DATASHEET

BIO-DOC

Cell Culture Media Exosome Extraction Kit
CAT#BDEP50P

For exosome extraction from cell culture media

Introduction

The Cell Culture Media Exosome Extraction Kit is specially designed for isolating the exosome, which contains the RNA and protein secreted by various types of cells, from the supernatant of cell culture media. Compared with traditional ultrahigh-speed centrifugation, the simple low-speed centrifugation process used in the aforementioned extraction kit results in lower centrifugal stress being exerted on the exosome; thus, the morphology of the exosome remains more intact when using low-speed centrifugation than when using ultrahigh-speed centrifugation. Moreover, this product can save time, requires a small number of input samples, and provides high isolation efficiency. The exosomes obtained using this product can be applied in various downstream applications, such as RNA analysis, high-throughput sequencing, and cell coculturing.

Kit Contents

Content	BDEP50P
Exosome extraction reagent	50 mL

Output
A total of 5–20 ng of total RNA is extracted using the following protocol: harvest 6 mL of the supernatant obtained from HeLa cell culture media (cultured for 48 h), use the exosome extraction reagent to isolate the exosome, and perform RNA isolation.

Protocol

1. Culture the cells.
   a For adherent cells, when cell density reaches approximately 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 the cell culture media when the adherent cell density reaches approximately 80%–95%.

   b For suspension cells, when cell density reaches approximately 50%–70%, collect cells by centrifuging the suspension at 4 ˚C and 300 × g for 10 min. Then, suspend and culture the cells by using 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 it at 4 ˚C and 300 × g for 10 min to separate the supernatant from the mixture.

2. Aspirate the supernatant of culture media with a syringe. Then, filter the supernatant with a 0.22-μm filter to remove cell debris and bacteria.
3. Transfer the filtrate to a new centrifuge tube and centrifuge it at 4 ˚C and 3000 × g for 20 min to remove the cell debris and bacteria.
4. Gently aspirate the supernatant into a new centrifuge tube without disturbing the cell sediment, and add the exosome extraction reagent with a volume that is one-third that of the supernatant to the mixture.

Supernatant volume of cell culture media	Reagent volume to be added
1 mL	0.33 mL
9 mL	3 mL

5. Gently invert the centrifuge tube several times until a homogenous solution is attained.
6. Incubate the centrifuge overnight for approximately 16 h at 4 ˚C in a refrigerator.
7. Centrifuge the overnight-cultured sample at 4 ˚C and 10 000 × g for 30 min, and aspirate and discard the supernatant by using 1-mL pipette tips to obtain the exosome sediment.

   Note: Because the sediment may be invisible, we recommend using a basket centrifuge or marking the direction of the centrifuge tube used in an angle-fixed centrifuge when performing centrifugation to facilitate exosome sediment recognition.

8. (Optional) Centrifuge the sediment from step 7 again at 4 ˚C and 1500 × g for 2 min to discard the extra supernatant.
9. Resuspend the exosome sediment in a convenient volume of 1× PBS or directly apply the sediment in the subsequent experiments.

   Note: Store the exosome at 4 ˚C for up to 1 week or at −20 or −70˚C for a long period.

Storage

The Cell Culture Media Exosome Extraction Kit should be stored at 2–8 ˚C.