

# Exosome Protein Extraction Kit

Cat. No.: EZB-exo-PRO1

## Description

Exosome Protein Extraction Kit uses a unique lysate formulation that can fully lyse exosomes in a short time and can fully inhibit the function of degradation of proteases. This kit can extract exosome proteins to the maximum extent within 10 minutes, which is an excellent tool for exosome research.

## Components

Components	Cat.No. (Size)	EZB-exo-PRO1 (100 Preps)
Exosome Protein Extraction Buffer		35 ml

## Storage

This kit can be stored at 4°C for 2 years. See the expiration date on the product label for details.

## Notice

**Avoid Protease contamination.** Laboratory clothing, disposable gloves, and disposable masks should be worn during the experiment. Centrifuge tubes, tips, and other supplies used in the experiment must be Protease free.

## Protocol

**1. Before use, take an equal amount of sample (such as plasma, serum or cell culture supernatant or other body fluids) to extract exosomes.**

【If the exosome samples need to extract RNA in addition to protein extraction, add 40  $\mu$ l PBS to the extracted exosomes, mix by pipetting up and down, then take 20  $\mu$ l for protein extraction, and the remaining samples for RNA extraction (EZB-exo-RN1 kit can be used for extraction). 】

The extracted exosomes, if all are used to extract proteins, the corresponding volume of Exosome Protein Extraction Buffer is directly added according to the following table; if a part is used for protein extraction, the corresponding volume of Exosome Protein Extraction Buffer is added in proportion:

Sample types	Serum/plasma	Seminal fluid	Follicular fluid	Cell culture supernatant	Urine	Cerebrospinal fluid	Alveolar lavage fluid
Sample volume (ml)	1	1	1	10	10	5	10
Volume of Exosome Protein Extraction Buffer added ( $\mu$ l)	100 ~ 200			60 ~ 120			

**2. According to the described above, add Exosome Protein Extraction Buffer to the exosome precipitations, and then vortex vigorously for 30 seconds using a vortex oscillator to mix thoroughly.**

**3. Add 0.2 times the volume of 6 $\times$  SDS Protein Loading Buffer (or 0.25 times the volume of 5 $\times$  SDS Protein Loading Buffer) to the previous solution, and mix thoroughly.**

**4. Heat in a metal bath at 95°C for 5 ~ 10 minutes.**

**5. Vortex again for 10 seconds.**

**6. Extracted protein can be directly used as samples to run electrophoresis in Western Blotting, or directly frozen at -20°C.**

## Trouble Shooting

### 1. How to get as much exosome protein as possible for Western Blotting experiments?

- a. Maximize the amount of initial samples used for exosome extraction;
- b. Use ultracentrifugation, or a reliable exosome extraction kit (such as EZB-exo1, EZB-exo2, etc.) to ensure the extraction efficiency of exosomes.

### 2. If the content of exosomes is very little, how to adjust the amount of Exosome Protein Extraction Buffer to optimize the experiment?

For samples with little exosome content, as little as 60  $\mu$ l of Exosome Protein Extraction Buffer can be added, shaken well, and mixed thoroughly, and doing the subsequent steps.

### 3. What should I do if the experiment needs to quantify the protein concentration?

If the extracted sample needs to be used for protein quantification, it needs to be detected by the BCA protein assay kit. Then adjust the volume with the Exosome Protein Extraction Buffer to make the samples to the same concentration. Then mix it with 5 $\times$  or 6 $\times$  SDS Protein Loading Buffer, vortex vigorously, and then heated and denatured (for sample extracted from this kit, it is recommended to control the initial sample volume to ensure the same amount of sample).