
5. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, transfer the remaining mixture from step 3 to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.
* If RNA is extracted from a volume greater than 200 µl exosome solution, centrifuge the mixture several times in the same way until all the mixture has passed through the purification column.

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube. Add 700 µl Buffer WA to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WA.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube. Add 800 µl Buffer WBR to the Spin Column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WBR.

8. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at its full speed for 2 min.
* This step of high-speed emptying is to remove the residual ethanol, please do not omit, otherwise the subsequent experimental effect may be affected due to the residual ethanol in the extracted nucleic acid.

9. **Discard the 2 ml tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 30-50 µl RNase-free Water, close the lid, incubate at room temperature for 1 min, and centrifuge at 12,000 rpm for 30 sec to elute RNA.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm centrifugation for 1 min to avoid the tube cap coming off and damaging the centrifuge.

10. **Discard the Spin Column, the eluted RNA can be immediately used in a variety of molecular biology experiments or stored below -70°C for later use.**