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ExoCap™ Ultracentrifugation/Storage Booster

Code No.	Quantity
MEX-USB	50 mL

Product description

Exosomes are extracellular vesicles secreted by most cell types and contain various marker proteins and RNAs, such as microRNA and fragmented mRNA. **ExoCap™ Ultracentrifugation/Storage Booster** (**ExoCap™ USB**) is an additive for exosome-conditioned media specifically designed to 1) improve the recovery rate of ultracentrifugation and 2) stabilize and extend 4°C exosome storage. The advantages of this reagent are as bellow:

- Improving exosome recovery rate from cell culture supernatant by ultracentrifugation
- Improving stability at 4°C storage for exosomes purified from cell culture supernatant
- Acceptable at -80°C storage for exosome
- No complicated troublesome work

Product contents

Component	Amount	Storage
ExoCap™ Ultracentrifugation/Storage Booster	50 mL	2-8°C

Note: This reagent contains no preservative; handle under aseptic conditions.

Storage and Stability

Stable for 2.5 years from the date of receipt when stored at 2-8°C. Do not freeze.

Note: **If precipitation occurs during storage, equilibrate the reagent to room temperature and mix well to re-dissolve the precipitation.**

Materials required but Not Provided

1. Refrigerator (2°C to 8°C)
2. Ultra low temperature freezer (-80°C)
3. Ultracentrifuge capable of 100,000 × *g*
   (*e.g.*, Optima XE-90, Rotor: SW32Ti and SW40Ti (Beckman Coulter))
4. Ultracentrifuge tubes (*e.g.*, Beckman Coulter; code no. 344058 and 344060)
5. Centrifuge tubes [Recommendation; use low-adhesion tube]
6. Pipette tips [Recommendation; use low-adhesion pipette tip]
7. Pipettes (Micropipettes)
8. 0.22 μm filter
9. MilliQ water

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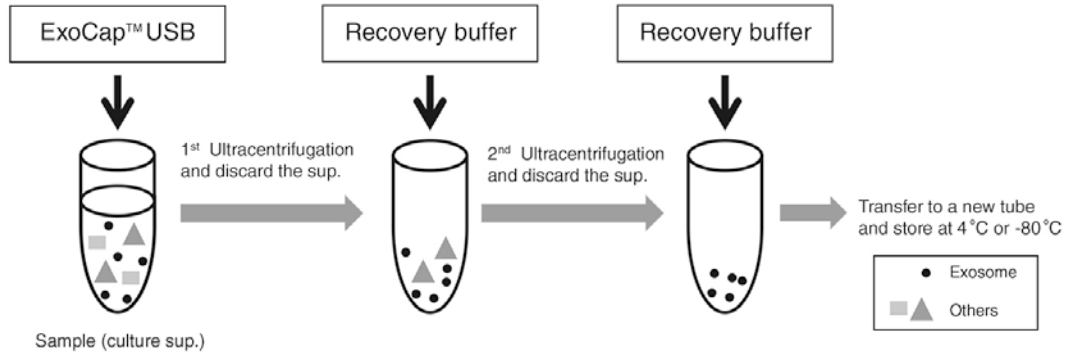
ExoCap™ Ultracentrifugation/Storage Booster Procedure

A. Ultracentrifugation for exosome isolation

1. Procedure summary



2. Buffer preparation

Recovery buffer (for the 2nd ultracentrifugation and the final re-dispersion of exosomes)
Mix 1 part of the **ExoCap™ USB** with 9 parts of MilliQ water before use.
Adjust the Recovery buffer preparation according to the size of ultracentrifugation instruments and apparatuses.

3. Protocols

◆ Pre-clearance

1. Collect the serum-free exosome-conditioned cultured medium from the cell culture dish into the centrifugation tube.
2. Centrifuge the tube at 300 × *g* at 4°C for 10 minutes.
3. Transfer the supernatant to a new centrifugation tube and discard cell pellet.
4. Centrifuge the tube at 2,000 × *g* at 4°C for 10 minutes.
5. Transfer the supernatant to a new tube and discard dead cell pellet.
6. Filter the final supernatant with 0.22 μm filter.

◆ Ultracentrifugation and storage

The required volume of ExoCap™ USB is described in the table below.

Sample	ExoCap™ USB	Recovery Buffer		Total use of ExoCap™ USB /sample
	1st UC	2nd UC†	Storage†	
10 mL	1.1 mL	10 mL (1.1 mL)	0.1 mL (0.01 mL)	2.21 mL
30 mL	3.3 mL	30 mL (3.3 mL)	0.3 mL (0.03 mL)	6.63 mL
50 mL	5.5 mL	50 mL (5.5 mL)	0.5 mL (0.55 mL)	11.65 mL

†Numbers in parentheses shows the amount of ExoCap™ USB.

Note: The usage depends on the ultracentrifugation scale to use. When smaller size tube is used for the 2nd ultracentrifugation (2nd UC), the total amount of ExoCap™ USB can be decreased.

1. Add 1 part of the **ExoCap™ USB** to 9 parts of the supernatant (prepared by pre-clearance step) and mix well.
   (Example) Supernatant 30 mL + ExoCap™ USB 3.3 mL

2. Transfer the sample to the ultracentrifugation tube (*e.g*. Beckman Coulter; code no. 344058).
3. 1st Ultracentrifugation.
   (Example) Apparatus: Optima XE-90, Rotor: SW32Ti (Beckman Coulter), 100,000 × *g* at 4°C for 70 minutes.
   Note: During the 1st ultracentrifugation, prepare the **Recovery buffer.**
   (Example) ExoCap™ USB 1.2 mL + MilliQ water 10.8 mL
4. Discard the supernatant.
5. Suspend the pellet by Recovery buffer and transfer it to the new ultracentrifugation tube (*e.g*. Beckman Coulter; code no. 344060).
   (Example) The pellet is suspended by 11 mL of **Recovery buffer**. Transfer it to the new ultracentrifugation tube.
6. 2nd ultracentrifugation.
   (Example) Apparatus: Optima XE-90, Rotor: SW40Ti (Beckman Coulter), 100,000 × *g* at 4°C for 70 minutes.
7. Discard the supernatant.
8. Suspend the pellet with **Recovery buffer**.
   (Example) The pellet is resuspended with 0.3 mL **Recovery buffer** (100 times concentrated).
9. Storage at 4°C or -80°C in portions.

B. Storage of isolated exosomes

Protocols

1. Purify exosome from cell culture supernatant by proper method such as ultracentrifugation, size exclusion chromatography, or Beads.
2. Disperse the exosomes in proper buffer (*e.g*. PBS).
3. Add 1 part of the ExoCap™ USB to 9 parts of the sample and mix well.
4. Storage at 4°C or -80°C in portions.

Note: Even if ExoCap™ USB is used for storage, repeated freeze-thaw or storage at 4°C will occasionally cause a deterioration of the collected exosomes. Therefore, for long-term storage, preparation of small aliquots for -80°C storage is recommended. The exosome solution with the additive at 4°C should be used within a few days.

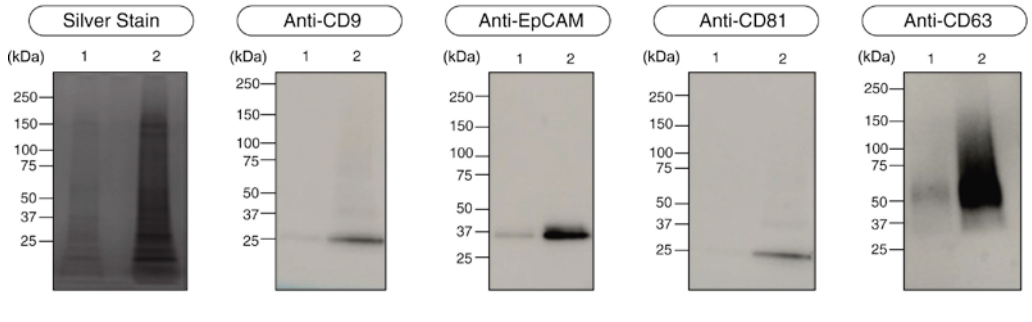
Attention

- The efficiency of ExoCap™ USB depends on the sample. Preliminary tests are strongly recommended.
- According to the purpose of use, prepared exosome solution should be sterilized before use.

Examples of ExoCap™ USB

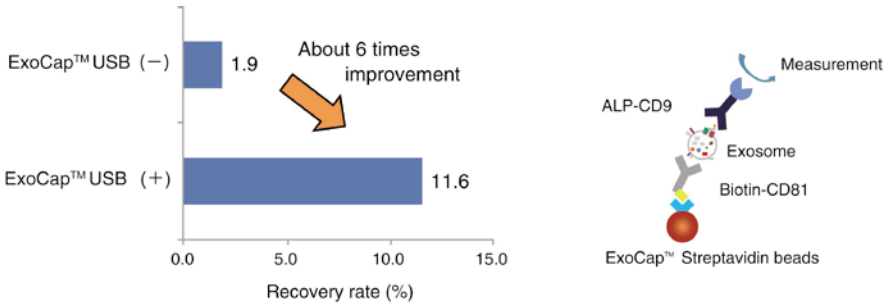
Exosome Purification by Ultracentrifugation

1) Evaluation of the ExoCap™ USB effect for ultracentrifugation by Western blotting (Non-reduced)



HT29 cells were cultured up to 60-80%. Subsequently, wash the cells and change the new medium without FBS. 3 days later, recover the supernatant. The cell density was reached from 90% to confluent. After the pre-clearance by centrifugation at 300-3,000 × *g*, this supernatant was filtrated by 0.22 μm filter and then used for the experiment. Exosome was isolated from the supernatant by ultracentrifugation with/without ExoCap™ USB. The intensity of the bands of exosome markers with the ExoCap™ USB was denser than those of without ExoCap™ USB. Exosome recovery was boosted by using the ExoCap™ USB.

2) Evaluation of the ExoCap™ USB effect for ultracentrifugation by CLEIA



Exosome recovery rate was improved as a result of the ExoCap USB addition. For beads CLEIA (Chemiluminescent Enzyme Immuno Assay) ExoCap™ Streptavidin Kits, biotinylated CD81 antibody and alkaline phosphatase (ALP) labeled CD9 antibody were used.

Manufacturer

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