

Exosome Isolation and Purification Kit (from plasma and serum)

1 Contents

| Components | HY-K1063-30T |
|-----------------------------------|--------------|
| Blood PureExo Solution (BPS) | 30 mL |
| Exosome Purification Filter (EPF) | 30 Tubes |

2 Introduction

Exosomes are small vesicles (30-150 nm) containing RNA and protein that are secreted by various types of cells in culture, and found in abundance in body fluids including blood, saliva, urine, and breast milk. Exosomes are thought to function as intercellular messengers, delivering their cargo of effector or signaling macromolecules between specific cells.

MCE Exosome Isolation and Purification Kit provides a simple and effective method to isolate and purify intact exosomes from plasma and serum that can be used for electron microscope analysis, NTA analysis, WB, qPCR, etc. This product contains sufficient reagents for processing 30 mL of plasma or serum.

3 General Protocol

Sample preparation

1. For newly prepared sample, place on ice for later use. For frozen samples, defrost in 25°C water bath and then place on ice.

Note: Dilute or concentrate appropriately when the concentration of the sample is too high or too low.

2. Centrifuge 1 mL of plasma or serum at 3,000 g at 4°C for 10 minutes to remove cells and debris. Transfer the supernatant to a new tube without disturbing the pellet.

3. Centrifuge at 10,000 g at 4°C for 10 minutes and collect the supernatant.

Note: If additional debris remains detectable, centrifuge for additional 10 minutes and collect the supernatant.

| Plasma/Serum | PBS volume | BPS volume |
|--------------|------------|------------|
| 1 mL | 3 mL | 1 mL |

Isolation of exosomes

1. Add 3 mL of pre-cooled 1× PBS and 1 mL of Blood PureExo Solution (BPS) per 1 mL of the supernatant.

Note: a) Mix the Blood PureExo Solution thoroughly before use.

b) In order to improve the purity of exosomes, the dosage of PBS and BPS can be appropriately increased 2-3 times, eg: $V_{\text{serum/plasma}}: V_{\text{PBS}}: V_{\text{BPS}} = 1:6:2$ or $1:9:3$.

2. Mix by vortexing for 1 minute, and then incubate at 4°C for more than 2 hours.

Note: The yield of exosomes could be increased with longer incubate time. However, it is recommended not to exceed 24 hours.

3. Centrifuge at 10,000 g at 4°C for 60 minutes, discard the supernatant. Exosomes are contained in the pellet at the bottom of the tube (not visible in most cases).

4. Add 0.5 mL of 1× PBS per 1 mL of plasma or serum to resuspend the pellet. Centrifuge at 12,000 g at 4°C for 2 minutes, and the supernatant is crude exosomes.

Note: If additional debris remains detectable, centrifuge for additional 2 minutes and collect the supernatant.

Purification and storage of exosomes

1. Add the crude exosomes to the Exosome Purification Filter, centrifuge at 3,000 g at 4°C for 10 minutes and collect the purified exosomes.

2. Aliquot the purified exosomes into multiple tubes and store at -80°C for downstream analysis. Avoid repetitive freeze-thaw cycles.

4 Storage

Store at room temperature for two years

5 Precautions

1. The protein content in serum and plasma is relatively high. In order to improve the purity of exosomes, the dosage of PBS and BPS can be appropriately increased 2-3 times, eg: $V_{\text{serum/plasma}}: V_{\text{PBS}}: V_{\text{BPS}} = 1:6:2$ or $1:9:3$.
2. To ensure that isolated exosomes originated from your cells of interest, it is recommended to culture the cells with exosome depleted fetal bovine serum (FBS).
3. This product is free of RNase and DNase. Please avoid the contamination of RNase and DNase during use.
4. This product is for R&D use only, not for drug, household, or other uses.
5. For your safety and health, please wear a lab coat and disposable gloves to operate.