

VEX Exosome Isolation Reagent (from cell culture media)

R601



Version 8.1

Vazyme biotech co., Ltd.

Introduction

VEX Exosome Isolation Reagent (from cell culture media) is specially designed for isolating the exosome, containing RNA and protein secreted by various types of cells, from the supernatant of cell culture media. Compared with the traditional ultra-speed centrifugation, the simple low-speed centrifugation involved in this reagent makes the exosome less affected by centrifugal stress, therefore more intact in morphology. Meanwhile, VEX Exosome Isolation Reagent can save the experiment time, requires less input amount of sample, and with high isolation efficiency. The exosomes obtained by this product can be applied to a variety of downstream applications, such as RNA analysis, high-throughput sequencing, cell co-culture, etc.

Components

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VEX Exosome Isolation Reagent (from cell culture media)	50 ml

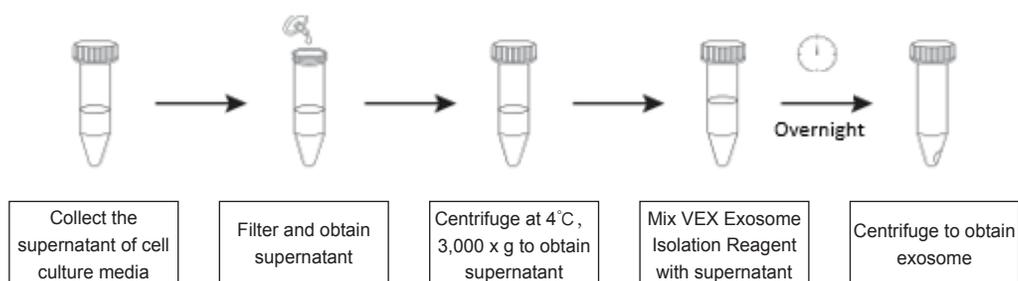
Storage

Store at 4°C

Quality Control

Function assay: harvest 6 ml of supernatant of HeLa cell culture media (cultured for 48h), use VEX Exosome Isolation Reagent (from cell culture media) to isolate exosome, from which followed by a RNA isolation, and lastly, take 5 - 20 ng of that RNA as template to detect expressions of two small RNA by qPCR.

Workflow



- Culture the cells in normal serum media for a period of time.
 - For adherent cells, when cell density reaches to about 50% - 70%, replace the original serum-containing media with fresh serum-free media or exosome-free serum media for further cultivation. Harvest the supernatant of cell culture media when adherent cell density reaches approximately 80% - 95%.
 - For suspension cells, when cell density reaches to around 50% - 70%, collect cells by centrifuging at 4°C, 300 x g for 10 min, suspend and culture the cells by fresh serum-free media or exosome-free serum media. When the suspension cell density reaches 80% - 95%, collect the mixture of cells and culture media and centrifuge at 4°C, 300 x g for 10 mins for the following supernatant separating.
- Aspirate the supernatant of culture media with syringe, then filter with a 0.22 μm filter to remove cell debris and bacteria.
- Transfer the filtrate to a new centrifuge tube and centrifuge at 4°C, 3,000 x g for 20 mins to remove the cell debris and bacteria.



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- Gently aspirate the supernatant into a new centrifuge tube without disturbing the cell sediment and add 1/3 volumes of the VEX Exosome Isolation Reagent (from cell culture media).

Supernatant volume of cell culture media	Reagent volume to be added rxn (20 µl/rxn)
1 ml	0.33 ml
9 ml	3 ml

- Gently invert several times to mix well until there is a homogenous solution.
- Incubate overnight (about 16h) at 4°C (in refrigerator).
- Centrifuge the overnight-cultured sample at 4°C, 10,000 x g for 30 mins, aspirate and discard the supernatant by 1ml pipette tips to obtain the exosome sediment.

*Due to the sediment that may be invisible, we recommend to use basket centrifuge or to mark the direction of centrifuge tube used in angle-fixed centrifuge when performing centrifugation, so as to facilitate the exosome sediment recognition.
- (Optional) centrifuge at 4°C, 1,500 x g for 2 mins, and discard the supernatant thoroughly.
- Resuspend the exosome sediment in a convenient volume of 1X PBS or directly apply the sediment to subsequent experiment.

Store the exosome at 4°C for up to 1 week, or -20°C/-70°C for long term if necessary.

Troubleshooting

◇ What is the requirement for samples?

VEX Exosome Isolation Reagent (from cell culture media) is suitable for the exosome isolation from all kinds of cell culture media. To prevent bovine exosomes contamination from FBS, serum-free culture or exosome-free serum media can be used to replace the old culture media when cells density reaches 50% - 70%; after a further cell cultivation for 12 h, the supernatant can be collected and used. The cells can also be cultured by initially using the exosome-free serum media, and exosomes can be directly extracted from the collected culture media supernatant without changing the culture media.

◇ How much media does a single experiment need?

The input volume of sample varies according to different experiment demand. The amount of exosomes in cell culture media are influenced by cell type, cell state and cell number.

◇ Why marking direction of the centrifuge tube when using angle-fixed centrifuge?

Since the sediment are not visible in most cases, marking centrifugation tube will help to locate and resuspend the exosome sediment.

◇ How to apply the isolated exosome to downstream experiment?

The isolated exosome sediment can be resuspend by 1X PBS or directly by the reagent subsequently used in the downstream experiment.



ISO 9001: 2015