Kimia Andisheh Gene Pouyan

Knowledge based, technology- and research

Technical Manual

Exosome Extraction Kit

- ***Catalogue Code: 106-Exokp***
- ***Size: R1:25 ML R2:5ML***
- ***Research Use Only***

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Introduction

Exosomes are extracellular vesicles with a nano-size (30 to 150 nm) that have a phospholipid bilayer membrane and a hydrophilic core (the vesicle interior). Different cells are always able to secrete these nano-vesicles that contain different cargoes such as DNA, RNA, lipids, proteins, etc. Depending on the type of producing cell and their mission, exosomes play a role in different pathways such as intercellular communication, signal transduction, antigen presentation, and tumor progression. Therefore, the biological .content of exosomes will be different.

The *Exokp*" kit is a product that offers a simple, reliable, and reproducible method for extracting intact exosomes from cell culture media. It is capable of isolating exosomes from any amount and volume of the original sample in a short time, with high efficiency, without the use of high-speed centrifuges or other advanced equipment, and in a completely sterile manner. Separated exosomal vesicles The product is suitable for various downstream applications such as electron microscopy analysis, NTA analysis, .Western blot, quantitative PCR and high-throughput sequencing, etc

How to store the kit:

The kit should be stored at 5-8°C (refrigerator) and away from light. The buffers are stable .for at least 6 months in unopened containers

Note that all buffers are sterilized. For each use of the buffers, use a sterile pipette or sampler tip (it is better that all equipment used in exosome isolation is RNase free) and .then close the buffer caps tightly.

How to use the kit:

1- Centrifuge the sample (usually cell culture medium) for 10 minutes at 4× 1000 RPM (room temperature) to remove particles and cellular debris.

2-For best results, pass the supernatant from step 1 through a 0.22 µm filter

3- Before use, vortex the buffer 1 and heat it to 37°C to eliminate any crystals that may have formed before use.

4- Mix the sample from step 2 with buffer 1 in a 4:1 ratio (e.g. 4 mL sample + 1mL buffer). For best results, perform this step in a new Falcon (preferably a new Falcon.

5- Vortex the resulting mixture for 5 minutes to ensure homogeneity of the mixture. At this point, a cloudy appearance may be visible depending on the concentration of the original sample.

6- Close the Falcon containing the mixture tightly and Incubate for 12 hours at 4°C.

*** For better results, an automatic shaker can be used during incubation or the flask can be shaken manually (up and down) every hour.**

7- After the incubation time is over, vortex the flask for 1 minutes to ensure homogeneity of the mixture.

8- Then centrifuge for 40 minutes at 4000 × RPM at 4°C.

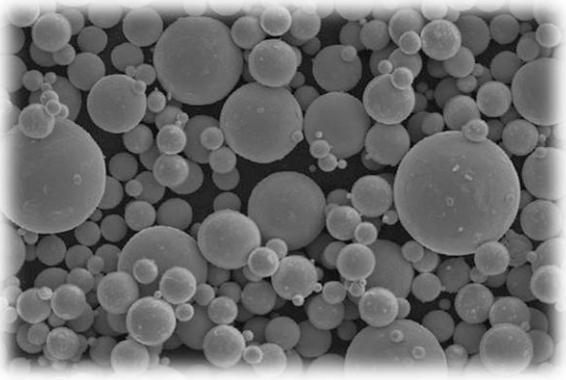
9- Then completely drain and discard the supernatant.

10-Before use, vortex Buffer 2 and make sure that it has not settled. Add an appropriate amount of Buffer 2 to the precipitate formed in Step 9. Slowly mix Buffer 2 with the precipitate, taking care to avoid bubble formation.

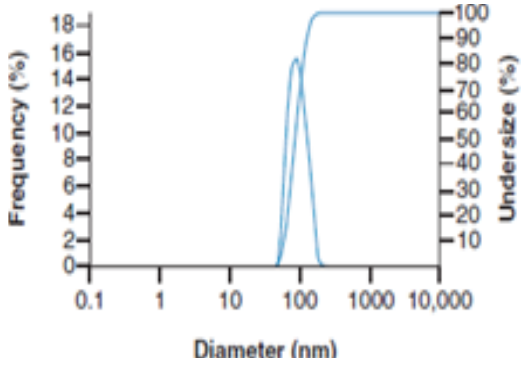
The amount of Buffer 2 used in this step depends on the concentration of the original sample (usually the cell culture medium used in Step 0 for purification). It depends on the exosome used, which can vary from 50 to 100 microliters. Note that the resulting precipitate contains pure exosome and the amount of buffer 5 used should be such that both the precipitate dissolves uniformly and does not lead to excessive dilution of the sample and a decrease in its quality.

11- Purified exosome can be stored at 4°C for several days and at -20 to -80°C for a long time.

To prevent intermittent freezing and thawing of the sample containing exosome and consequently a decrease in the quality of the exosome, it is better to divide the resulting sample into microtubes at this stage and thaw and use one of the microtubes for each use and for performing quantitative and qualitative tests such as concentration measurement, etc.



Exosome sample extracted from animal



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-Expert working group and different orientations.

-Up-to-date equipment for research and knowledge development.

-Environmentally friendly and compliant products.

-Production of laboratory kits with high measurement accuracy and in accordance with global standards.

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