FUJIFILM
Value from Innovation

Wako

## To suppress adsorption of extracellular vesicles to laboratory tools

# EV-Save™ Extracellular Vesicle Blocking Reagent

EV-Save™ Extracellular Vesicle Blocking Reagent protects extracellular vesicles (EVs) against freezing and suppresses their adsorption to labware such as test tubes and pipette tips. Our product lineup also includes EV-Save™ for *in vivo* use, which consists only of ingredients that have been successfully used as pharmaceutical additives.

### Features

- **Protects EVs against freezing**
⇒Allowing storage of extracellular vesicles at −80°C!

- **Suppresses the adsorption to labware of EVs**
⇒Efficiency of EV recovery is improved!

- **Simple operation**
⇒ simply add to the sample.

## Product Information

| Code No. | Product name | Grade | Pkg.size |
|---|---|---|---|
| 058-09261 | EV-Save™ Extracellular Vesicle Blocking Reagent | For genetic reseach | 1mL |
| 050-09461 | EV-Save™ Extracellular Vesicle Blocking Reagent For *in vivo* | | |

## Application available

It has been confirmed that this product does not interfere with EV analysis in the following experiments:

1. Nanoparticle Tracking Analysis (NTA)
2. ELISA
3. Experiments involving the addition of EVs to cells
4. Western blotting*1
5. Microarray analysis*1

*1 Applicability confirmed for EV-Save™ (product code No. 058-09261) only.

- If this product is used for samples that are rich in serum, plasma, or impurities, it is unlikely the desired anti-adsorption effect will be obtained.
- EV-Save™ contains a polymer. If there is a concern about the possible influence of the polymer on experimental results in any subsequent steps, please avoid the use of this product.
- Using EV-Save in conjunction with a fluorescent dye could raise the background level.

## Comparing EV-Save™ and EV-Save™ for *in vivo*

| | EV-Save™ (Code No. 058-09261) | EV-Save™ for *in vivo* (Code No. 050-09461) |
|---|---|---|
| Excretion to outside the body | No data | Only ingredients with molecular weights that allow the product to be excreted outside the body |
| Published use as pharmaceutical additives | No | Yes |
| Use in animal experiments | No data | Possible |
| Ultrafiltration application | Possible | Impossible |

## How to Use (applicable to both EV-Save and EV-Save for *in vivo*)

### ■Using MagCapture™ Exosome Isolation Kit PS Ver.2 (Code No. 290-84103)

Isolation of EVs from the cell culture supernatant

1. Add EV-Save™ or EV-Save™ for *in vivo* to the cell culture supernatant to make a 100-fold dilution, and mix by inverting or tapping.
2. Proceed to Step 5. "Washing of EVs-binding beads" as specified in the instruction manual for MagCapture™ Exosome Isolation Kit PS Ver.2 (Ver.2).
3. Add EV-Save™ or EV-Save™ for *in vivo* to the "Exosome Elution Buffer" included in the Ver.2 to make a 100-fold dilution, and mix by inverting or tapping.
4. Restart the experiment at Step 6. "Elution of Extracellular Vesicles" as specified in the instruction manual for the Ver.2, to elute extracellular vesicles.
5. Store the eluted extracellular vesicles*2, or proceed to the next experiment.

Isolation of EVs from serum and plasma

1. Proceed to Step 5. "Washing of EVs-binding beads" as specified in the instruction manual for the Ver.2.
2. Add EV-Save™ or EV-Save™ for *in vivo* to the "Exosome Elution Buffer" included in the Ver.2 to make a 100-fold dilution, and mix by inverting or tapping.
3. Restart the experiment at Step 6. "Elution of Extracellular Vesicles" as specified in the instruction manual for the Ver.2, to elute extracellular vesicles.
4. Store the eluted extracellular vesicles*2, or proceed to the next experiment.

### ■Using another method of isolation

Isolation of EVs from the cell culture supernatant

1. Add EV-Save™ or EV-Save™ for *in vivo* to the cell culture supernatant to make a 100-fold dilution, and mix by inverting or tapping.
2. Add EV-Save™ or EV-Save for *in vivo* to isolated extracellular vesicles to make a 100-fold dilution, and mix by inverting or tapping.
3. Store the eluted extracellular vesicles*2, or proceed to the next experiment.

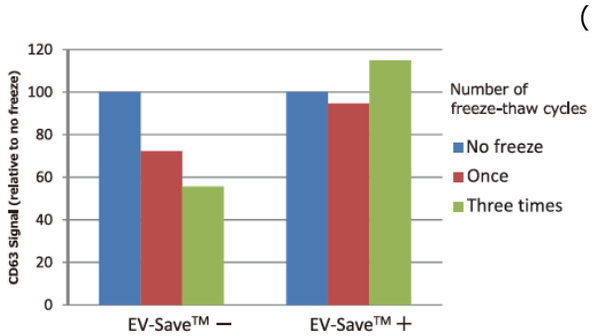
*2 By adding this product, the sample can be stored frozen at under −20°C. However, please avoid a large number of freeze-thaw cycles.

## EV-Save™ application data

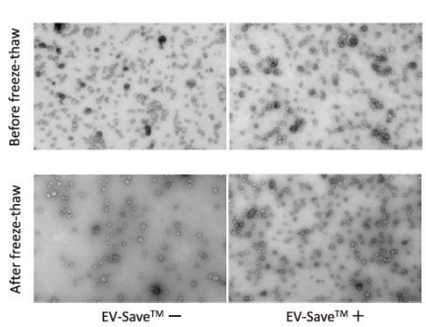
### (1) Protective effect against exosome freezing

Exosomes are known to be damaged by repeated cycles of freezing and thawing (Steffi, B. et al. Scientific Reports, 6, 36162 (2016)). After freeze-thawing, a COLO201-cell-derived exosome, previously purified using a PS affinity method*3, was assayed by measurement using the PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD) (product code No. 297-79201) (A) and analyzed using a transmission electron microscope (TEM) (B), and data were evaluated to determine whether addition of EV-Save™ suppresses exosome damage.

(A)




(B)

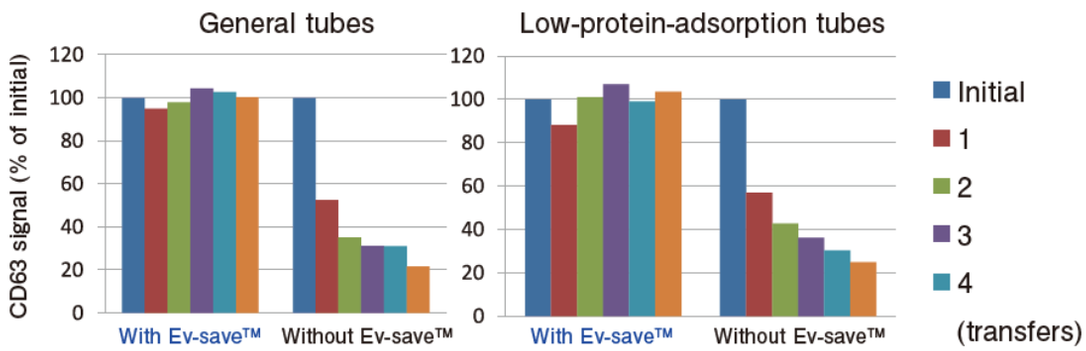



The exosome marker CD63 signal was decreased by freeze-thawing, and this signal reduction was suppressed by adding EV-Save™ (A). Furthermore, electron microscopic analysis showed that freeze-thawing markedly reduced particle counts, and this phenomenon was also suppressed by adding EV-Save™ (B).

*3 A method in which EVs are captured using a molecular species that binds to phosphatidylserine (PS), in a metal-ion-dependent manner, after which they are eluted with a chelating agent. MagCapture™ Exosome Isolation Kit PS Ver.2 allows you to use this method to purify EVs.

### (2) Suppressive effect against exosome adsorption to the tube

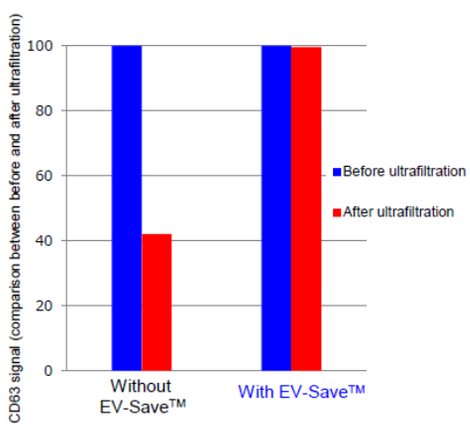
A COLO201-cell-derived exosome, previously purified using a PS affinity method*3, was added to a test tube, allowed to stand, and then transferred to another tube. This operation was repeated four times. For "With EV-Save™" conditions, EV-Save™ was added first to achieve dilution of 1 to 100 of liquid volume. CD63 signal level was measured using the PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD), and exosome reduction rates associated with the number of tube transfers were determined. The signal values relative to the 100% level obtained before transfer are shown graphically.




Regardless of whether the test tube used was a standard test tube or a low protein binding tube, the exosome content (CD63 signal) was decreased by transfer to another tube. However, exosome reductions due to tube transfer were suppressed nearly completely by adding EV-Save™.

### (3) Suppressive effect against exosome adsorption during ultrafiltration

Human iPS cell culture supernatant was examined for exosome loss by comparing exosome levels obtained before and after concentration by ultrafiltration (Vivaspin 20 fraction, molecular weight 100 K).

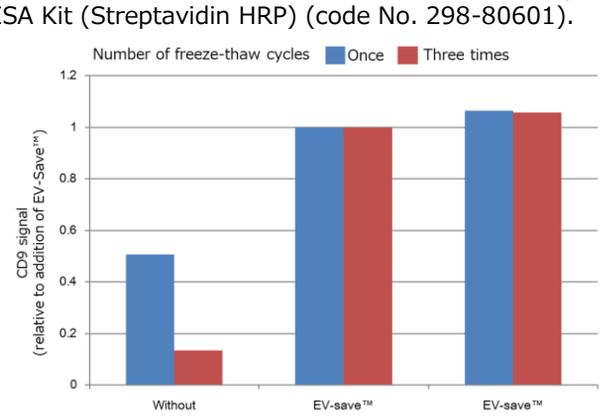



The exosome content (CD63 signal) in the culture supernatant was decreased approximately 60% by ultrafiltration, and this reduction was suppressed nearly completely by adding EV-Save™.

## EV-Save™ for *in vivo* application data

### (1) Protective effect against exosome freezing

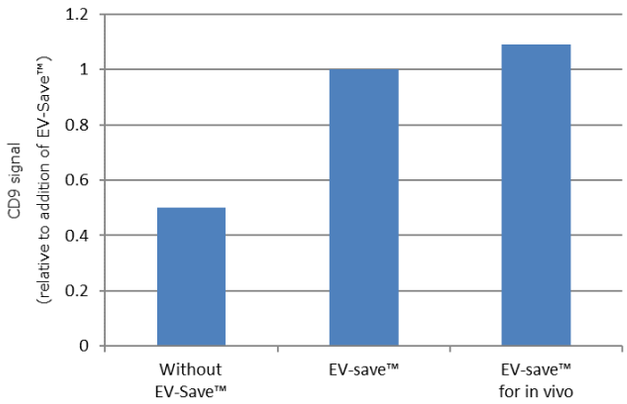
EV-Save™ and EV-Save™ for *in vivo* were each added to COLO201-cell-derived exosomes, previously purified using a PS affinity method*3, and freeze-thawing was performed either once or for three repeated cycles. Thereafter, the exosome content in the sample tube was measured using the PS Capture™ Exosome ELISA Kit (Streptavidin HRP) (code No. 298-80601).




Without addition of EV-Save™, the exosome marker CD9 signal was decreased by freeze-thawing, and this signal reduction was suppressed by adding EV-Save™ and EV-Save™ for *in vivo*.

### (2) Suppressive effect against exosome adsorption

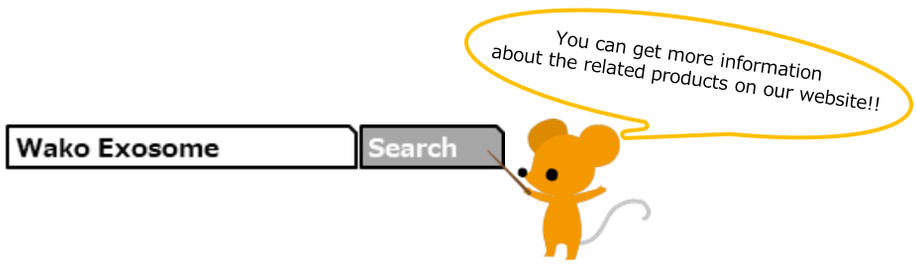
EV-Save™ and EV-Save™ for *in vivo* were each added to COLO201-cell-derived exosomes, previously purified using a PS affinity method*3, and the sample was stored at 4°C for 16 hours. Thereafter, the exosome content in the sample tube was measured using the PS Capture™ Exosome ELISA Kit (Streptavidin HRP).




Without addition of EV-Save™, the exosome content (CD9 signal) decreased, and this was suppressed by adding EV-Save™ and EV-Save™ for *in vivo.*

## Related products

| Code No. | Product Name | Grade | Pkg. size |
|---|---|---|---|
| 294-84101 | MagCapture™ Exosome Isolation Kit PS Ver.2 Ref | For genetic reseach | 2 tests |
| 290-84103 | | | 10 tests |
| 297-79201 | PS Capture™ Exosome ELISA Kit (Anti Mouse IgG POD) Ref | For genetic reseach | 96 tests |
| 298-80601 | PS Capture™ Exosome ELISA Kit (Streptavidin HRP) Ref | For genetic reseach | 96 tests |






**FUJIFILM Wako Laboratory Chemicals site**
**https://labchem-wako.fujifilm.com**

**FUJIFILM Wako Pure Chemical Corporation**
1-2, Doshomachi 3-Chome, Chuo-ku, Osaka 540-8605, Japan
Tel: +81 6 6203 3741 Fax: +81 6 6203 1999
ffwk-cservise@fujifilm.com

**FUJIFILM Wako Chemicals U.S.A. Corporation**
1600 Bellwood Road, Richmond, VA 23237, U.S.A.
Toll-Free (U.S. only): +1 877 714 1920
Tel: +1 804 271 7677 Fax: +1 804 271 7791
wkuslabchem@fujifilm.com

**FUJIFILM Wako Chemicals Europe GmbH**
Fuggerstr 12, 41468 Neuss, Germany
Tel: +49 2131 311 0 Fax: +49 2131 311 100
labchem_wkeu@fujifilm.com

**FUJIFILM Wako Chemicals (Hong Kong) Limited**
Room 1111, 11/F, International Trade Centre, 11-19 Sha Tsui Road, Tsuen Wan, N.T., Hong Kong
Tel: +852-2799-9019 Fax: +852-2799-9808
wkhk.info@fujifilm.com

**FUJIFILM Wako (Guangzhou) Trading Corporation**
Room 3003, 30/F., Dong Shan Plaza 69, Xian Lie Zhong Road, Guangzhou, 510095, China
Tel: +86-20-8732-6381(Guangzhou) Tel: +86-21-6288-4751(Shanghai)
Tel: +86-10-6413-6388(Beijing)
wkgz.info@fujifilm.com