

EXOMED

Exosome Isolation Kit



Exosomes are nano-sized extracellular vesicles (30 to 150 nm) that have a doublelayered phospholipid membrane and a hydrophilic core (vesicle interior). Different cells are constantly able to secrete these nano-vesicles, which contain a variety of cargoes such as DNA, RNA, lipids, proteins, and others. Exosomes play different roles in various pathways such as intercellular communication, signal transduction, antigen presentation, and tumor progression, depending on the type of producing cell and their mission. Therefore, the biological content of exosomes will be different.

The "**EXOMED**" kit is a product that provides a simple, reliable, and reproducible method for the extraction of intact exosomes from cell culture. It is capable of separating exosomes from any amount and volume of the initial sample with a fast procedure, high yield, without the use of high-speed centrifuges or other advanced equipment, and completely sterile. The isolated exosomes would be suitable for various downstream applications such as electron microscopy analysis, NTA analysis, Western blotting, quantitative PCR, and high-throughput sequencing, etc.

Kit contents:

Buffer 1: 25 ml Buffer 2: 5 ml





Transmission Electron Microscopes (TEM)

Storage of the kit:

- Store the kit at 2-8 degrees Celsius (refrigerator) and away from light.
- The buffers are stable for at least 6 months in unopened containers.
- Please note that all buffers are sterile. For each use of the buffers, use sterile pipette or sampler tip (it is better to use RNase free equipment in all steps of the exosomes separation) and then tighten the buffer lids.

Instructions to use the kit:

- 1. Centrifuge the sample (usually cell culture medium) at $4000 \times \text{RPM}$ for 10 minutes at room temperature to remove particles and cellular debris.
- 2. For better results, pass the supernatant from step 1 through a 0.22-micron filter.
- 3. Vortex the buffer 1 before use and heat it to 37 degrees Celsius to dissolve any crystals that may have formed.
- 4. Mix the sample from step 2 with buffer 1 at a ratio of 4 to 1 (for example, 4 ml of sample + 1 ml of buffer 1). For better results, preferably do this step in a new falcon tube.
- 5. Vortex the mixture for 5 minutes to ensure homogeneity. At this stage, a cloudy appearance may be visible, depending on the concentration of the original sample.
- 6. Tightly close the lid of the falcon tube containing the mixture and incubate at 4 degrees Celsius for 12 hours. For better results, you can use an automatic shaker during incubation or shake the falcon tube manually (upside down) every hour.
- 7. When the incubation time has elapsed, vortex the falcon tube for 1 minute to ensure homogeneity.
- 8. Then centrifuge at $4000 \times \text{RPM}$ for 40 minutes at 4 degrees Celsius.
- 9. Completely discard the supernatant.
- 10. Vortex buffer 2 before use and make sure it is not precipitated. Add the appropriate amount of buffer 2 to the precipitate formed in step 9. Slowly mix the buffer 2 with the precipitate, be careful to avoid bubbles. The amount of buffer 2 used in this step depends on the concentration of the original sample (usually cell culture medium that was used to extract exosomes in step 1), which can vary from 50 to 200 microliters. Note that the obtained precipitate contains pure exosomes and the amount of employed buffer 2 should be sufficient to dissolve the precipitate evenly without diluting the sample too much and reducing its quality.
- 11. The extracted exosomes can be stored at 4 degrees Celsius for several days and at -20 to -80 degrees Celsius for a long time. To prevent repeated freezing and thawing of the exosome-containing sample, which can reduce the quality of the exosomes, it is better to divide the resulting sample into appropriate number of microtubes at this stage and thaw one of the microtubes for each use to perform quantitative and qualitative tests such as concentration measurement, etc