

# For Lyophilization of Exosomes

## What is exosomes ?

- Exosomes are membrane vesicles of about 30 nm to 200 nm in diameter secreted by most cells and are observed *in vivo* in body fluids such as saliva, blood, urine, and milk, and are also secreted by cultured cells.
- Utilizing the properties of exosomes, research is being conducted on their use as drug delivery systems (DDS), and exosomes derived from bovine milk are attracting attention as a means of orally administering anti-cancer drugs.

## Characterization of bovine milk exosomes derived by ultracentrifugation

### Materials & Methods (1)

#### Non-fat bovine milk

- ↓ Addition of acetic acid (final conc. 1%) and mix well  
Centrifugation at  $1500 \times g$  for 30 min at  $4^{\circ}C$   
Filtration using  $0.22 \mu m$  filter

#### Whey

- ↓ Ultracentrifugation at  $150,000 \times g$  for 70 min at  $4^{\circ}C$   
Pellet (exosomes) was suspended in PBS  
Ultracentrifugation at  $150,000 \times g$  for 70 min at  $4^{\circ}C$   
Pellet was resuspended in PBS  
Centrifugation at  $10,000 \times g$  for 5 min at  $4^{\circ}C$

#### Exosome (Western blotting)

- ↓ Protein concentration was measured by BCA method  
Seventy  $\mu g$  of whey or 5  $\mu g$  of bovine milk exosomes were applied on SDS-PAGE  
Blotted onto PVDF membrane  
Staining with anti-CD9, CD63, or CD81 antibody, respectively  
(1:1,000, SBI System Biosciences)  
Washing the membrane 3 times for 5 min  
Staining with HRP-conjugated goat anti-rabbit antibody  
(1:10,000, SBI System Biosciences)  
Washing the membrane 3 times for 5 min  
Detection using ECL Select™ Western Blotting Detection System (GE Healthcare).

### Results (1)

- ▶ The milk exosomes derived from whey by ultracentrifugation were positive for CD9, CD63 and CD81.
- ▶ As far as tested by Western blotting, respective positive band detected by anti-exosome related antibody was not found in the whey.

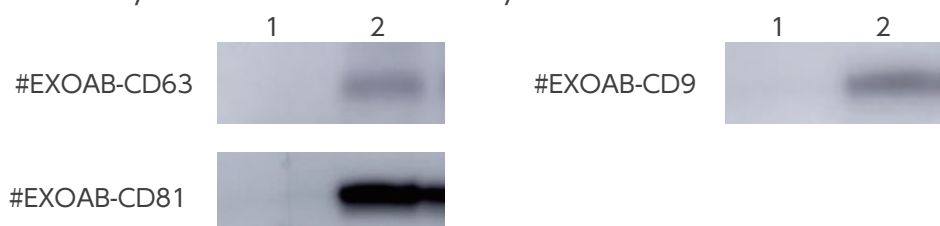


Fig.1. Characterization of bovine milk exosomes by Western blotting.  
No.1; whey (70  $\mu g$ ), No. 2; bovine milk exosomes (5  $\mu g$ )

**Inhibitory effect of TREHALOSE SG on exosome aggregation by lyophilization**

**Materials & Methods (2)**

1. Bovine milk exosomes were prepared as described in Materials & Methods (1). The exosomes (70 μg) were osmotically adjusted with PBS and lyophilized overnight with or without trehalose. The exosomes were then dissolved in distilled water.
2. The exosome solution was analyzed by Dynamic Light Scattering (DLS) using a Zetasizer Nano ZS.

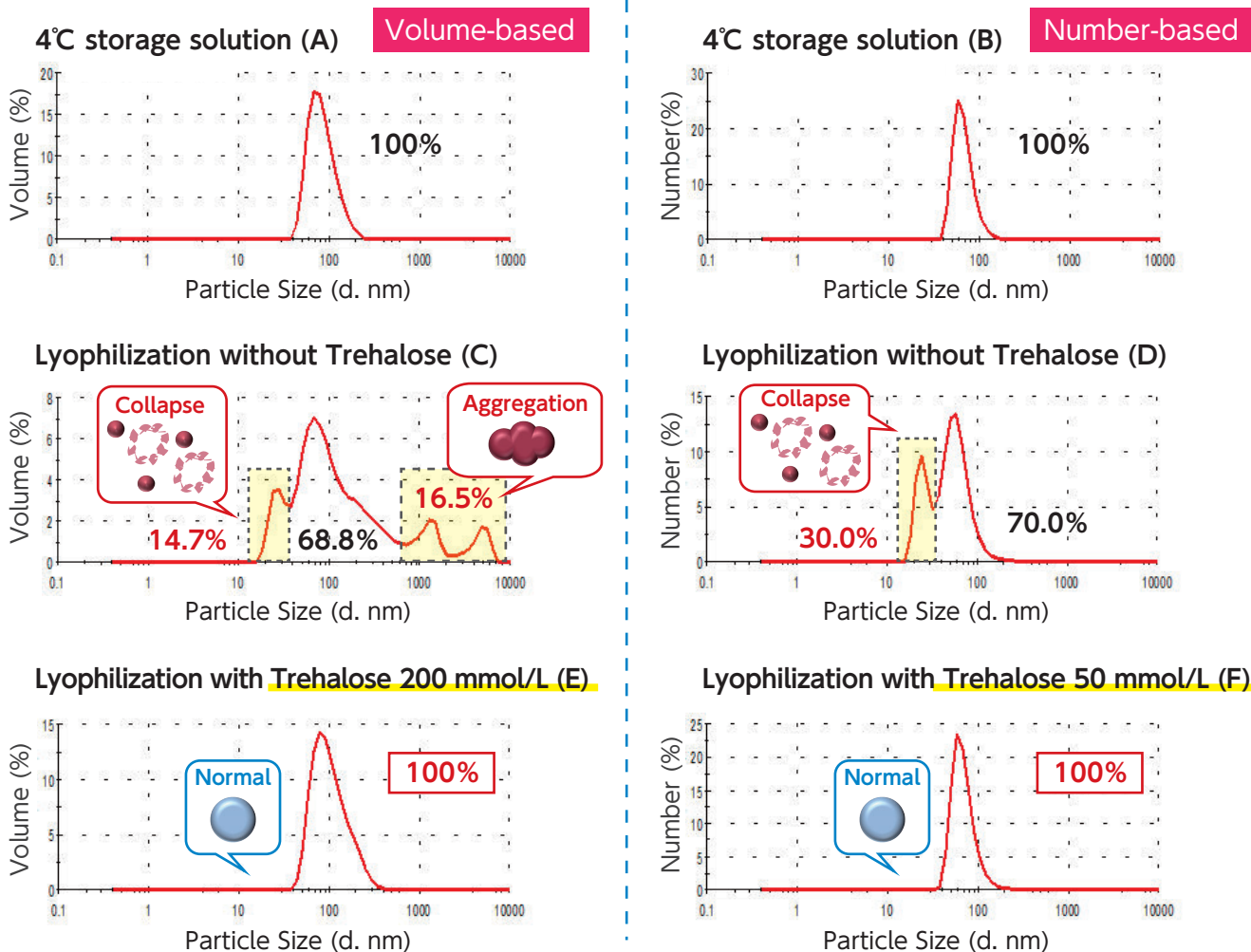


Fig. 2. DLS analysis of exosomes after lyophilization with or without TREHALOSE SG (Trehalose)

**Results (2)**

- ▶ Aggregation or collapse of exosomes was largely caused by lyophilization (Fig. 2C, D) compared to the pattern in the 4°C storage solution (Fig. 2A, B).
- ▶ Before lyophilization, addition of TREHALOSE SG inhibited the aggregation and collapse of exosomes (Fig. 2E, F).

**Reference**

- Trehalose significantly enhances the recovery of serum and serum exosomal miRNA from a paper-based matrix. Neo SH, Chung KY, Quek JM & Too H-P, *Sci. Rep.* 7(1):16686 (2017).
- Milk-derived exosomes for oral delivery of paclitaxel, Agrawal AK, *et al.*, *Nanomedicine.*13(5):1627 (2017).

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