SOLBIOTE™

TREHALOSE SG
MALTOSE PH

Bringing Sustainable Solutions to Biopharmaceuticals

Our high-purity and low-endotoxin saccharide products provide you solutions for biologics, vaccines, and cell-based therapeutics development.

Discover our philosophy:
Pharmaceutical Ingredients x Sustainability

PLATINUM Top 1%
ecovadis
Sustainability Rating
MAR 2024

Achieving equitable well-being for all; no one shall be left behind

We are dedicated to the sustainable production of life-changing excipients, SOLBIOTE™, to create a prosperous future for both people and the planet. We aspire to go beyond simply providing pharmaceutical solutions to deliver universal health; we ensure equitable access to quality healthcare for all.

Product inquiries to:

Nagase & Co., Ltd.

Life & Healthcare Products Department
dnfct@ex.nagase.co.jp

Nagase Viita

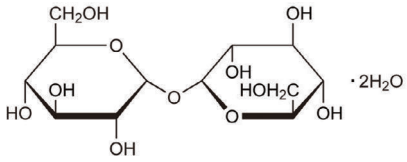
Injectable Grade of Trehalose

General

Trehalose is a dihydrous crystalline and non-reducing disaccharide consisting of two glucose molecules linked by an α,α-1,1 bond.

TREHALOSE SG is monographed as being low endotoxin and is intended mainly for injection. Because of its stability it can be autoclave or filter sterilized.

Chemical formula: $C_{12}H_{22}O_{11} \cdot 2H_2O$
Molecular weight: 378.33
CAS RN® : 6138-23-4

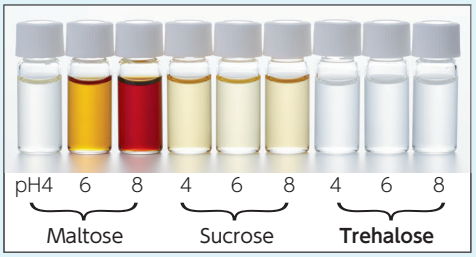


Properties

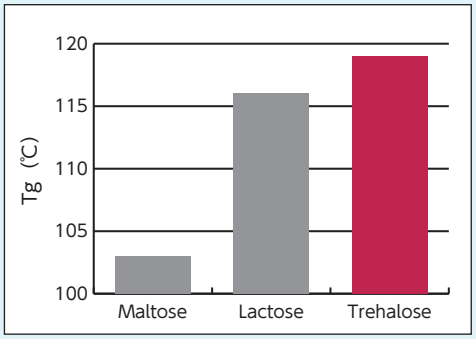
- Protects the quality of products during processing due to its non-reactivity. TREHALOSE SG does not participate in the Maillard reaction, preventing the development of undesired colors, odors and flavors.
- Heat and acid resistant (pH 2 and 100˚C for 24 hours)
- Stable amorphous phase under high temperature due to its high glass-transition temperature (Tg:approximately 120℃). TREHALOSE SG can be used as a stabilizer for biomaterials due to its protective effect against environmental temperature variations.

Reaction of glycine with saccharide solutions

Saccharides (12.5%) with glycine (0.5%) at 120℃ for 1 hour



Glass-transition temperature of disaccharides

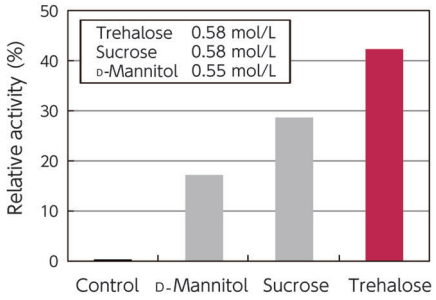


Stabilization of biomaterials

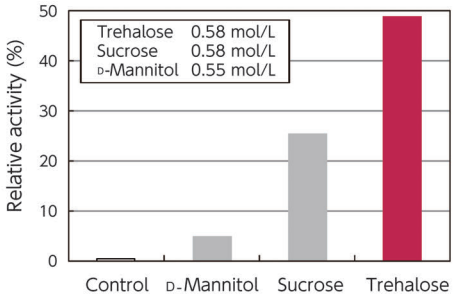
- Proteins can be denaturized by stressors such as heat, shear, and phase change during processing or storage. TREHALOSE SG can replace the water molecules that is closely associated with proteins, stabilizing the higher-order structure to prevent denaturation, especially during heating, freezing or lyophilization.
- TREHALOSE SG modifies ice crystal development to reduce damage to cells and proteins during freezing.

Liquid phase

mAb-IFN-γ (5 mg/mL, 60℃ • 28-hour storage)

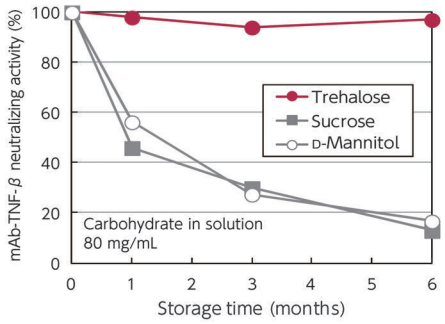


TNF-α (1 mg/mL, 60℃ • 18-hour storage)

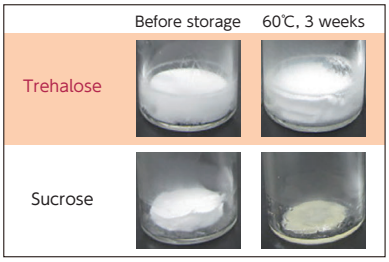


Lyophilization phase

mAb-TNF-β (0.1 mg/mL, 40℃)



Physical change of lyophilized samples of mAb-TNF-β before and 3 weeks after storage



Packaging

20 kg (PE bag in plastic container or carton box)
1 kg (PE bag in carton box)

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PTSG1·3·2024·04

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For Culture Media Use

What is TREHALOSE SG ?

- TREHALOSE SG is a dihydrous, crystalline and non-reducing disaccharide consisting of two glucose molecules linked by an α,α-1,1 bond.
- TREHALOSE SG is white crystalline powder.
- TREHALOSE SG is very soluble in water and very heat stable.
- TREHALOSE SG is an injectable grade of pharmaceuticals and is monographed as being low endotoxin.

Dihydrous crystalline trehalose

Evaluation of the suppression of protein aggregation in CHO cell culture ①

Materials & Methods (1)

1. Protein solution of a bispecific antibody (bispecific single-chained diabody with Fc, scDb-Fc, 0.8 mg/mL) was prepared with or without addition of trehalose (200 mM) in culture medium.
2. Circular dichroism (CD) spectroscopy of the scDb-Fc protein contained in the supernatant was measured.

Materials & Methods (2)

1. The scDb-Fc (0.8 mg/mL) solution prepared in the same manner as described in M & M (1) was heat-treated at 60℃ for 5 minutes.
2. The precipitate was removed by centrifugation.
3. The soluble fraction of scDb-Fc protein in the supernatant was measured.

Fig.1 CD spectrum analysis

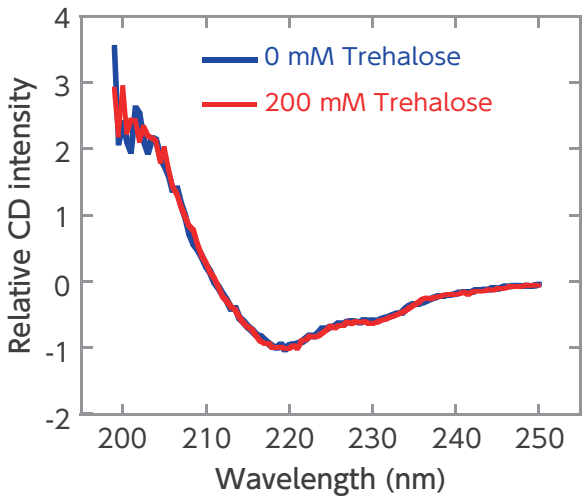
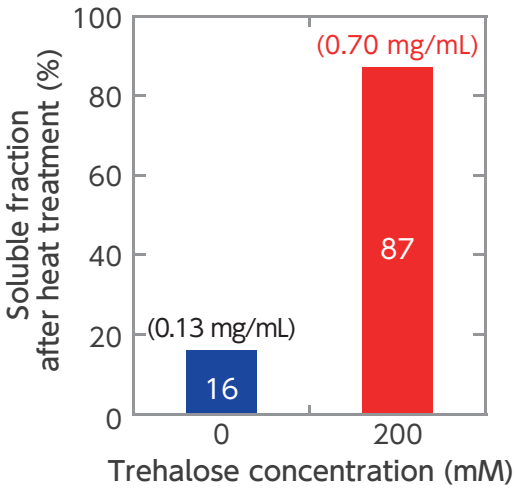


Fig.2 Effect on heat denaturation



Results

▲ Addition of trehalose (200 mM) had no effects on the structure of scDb-Fc protein (Fig. 1).
▲ Protein aggregation caused by heat denaturation was greatly reduced by trehalose (Fig. 2).

Evaluation of the suppression of protein aggregation in CHO cell culture ②

Materials & Methods

1. The CHO Top-H cell line producing a bispecific antibody (single-chained diabody with Fc: scDb-Fc) was grown in cell culture media containing 150 mM trehalose. The cells were then cultured in an animal cell culture bioreactor (1 L scale, medium capacity 0.7 L) with or without addition of 150 mM trehalose.
2. After purifying the scDb-Fc protein from the culture supernatant by protein A affinity chromatography, the secondary structures (monomer, dimer and large aggregates), and their cohesiveness were evaluated by circular dichroism (CD)/fluorescence spectroscopy and gel filtration column chromatography, respectively.

Results

- A decrease in the ratio of large aggregates was observed by adding trehalose to the culture medium as compared with the culture medium without trehalose. On the other hand, the ratio of the monomeric scDb-Fc protein, which is an indication of no aggregation, increased while no effect on the dimer was observed (Fig. 3).
- The secondary structure of the dimer was similar to that of the monomer with antibody-like β-strand structure (Fig. 4).
- The large aggregates had both a non-native β-strand structure and a misfolded structure in which a hydrophobic region is largely exposed (Fig. 5).

Trehalose concentration	Large Aggregates	Dimer	Monomer
0 mM	17.5%	10.3%	72.2%
150 mM	5.9% ↓	8.7%	85.4% ↑

Fig. 3 Gel filtration column chromatogram

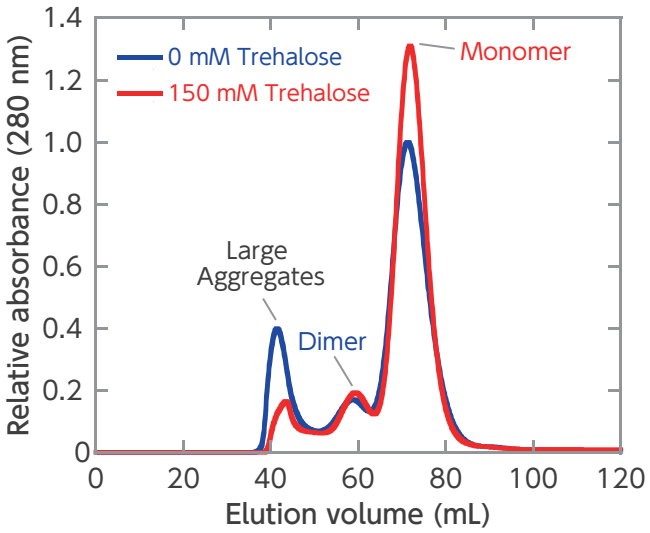


Fig. 4 CD spectrum analysis

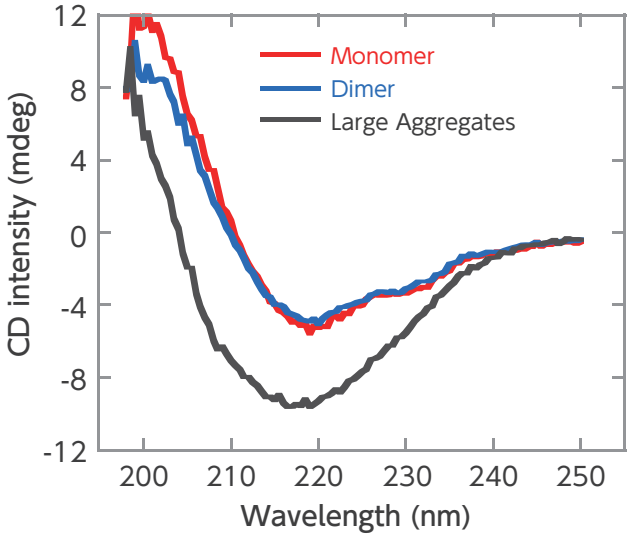
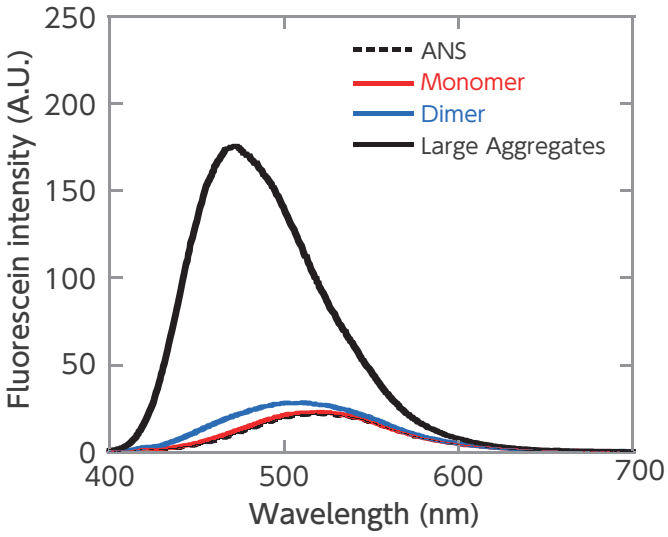


Fig. 5 Fluorescence spectrum analysis



ANS: 8-anilino-1-naphthalenesulfonic acid

Conclusion

- It was possible to culture the antibody-producing CHO cell line in the presence of TREHALOSE SG.
- TREHALOSE SG can suppress antibody aggregation, especially the formation of high-order aggregates, during the cell culture process.
- Use of TREHALOSE SG appears to increase efficiency of functional antibody production.

Reference

● Suppression of Antibody Aggregation in CHO Cell Culture by Trehalose Addition
Masayoshi Onitsuka and Takeshi Omasa: Institute of Technology and Science, The University of Tokushima. 16th Trehalose Symposium (2012)

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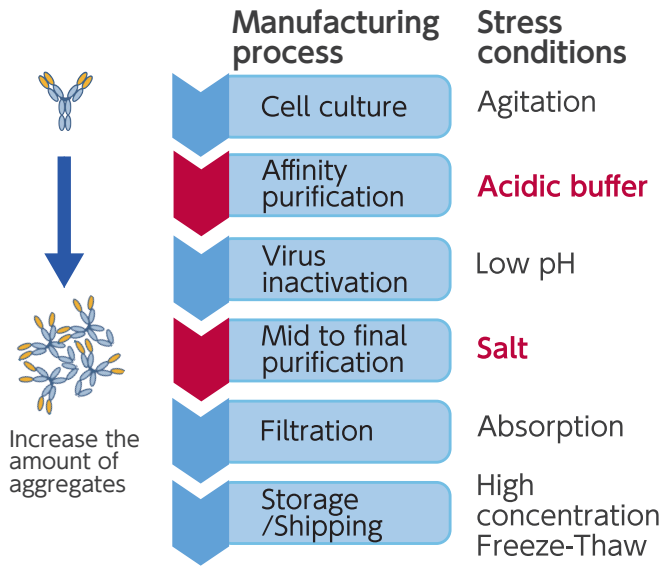
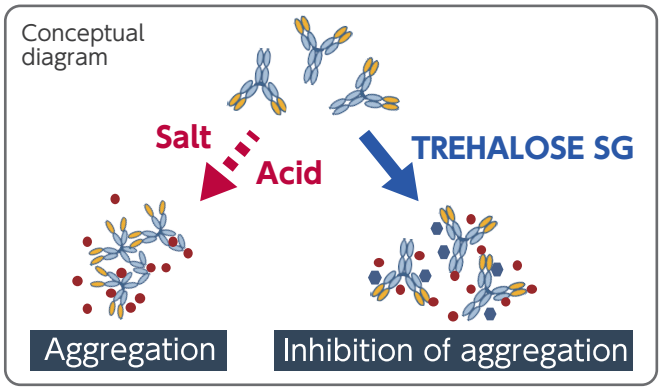
For Antibody Purification Use

Antibody manufacturing process and aggregate formation

- While there are various stressors that cause antibody aggregation during the antibody manufacturing process, use of acidic buffers and high concentrations of salt solutions are the major stressors leading to aggregate formation.
- The resulting aggregates may also act as nuclei for further aggregate growth, possibly creating antigenicity of the aggregates, raising quality and safety concerns.

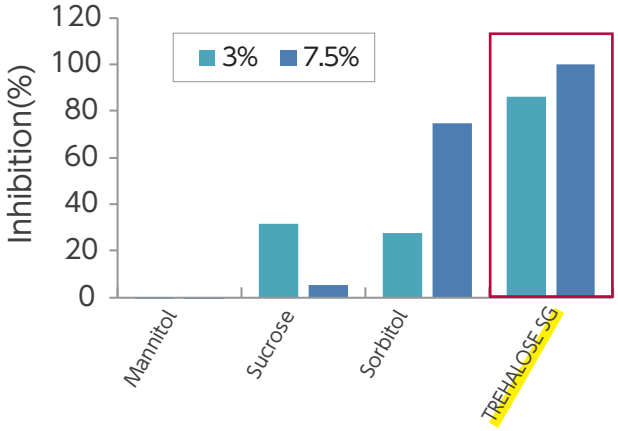


Effects of TREHALOSE SG

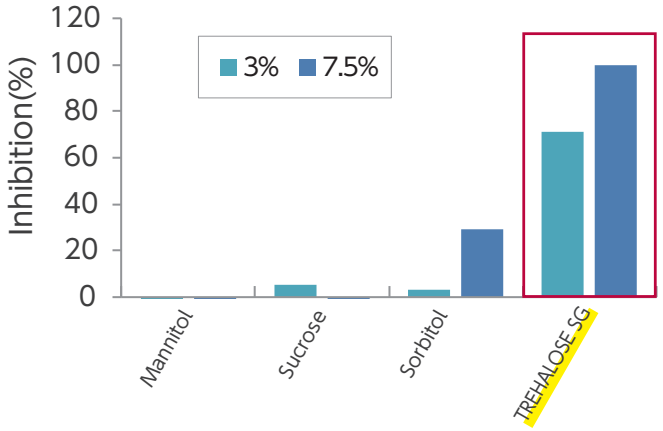


- TREHALOSE SG is suitable for use as an additive for antibody chromatography buffers because it suppresses antibody aggregation due to acids and salts, which is a particular problem during antibody purification.
- Addition of TREHALOSE SG in the purification buffer prevents loss of active antibody yield without affecting the interaction with the carrier used for chromatography.

Acidic buffer (glycine-HCl pH 2.7)



Salt (5 mol/L lithium chloride)



*Monoclonal antibody was incubated with TREHALOSE SG, sucrose, mannitol or sorbitol in glycine-HCl buffer (0.1mol/L, pH2.7) or lithium chloride buffer (5 mol/L) for 30 min at 25 ℃. The percent inhibition of aggregate formation in the antibody solutions were determined using dynamic light scattering and presented as a relative value when compared with the inhibition percentage when 7.5% TREHALOSE SG is added. It was shown that the addition of 7.5% TREHALOSE SG efficiently inhibits antibody aggregation in the chromatography buffers containing acid and salts.

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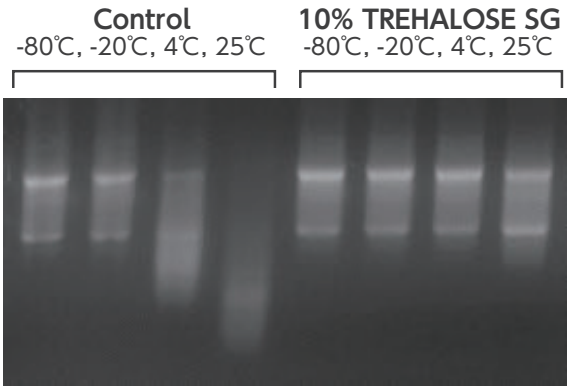
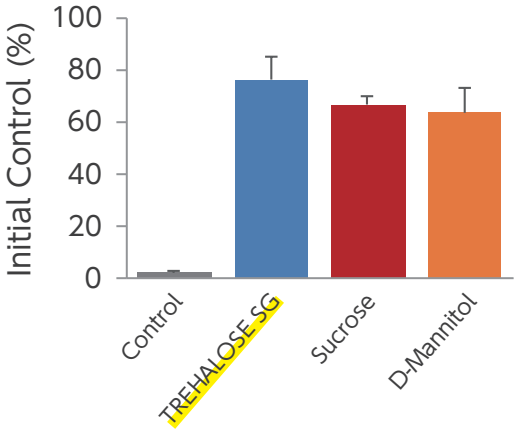
For DNA/RNA Stabilization

RNA Stabilization by TREHALOSE SG

- Stable storage of nucleic acids from clinical samples is important for accurate molecular diagnostics.
- RNA is relatively less stable than DNA against various physical stressors including RNase contamination during RNA extraction from the clinical samples and subsequent treatments including reverse transcription (RT).

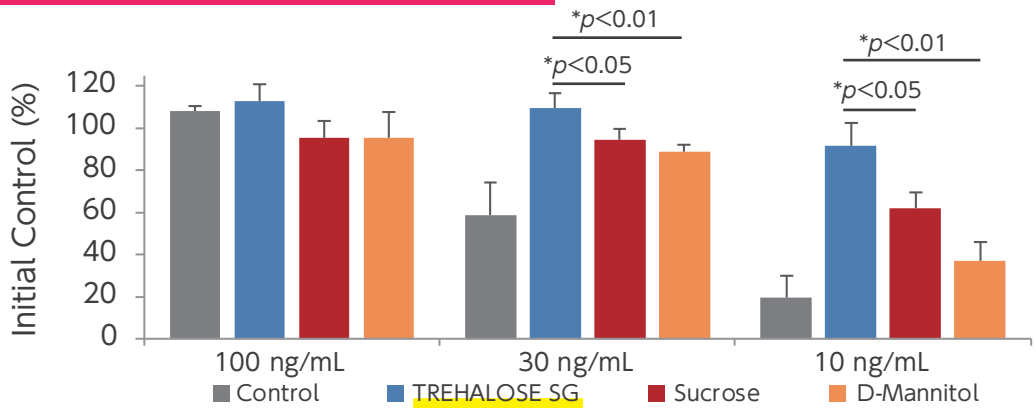


Total RNA was extracted from mouse liver in the presence or absence of 10% TREHALOSE SG and then dried. After storage at various temperatures for 2 weeks, the 28S and 18S ribosomal RNA bands were examined by agarose gel electrophoresis. A decrease in molecular weight due to RNA degradation was observed in the control samples when stored at 4 and 25℃. However, TREHALOSE SG protected the RNA from degradation.



Total RNA prepared with or without 10% of one of three saccharides were stored at 25℃ for 2 weeks. After RT reaction, quantitative polymerase chain reaction (qPCR) was performed to amplify the 18S ribosomal RNA gene. The percentage of the initial control (100%) using TREHALOSE SG was greater than the other saccharides, demonstrating that TREHALOSE SG is effective in preserving 18S ribosomal RNA. Results further imply that TREHALOSE SG is free of both RNase and DNase.

DNA Stabilization by TREHALOSE SG



Genomic DNA solutions prepared from mouse liver in the presence or absence of 5% concentrations of various saccharides were vacuum-dried and further heat-treated at 50℃ for 2 hrs. Then, samples were dissolved in DNase-free water and subjected to qPCR to amplify 18S ribosomal DNA to determine amplification rate. When the initial rate was set to 100%, no change was observed at 100 ng/mL DNA, but there was a decrease at lower concentrations (10-30 ng/mL), which was attributed to DNA fragmentation and insolubilization due to changes in the higher order structure. TREHALOSE SG was more effective than other sugars, indicating its stronger stabilizing effect on the structure of DNA.

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Nagase Viita

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 × g for 30 min at 4℃
Filtration using 0.22 μm filter

Whey
↓
Ultracentrifugation at 150,000 × g for 70 min at 4℃
Pellet (exosomes) was suspended in PBS
Ultracentrifugation at 150,000 × g for 70 min at 4℃
Pellet was resuspended in PBS
Centrifugation at 10,000 × g for 5 min at 4℃

Exosome (Western blotting)
↓
Protein concentration was measured by BCA method
Seventy μg of whey or 5 μg 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.

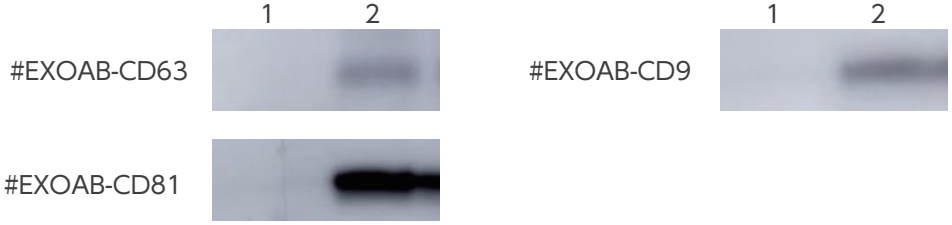

Fig.1. Characterization of bovine milk exosomes by Western blotting.
No.1; whey (70 μg), No. 2; bovine milk exosomes (5 μg)

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.

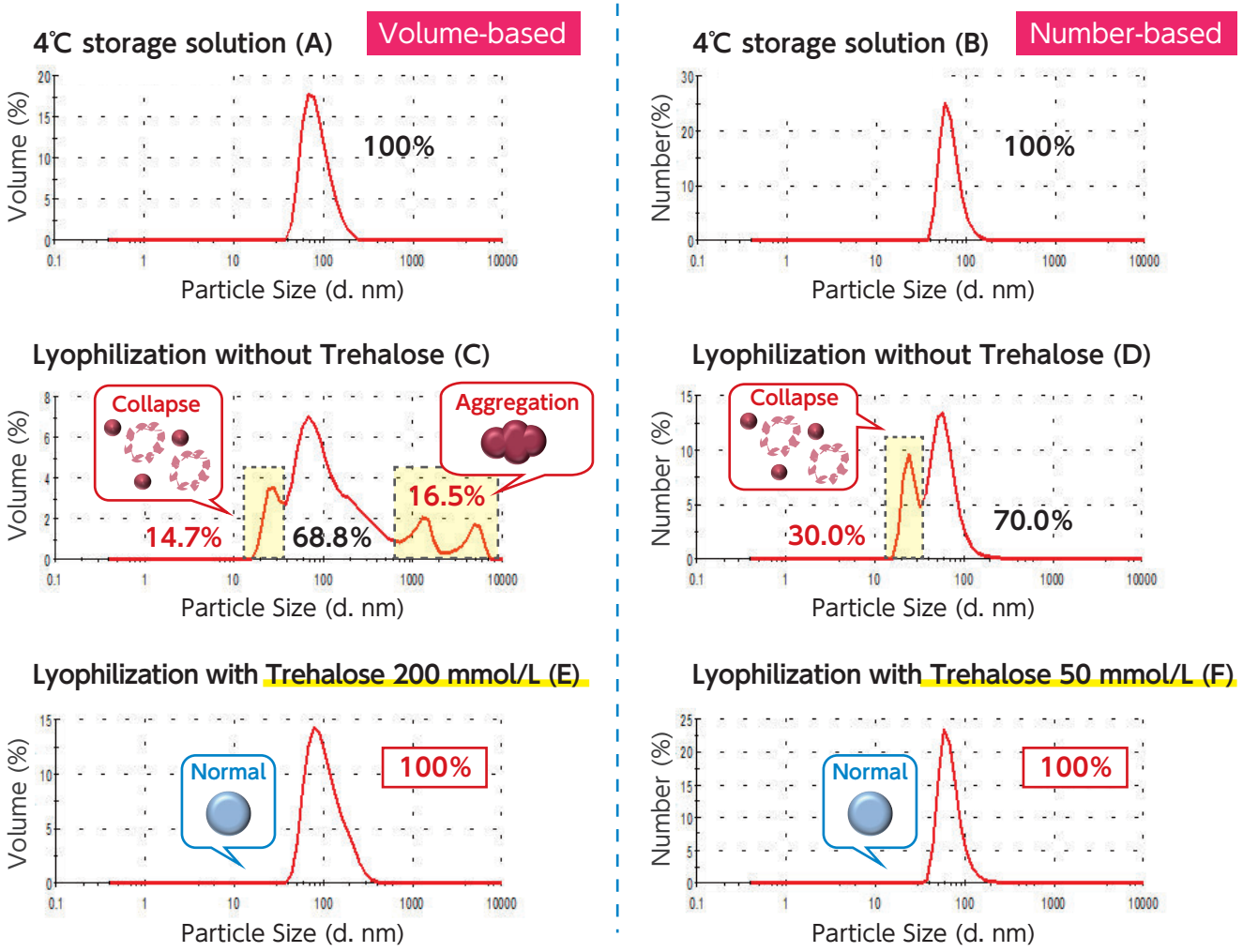

Fig. 2. DLS analysis of exosomes after lyophilization with or without TREHALOSE SG (Trehalose)

Results (2)

- Aggregation or collapse of exosomes was largely caused by lyophilization (Fig. 2C, D) compared to the pattern in the 4℃ 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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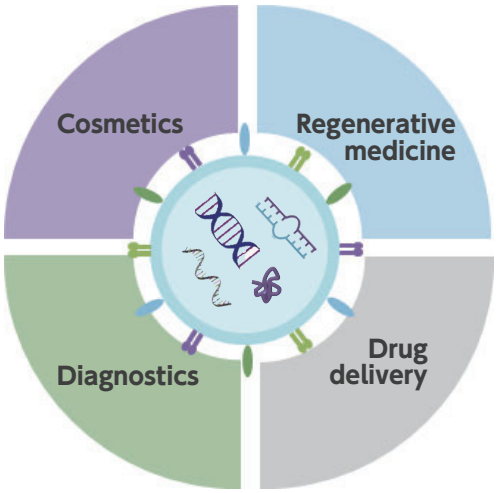
For Exosome Production and Storage

Mesenchymal Stem Cell (MSC)-derived Exosomes

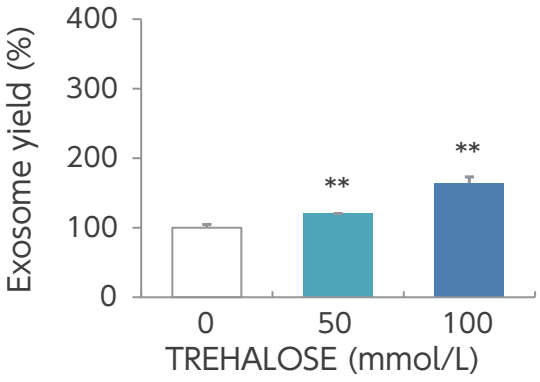
- MSC-derived exosomes are attracting attention for their application in regenerative medicine, drug delivery, diagnostics and cosmetics, and the need for a stable supply of exosomes is increasing.
- TREHALOSE SG is expected to be a powerful tool for industrial applications by increasing the exosome yield in the three processes of exosome production, purification and preservation.



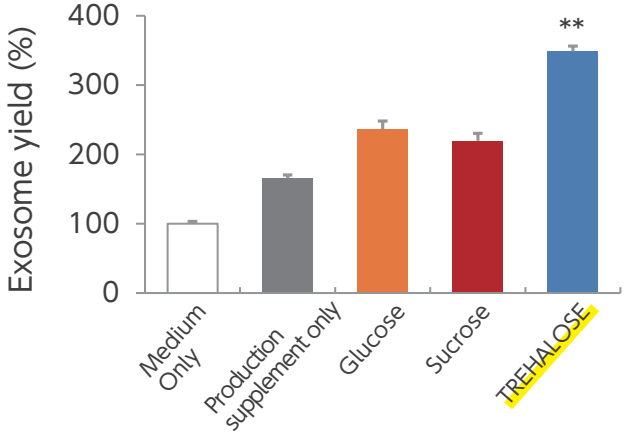
Improvement of Production Yield

- **The addition of TREHALOSE SG increases the production of MSC-derived exosomes via elevating Rab7 protein level.**
- **The combination of TREHALOSE SG with another supplement is more effective.**

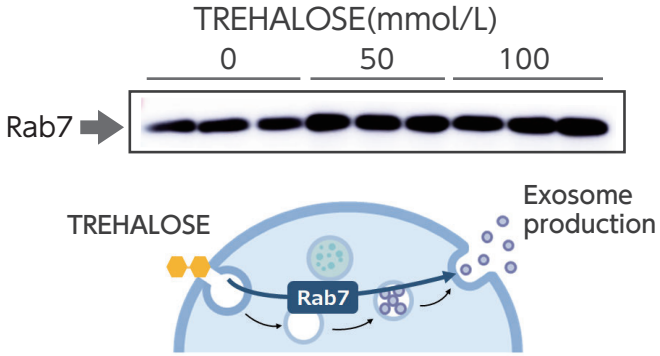
TREHALOSE alone

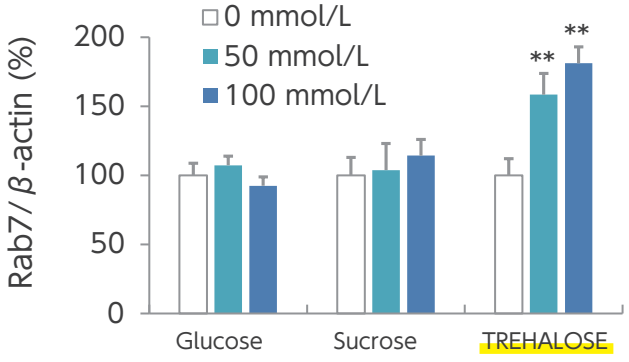


Combination Use



Mechanism

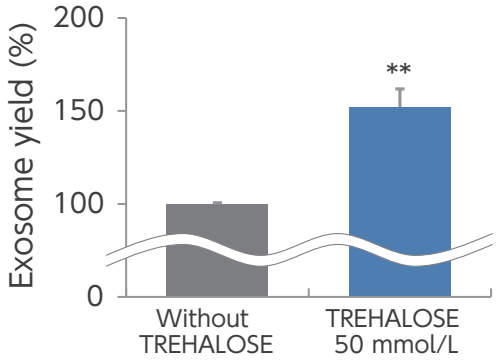




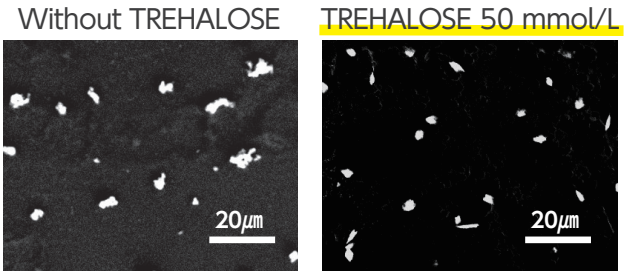
[Methods] Human adipose-derived MSCs (4×10^4 cells /2mL/well) were incubated with TREHALOSE SG in Messenchymal stem cell growth medium DXF (TAKARA) for 48 hrs, and exosome marker (CD9, CD63, CD81) positive particles in the culture supernatant were measured by flow cytometer. Effects of TREHALOSE SG were examined alone or in combination with exosome production supplements (EV-Up™, Fujifilm Wako Pure Chemical Corporation). Rab7 protein levels in MSCs were analyzed by Western blotting using anti-Rab7 antibody (Cell Signaling Technology, Inc.), and calculated relative to the β-actin signal (**$p<0.01$ vs. TREHALOSE 0 mmol/L).

SOLBIOTE™ TREHALOSE SG JP, USP-NF, Ph.Eur., CP

Improvement of Purification Yields



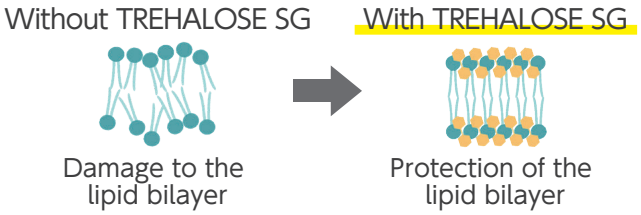
Addition of TREHALOSE SG suppresses exosome aggregation during purification.



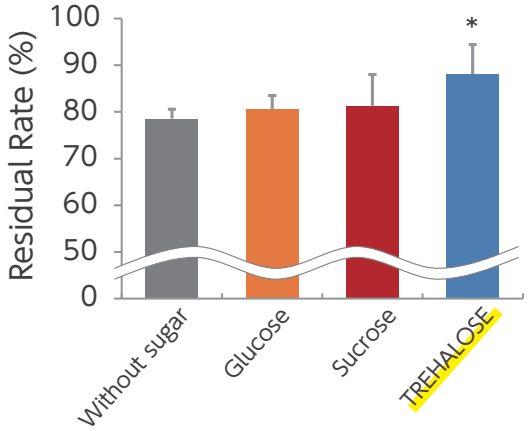
[Methods] MSC-derived culture supernatants were centrifuged using a 10-kDa ultrafiltration membrane (Amicon Ultra, Merck Millipore) at 9,000 g for 20 min at 4℃. Extracellular particles were collected and adjusted to 200 μL using 0.1 μm filtered PBS, and the number was measured using a flow cytometer. Results represent the exosome yield when the number of exosomes in the absence of TREHALOSE was settled as 100% (**p<0.01 vs. without TREHALOSE). The state of exosome was photographed using a scanning electron microscopy (× 500).

Improvement of Stability

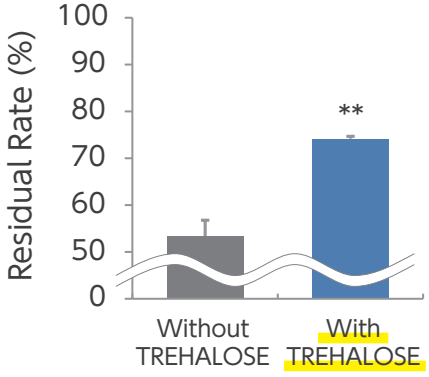
Addition of TREHALOSE SG improves the stability of exosomes during storage.



Storage at 4℃ (2 weeks)



Freeze-thawing (3 times)



[Methods] MSC-derived exosomes were suspended in 1 mL of PBS containing TREHALOSE SG or the other sugars (50 mmol/L), and the number of exosome was measured by flow cytometer after storage at 4℃ or freeze-thaw cycles (-80℃ to 4℃). Results represent residual rate of exosomes when the number of exosomes before storage was settled as 100%, and are expressed as the mean and standard deviation of three similar experiments (*p<0.05, **p<0.01 vs. without sugar or TREHALOSE).

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