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Exosome DNA Extraction Kit Instructions

Composition

Exosome DNA Extraction Kit Cat. No.	50 Preps 4022050
Spin Columns	50
2 ml Collection Tubes	50
Carrier RNA	180 µl
Buffer AC (concentrate)	37 ml
Buffer WB (concentrate)	17 ml
Buffer TE	5 ml
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Storage

1. Carrier RNA should be stored at -20℃.
2. Proteinase K can be stored for at least 1 year at room temperature (0~30℃). For longer storage, it is recommended to keep at 2~8℃.
3. Other reagents and items can be stored within 2 years at room temperature (0~30℃). For longer storage, it is recommended to keep at 2~8℃.(the product stored at 2~8℃ should be restored to room temperature before use).

Technical Support

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Introduction

This kit is designed to recover concentrated DNA from 200 to 400 µl isolated exosome solutions. Column purification technology combined with Carrier RNA can efficiently recover DNA fragments of about 100 bp, and lipids, proteins and other substances are filtered out. Exosome DNA can eventually be eluted to a microvolume of 30~50 µl and can be immediately used in various molecular biology experiments related to exosome DNA.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol, isopropanol.
2. 1.5 ml centrifuge tubes, pipettes, and tips (tips with filter are recommended to prevent cross-contamination between samples).
3. Disposable gloves, paper towels and protective supplies.
4. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml Collection Tubes).

Preparation Before Use

1. If the centrifuge has a refrigeration function, set the temperature to 25℃.
2. Add isopropanol to Buffer AC and absolute ethanol to Buffer WB according to the instructions on the bottle label and tick the box on the label to mark " Isopropanol Added" and "Ethanol Added".

Protocol

1. **Add 2.5 times the volume of Buffer AC and 3 µl Carrier RNA to the collected exosome solution, and vortex to mix well.**

* Ensure that isopropanol has been added to Buffer AC.
* For example, if you need to recover DNA from a 200 µl exosome solution, you need to add 500 µl Buffer AC.
* The volume ratio of exosome solution to Buffer AC must be strictly mixed with a volume ratio of 1:2.5, otherwise it will seriously affect the efficiency of subsequent exosome DNA recovery.
* When DNA is recovered from exosome solutions of different volumes, the amount of Carrier RNA added remains unchanged. Carrier RNA can greatly improve the recovery efficiency of trace nucleic acids and cannot be omitted.

2. **Transfer the mixture into a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid and centrifuge at 12000 rpm for 30 sec.**

* If DNA is recovered from an exosome solution larger than 200 µl volume, divide the mixture through the column in twice.

3. **Discard the filtration and place the Spin Column back into the 2 ml Collection Tube. Add 700 µl Buffer WB to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded. To avoid contamination of the centrifuge by the filtrate adhering to the nozzle of the centrifuge tube, the 2 ml Collection Tube can be slapped once on a paper towel.
* Ensure that absolute ethanol has been added to Buffer WB.

4. **Repeat step 3 once.**
5. **Discard the filtration, 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 14000 rpm, centrifuge at the full speed for 2 min.
* This step is to remove the remaining ethanol at a high speed. Please do not omit it, otherwise it may affect the subsequent experimental effect due to the residual ethanol in the purified nucleic acid.

6. **Discard 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 30~50 µl Buffer TE to the center of the Spin Column, close the lid, incubate at room temperature for 1 min, centrifuge at 12000 rpm for 30 sec to elute DNA.**

* If the centrifuge does not have a leak-proof cap, change the centrifuge condition to 8000 rpm for 1 min to avoid damage to the centrifuge due to the cap falling off.

7. **Discard the Spin Column, the eluted DNA can be immediately used in various molecular biology experiments or stored at -20℃ for later use.**