Exopresso® Exosome Isolation Kit- Cell Culture Media

For total exosome isolation from cultured cells media

Precautions

I. Handling Requirements
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

II. Equipment and Reagents to Be Supplied by User

- PBS
- 1.5 ml microcentrifuge tubes
- Pipet tips with aerosol barrier
- Vortexer
- Microcentrifuge (with rotor for 1.5 ml tubes) may be required for some samples

III. Waste Handling

- Treat waste with the country, federal, state and local regulations.

IV. Important points before use

- Do not use the product if it has expired.

Kit Contents

Content	Cat.No.
Exopresso® Exosome Isolation reagent-Cell Culture Media, 50ml	EPC-50

Storage and Stability:

This kit should be stored at 2-8°C.

Description

Exopresso® Exosome Isolation Kit is 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, using simple low-speed centrifugation equipment with this reagent makes the exosome less affected by centrifugal stress. Meanwhile, this product can save the experiment time, requires less input amount of sample, and with high isolation efficiency. The exosomes isolated by this kit can be applied to a variety of downstream applications, such as RNA analysis, high-throughput sequencing, cell co-culture, etc.

Exopresso® exosome Isolation Kit Test Data

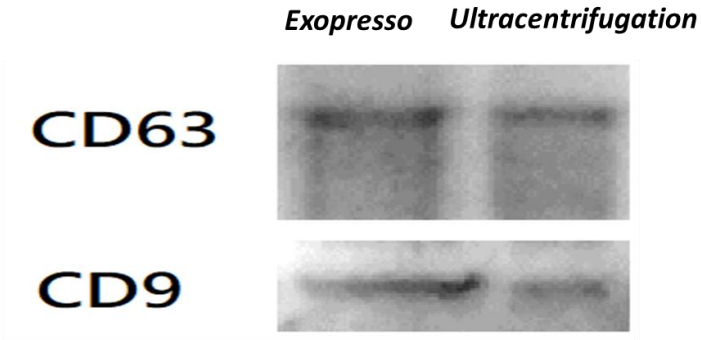

Fig. 1. Western blot analysis for the presence of exosomal marker protein CD63 and CD9 in samples recovered with Exopresso and ultracentrifugation method.

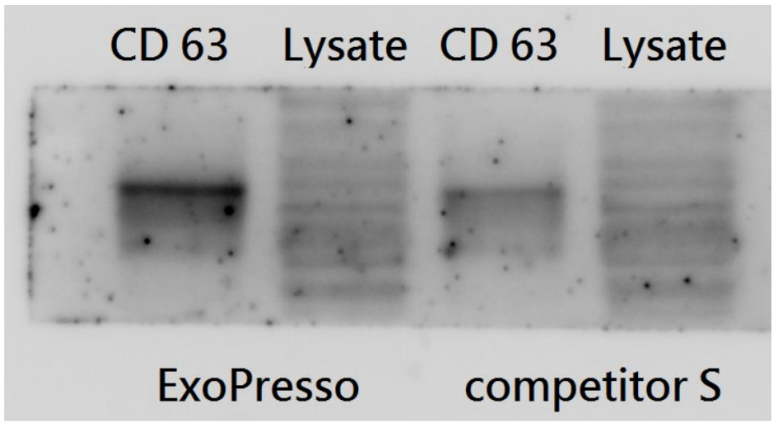

Fig. 2. Western blot analysis for the presence of exosomal marker protein CD63 in samples recovered with Exopresso and competitor S Exosome Isolation reagent.

Preparation before using

1. Exopresso® Exosome Isolation Kit 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.
2. Wear a lab coat and disposable gloves to prevent RNase contamination.
3. The input sample volume is according to different experiment conditions. The amount of exosomes in cell culture media are influenced by cell type, cell state and cell number.

Culture cell media Protocol Procedure

1. Collect cell culture media and centrifuge at 3000 × g for 15 minutes to remove cells and cell debris.
2. Transfer supernatant to a sterile vessel and add five fold volume of Exopresso to the culture media. Mix well by inverting or flicking the tube.
3. Refrigerate 1 hour at +4°C. The tubes should not be rotated or mixed during the incubation period and should remain upright.
4. Centrifuge Exopresso/culture media mixture at 1500 xg for 30 minutes. Centrifugation may be performed at either room temperature or +4°C with similar results. After centrifugation, the exosomes may appear as a beige or white pellet at the bottom of the vessel.
5. Aspirate supernatant. Add 5 ml PBS smoothly and centrifuge at 10000 × g for 20 minutes. Remove all traces of fluid by aspiration, taking great care not to disturb the precipitated exosomes in pellet.
6. Resuspend exosome pellet in 100-500 µl using sterile 1X PBS, or specific buffer according to your downstream application. We recommend using the precipitated exosomes immediately rather than freezing them for future use.

Troubleshooting

Problem	Possible Reasons/Solution
Invisible pellet after centrifuging	•Scale up the volume of culture media to precipitate more exosomes.
The exosome pellet is difficult to resuspend in buffer	•Try adding slightly more PBS to the pellet and allow the pellet to sit at room temperature for 5-10 minutes before resuspending.