

# **EXOBX® Serum Exosome Purification Kit Plus (Magnetic Beads-Based)**

---

**Cat. no. R6304E**

**Address:** SHT technology GmbH, Niederhöchstädter Str. 64, 61476 Kronberg im  
Taunus, Germany

**Tel.:** +49 176 34989414

**Website:** <https://sht-medino.de/>

# EXOBX® Serum Exosome Purification Kit Plus (Magnetic Beads-Based)

**Cat. no. R6304E**

| Catalog Number | Reagent In Kit | Size   | Storage |
|----------------|----------------|--------|---------|
| <b>R6304E</b>  | Buffer EXA     | 3 mLx5 | - 20 °C |
|                | Buffer EXC     | 10 ml  | 4 °C    |
|                | Buffer EXE     | 5 ml   | 4 °C    |
|                | Magnetic Beads | 2.5 ml | 4 °C    |
|                | Needle filter  | 10     | RT      |
|                | Manual         | 1      |         |

## 1. Transportation and Storage

This product is transported at room temperature, Each component shall be stored separately under specified conditions, with a shelf life of two years..

## 2. Important Information (Please read before using the kit)

- 1) The magnetic beads should be stored at 4 °C and are forbidden to freeze, or they become invalid.**
- 2) PBS and ddH<sub>2</sub>O used in the experiment must be filtered by a filter with a pore size of 0.2 µm.**
- 3) This kit is suitable for the extraction of exosomes from serum, plasma, and samples with high viscosity or abundant exosome content
- 4) Magnetic beads will sink to the bottom of the tube after standing for a long time, so vortex the magnetic beads before sucking them.
- 5) Freshly isolated exosomes are suitable for NTA detection.
- 6) The purified exosomes can be stored at -80 °C, and should avoid repeat freezing and thawing.
- 7) The exosome purification system provided in this kit is capable of purifying 20 ml of serum, plasma, or other highly viscous samples, meeting various experimental requirements such as exosomal protein extraction, RNA extraction, and miRNA extraction.
- 8) Please wear lab clothes, gloves and safety glasses during the experiment
- 9) For safety, wear a lab coat, latex gloves, and protective eyewear when handling lentivirus. Use a biosafety cabinet to prevent accidental viral exposure.**

## 3. Product Introduction

This product is an exosome purification kit that utilizes magnetic beads as the separation material. In a specific buffer system, the magnetic beads can adsorb exosomes, which are then eluted with an elution buffer, thereby achieving the

separation and purification of exosomes. The eluted exosomes are free of other compounds and can be directly used for cell culture experiments and in vivo animal injections..

#### 4. Features and Advantages

- 1). This reagent provides high purification efficiency and high exosome recovery rate.
- 2). The exosomes purified using this kit exhibit high purity with low levels of exogenous contaminant proteins.
- 3). The kit is easy to use and allows for rapid exosome purification within 2 hours.
- 4). The purification process is mild, with a short processing time, ensuring good integrity of the purified exosomes.
- 5). The purified exosomes are free of other compounds and can be directly used for cell culture experiments and in vivo animal injection studies.

#### 5. Experimental Protocol

- 1) Sample Preparation: Add 1 ml of serum or other sample into a centrifuge tube. Add an equal volume of EXC, invert and mix 10 times, and centrifuge at 10,000 × g, 4 °C for 20 minutes.
- 2) Magnetic Bead Preparation: Take out the bottled magnetic beads from the kit and mix thoroughly by vortexing for 30 seconds. Use a pipette to transfer 0.25 ml of magnetic beads into a 15 ml centrifuge tube, centrifuge at 3,000 × g, 4 °C for 2 minutes, and discard the supernatant.
- 3) Magnetic Bead Washing: Add 10 ml of pre-chilled PBS (4 °C), mix thoroughly by vortexing for 30 seconds, centrifuge at 3,000 × g, 4 °C for 5 minutes, and discard the supernatant.
- 4) Exosome Purification System: The exosome purification system has a total volume of 3.75 ml, which is suitable for purifying approximately 1 ml of serum sample. The components are listed in the table below.

**Table 1. Exosome purification system**

| Reagents                     | Volume(ml) | Ratio  |
|------------------------------|------------|--------|
| Buffer EXA                   | 1.5        | 40%    |
| Magnetic Bead                | 0.25       | 6.67%  |
| Sample (EXC-treated mixture) | 2          | 53.33% |
| Total                        | 3.75       | 100%   |

- 5) Exosome Purification System Preparation: Prepare the exosome purification system according to the exosome purification system table. Reagent volumes may be proportionally reduced to construct a mini-scale exosome **purification system; however, do not arbitrarily alter the ratios of the reagents, as this may result in purification failure.**
- 6) Incubation: After preparing the exosome purification system, place it on a rotator and incubate at 4 °C with gentle rotation for 20 minutes.

- 7) **Magnetic Bead Collection:** After incubation, place the centrifuge tube in a centrifuge and centrifuge at  $3,000 \times g$ ,  $4\text{ }^{\circ}\text{C}$  for 5 minutes to pellet the magnetic beads, and discard the supernatant; alternatively, place the centrifuge tube on a magnetic stand at  $4\text{ }^{\circ}\text{C}$  for 5–10 minutes to allow magnetic bead aggregation, and discard the supernatant.
- 8) **Residual Liquid Removal:** Transfer the centrifuge tube containing the magnetic bead pellet into a centrifuge and centrifuge at  $3,000 \times g$ ,  $4\text{ }^{\circ}\text{C}$  for 1 minute. Use a pipette to remove any residual liquid from the tube.
- 9) **Exosome Elution:** Add 0.5 ml of Buffer EXE (note that exosome concentration decreases with increasing elution volume) and vortex vigorously for 30 seconds.
- 10) **Exosome Collection:** Centrifuge at  $7,000 \times g$ ,  $4\text{ }^{\circ}\text{C}$  for 2 minutes. Place the centrifuge tube on a magnetic stand **(this effectively aggregates the magnetic beads and prevents bead dispersion in the solution, which could reduce exosome purity)**, and transfer the supernatant to a new centrifuge tube. This supernatant is the purified exosome solution.
- 11) **Filtration:** Rinse a syringe filter with PBS, then filter the exosome solution using the syringe filter to obtain a high-purity exosome solution.
- 12) **Storage:** The purified exosome solution can be used immediately for particle size analysis (NTA) or stored at  $-80\text{ }^{\circ}\text{C}$ .

## 6. Frequently Asked Questions and Analysis

| Questions                                                         | Reasons                                                                                                                  | Solutions                                                                                                                  |
|-------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|
| The concentration of isolated exosomes is low.                    | Limited sample volume                                                                                                    | Increase the sample volume in the purification system or scale up the purification system using fresh samples.             |
|                                                                   | Repeated freeze-thaw cycles of the sample leading to exosome degradation                                                 | Use reagents within their shelf life.                                                                                      |
|                                                                   | Expired kit                                                                                                              | Replace with new magnetic beads or a new kit.                                                                              |
|                                                                   | Frozen storage of magnetic beads resulting in reduced or lost exosome-binding capacity                                   | Use the correct volume of magnetic beads according to the requirements of the purification system.                         |
|                                                                   | Insufficient volume of magnetic beads added                                                                              | Add the components from the kit to the purification system in the specified proportions as required.                       |
|                                                                   | Arbitrary alteration of reagent ratios in the purification system contrary to specified requirements                     | Strictly follow the instructions for procedures such as incubation and elution strength.                                   |
|                                                                   | Failure to follow the instructions                                                                                       | Extend the incubation time.                                                                                                |
|                                                                   | Insufficient incubation time between magnetic beads and sample; inadequate elution efficiency                            | Increase the intensity and duration of vortexing.                                                                          |
|                                                                   | Excessive elution volume                                                                                                 | Reduce the volume of elution buffer.                                                                                       |
| The concentration of exosome protein is low.                      | Low concentration of purified exosomes                                                                                   | Refer to the above solutions.                                                                                              |
|                                                                   | Weak lysis efficiency of the exosomal protein extraction reagent                                                         | Use a strong lysis protein extraction reagent or our exosome protein extraction kit (R6306).                               |
|                                                                   | The exosomes purified using this kit exhibit high purity with minimal contaminant proteins                               | Increase exosome concentration.                                                                                            |
| Increased contaminant proteins in the extracted exosomal protein. | Overextended incubation time between sample and magnetic beads leading to nonspecific adsorption of contaminant proteins | Shorten incubation time between sample and magnetic beads.                                                                 |
| Low concentration of extracted exosomal miRNA.                    | Low exosome concentration                                                                                                | Refer to the above solutions.                                                                                              |
|                                                                   | Insufficient exosome input                                                                                               | Increase exosome input or scale up the RNA extraction system.                                                              |
|                                                                   | The RNA extraction kit is not suitable for trace RNA extraction                                                          | As exosomal RNA is mainly low-abundance miRNA, use a micro RNA extraction kit or our exosome miRNA extraction kit (R6310). |