2nd edition

Date of rescheduling: October 1, 2024

Product No. 131-19491
Product No. 137-19493

[Research Reagent]

MassivEV™ EV Purification Column PS

[Product Information]

Product No.	Product Name	Volume	Storage
131-19491	MassivEV™ EV Purification Column PS	1 mL	Refrigerated
137-19493		5 mL	

[Overview]

This product is a column for the purification of extracellular vesicles (EVs). High-purity EVs can be easily purified from cell culture supernatants using the PS affinity method. The product utilizes a substance that binds to phosphatidylserine (PS) on the EV surface in a calcium-dependent manner, allowing the intact EVs to be eluted using a chelating agent. The approximate dynamic binding capacity* of this product is as follows:

1-mL column: $5x10^{11}$ particles/1 mL resin

5-mL column: $2.5x10^{12}$ particles/5 mL resin

*The values were measured under the conditions set by FUJIFILM Wako using EVs derived from mesenchymal stem cells (MSCs) and these values may vary depending on the conditions.

[Materials to be Prepared (□: Checkboxes)]

1. Reagents

□ 295-96601 MassivEV™ Purification Buffer Set

□ Culture media (cell expansion media/EV production media)

Examples: 132-19345 MSCulture™ High Growth Basal Medium

133-19331 MSCulture™ High Growth Supplement

053-09451 EV-Up™ EV Production Basal Medium for MSC

298-84001 EV-Up™ MSC EV Production Supplement

□ 99.5% Ethanol

□ Ultrapure water

2. Equipment

Essential Items:

□ Peristaltic pump (example: Repligen #ACJR-U10-R)

□ Connectors (example: Cytiva #18111251)

□ Tubing for liquid transfer (example: Yamato Scientific #06435-14, Bio-Rad #7318215, Bio-Rad #7318215)

□ Two types of Luer fittings (example: Bio-Rad #7318222 and Bio-Rad #7318225)

□ Collection tubes (example: Corning #430791)

- For more details, please refer to the support documents available on the product website.

  FAQ support documents: Example of a purification system for the MassivEV EV Purification Column PS

  (https://labchem-wako.fujifilm.com/jp/product/detail/W01W0113-1949.html)

- The following equipment may or may not be necessary depending on the method used. Please check the Operating Procedure.

□ Centrifuge

□ Cell strainer (example: FALCON #352340)

□ 5-μm filter (example: GVS #1215396)

□ 0.8-μm filter (example: GVS #1214568)

□ 0.22-μm filter (example: Corning #431118)

□ Incubator or water bath (capable of heating to 37°C)

□ Reusable filter units (required when using the 5-μm/0.8-μm filters mentioned above, example: Thermo#300-4050PK)

□ Ultrafiltration membrane [100 kDa/PES material] (example: Sartorius #VS0141)

□ Gel filtration column (example: PD SpinTrap G-25)

[Preparation of Reagents]

The 1 column volume (CV) is as follows:
For a 1-mL column: 1 CV = 1 mL
For a 5-mL column: 1 CV = 5 mL

1. Preparation of Washing Buffer (1x)

Add 4 CVs of Washing Buffer (10x) to 36 CVs of ultrapure water.

Example: For a 1-mL column, add 4 mL of Washing Buffer (10x) to 36 mL of ultrapure water.

2. Preparation of EV Binding Enhancer/Washing Buffer (1x)

Add 1/100 volume of EV Binding Enhancer (100x) to 20 CVs of Washing Buffer (1x).

Example: For a 1-mL column, add 200 µL of EV Binding Enhancer (100x) to 20 mL of Washing Buffer (1x).

3. Preparation of EV Elution Buffer (1x)

Add 0.4 CV of EV Elution Buffer (10x) to 3.6 CVs of ultrapure water.

Example: For a 1-mL column, add 400 µL of EV Elution Buffer (10x) to 3.6 mL of ultrapure water.

4. Preparation of EV-Stabilizer/Elution Buffer (1x)

Depending on the buffer exchange method after EV elution, add 1/100 volume of EV-Stabilizer A or B to 4 CVs of EV Elution Buffer (1x).

Example: For a 1-mL column, add 40 µL of EV-Stabilizer A or B to 4 mL of EV Elution Buffer (1x).

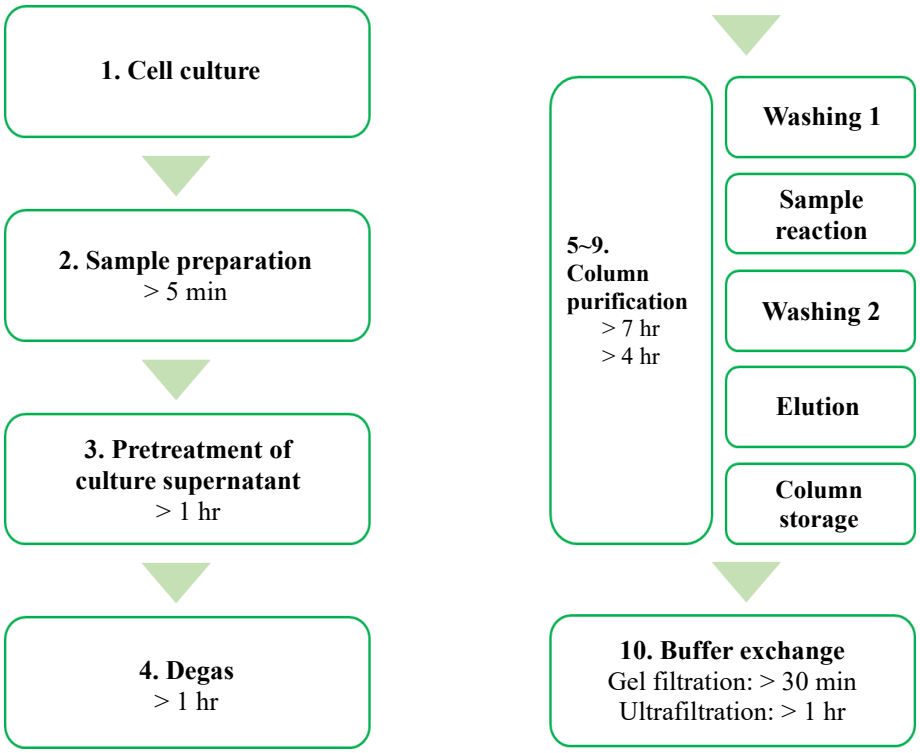
Buffer exchange method	Capability	EV-Stabilizer to be added	Administration to animals
(A) Gel filtration	Buffer exchange only	A	Possible
(B) Ultrafiltration	Buffer exchange, concentration	B	Not recommended

5. Preparation of 20% ethanol/Storage Buffer (1x)

Add 0.2 CV of Storage Buffer (10x) and 0.4 CV of 99.5% ethanol to 1.4 CVs of ultrapure water.

Example: For a 1-mL column, add 200 µL of Storage Buffer (10x) and 400 µL of 99.5% ethanol to 1.4 mL of ultrapure water.

[Process Flow]



[Operating Procedure]

1. Cell Culture

(1) Perform proliferation culture and EV production culture of the desired cells.

(2) Collect the cell culture supernatant.

(3) Store the supernatant as needed, either refrigerated (2-10°C) or frozen (below -20°C).

- If serum-containing media is used for EV production, impurities may adhere to the resin, potentially accelerating the degradation of resin performance. Therefore, serum-free media is recommended for EV production.

2. Addition of EV Binding Enhancer

(1) Add 1/100 volume of EV Binding Enhancer (100x) to the culture supernatant from step 1.

- If the media contains 2 mM or more calcium, the addition of EV Binding Enhancer is not necessary.
- If the calcium concentration in the media is unknown, the addition of EV Binding Enhancer is recommended.
- Excessive calcium addition may result in precipitates, which can be removed in a

subsequent filtration step.

3. Pre-treatment of Culture Supernatant

To remove impurities, perform pre-treatment using one of the following methods:

A. Pre-treatment by centrifugation

(1) Centrifuge the culture supernatant from step 2 at 5,000xg for 20 min.

(2) Collect the supernatant.

(3) Filter the supernatant from (2) using a 0.8-μm filter.

- Filters made of PES material are recommended.
- The centrifugation speed mentioned is based on experiments conducted at FUJIFILM Wako. If 0.22-μm filtration is feasible in the subsequent degassing step, centrifugation can also be performed at other speeds (e.g., 10,000xg for 60 min).

B. Pre-treatment by filtration

(1) Filter the culture supernatant from step 2 using a 40-μm cell strainer.

(2) Filter the filtrate from (1) using a 5-μm filter.

(3) Filter the filtrate from (2) using a 0.8-μm filter.

- Filters made of PES material are recommended.

4. Degassing the Culture Supernatant

(1) Heat the pre-treated culture supernatant from step 3 in a 37°C incubator or water bath until it reaches a temperature above room temperature (25-28°C).

(2) Filter the supernatant using a 0.22-μm filter.

- When returning refrigerated samples to room temperature, dissolved air in the liquid may be released, potentially introducing air bubbles into the column. To prevent this, always ensure that the sample temperature is slightly above room temperature after heating.
- Filters made of PES material are recommended.

5. Column Purification –Washing 1

To avoid introducing air bubbles into the column, ensure that all buffers are brought to room temperature before use in the subsequent column purification steps 5-9.

(1) Remove the column from the refrigerator and allow it stand for about 10 min to reach room temperature.

(2) Using a peristaltic pump, pass 10 CVs of Washing Buffer (1x) through the column.

- The recommended flow rates are as follows:
  For a 1-mL column: ≤ 0.85 mL/min
  For a 5-mL column: ≤ 4.25 mL/min
- To prevent air from entering the column, fill the column connector with buffer before connecting it to the peristaltic pump. Additionally, ensure that all buffers are brought to room temperature before use.

6. Column Purification – Sample Reaction

(1) Using a peristaltic pump, pass the degassed sample from step 4 through the column at room temperature, allowing it to react with the resin.

- The recommended flow rates are as follows:
  For a 1-mL column: 0.6 mL/min
  For a 5-mL column: 3 mL/min
- If there are concerns about the deterioration of extracellular vesicles (EV) or that of the sample, the reaction can be performed at a low temperature (2-10°C). In that case, reduce the flow rate compared to the rate at room temperature and allow the reaction to proceed overnight.

7. Column Purification – Washing 2

(1) Using a peristaltic pump, wash the resin in the column with 20 CVs of EV Binding Enhancer/Washing Buffer (1x).

- The recommended flow rates are as follows:
  For a 1-mL column: ≤ 0.85 mL/min
  For a 5-mL column: ≤ 4.25 mL/min

8. Column Purification – Elution

(1) Using a peristaltic pump, pass 0.4 CV of EV-Stabilizer/Elution Buffer (1x) through the column to replace more than 40% of the EV Binding Enhancer/Washing Buffer in the column.

(2) Place a collection tube under the column.

(3) Using a peristaltic pump, pass 3.6 CVs of EV-Stabilizer/Elution Buffer (1x) through the column and collect the eluate.

- The recommended flow rates are as follows:
  For a 1-mL column: ≤ 0.2 mL/min
  For a 5-mL column: ≤ 1 mL/min
- Set the flow rate lower than that in step 6 (sample reaction).

9. Column Purification – Column Storage

(1) Pass 10 CVs of Washing Buffer (1x) through the column using a peristaltic pump

(2) Pass 2 CVs of 20% ethanol/Storage Buffer (1x) through the column, ensuring it is filled with 20% ethanol/Storage Buffer (1x) (stop after passing 2 CVs).

(3) Wrap the top of the column with parafilm and store it in a refrigerator at 2-10°C.

- It is recommended to set the flow rate in (1) lower than that of step 6 (sample reaction).
- The recommended flow rates are as follows:
  For a 1-mL column: ≤ 0.6 mL/min
  For a 5-mL column: ≤ 3 mL/min
- The column can be reused up to 5 times.
- Wrap the parafilm around the area indicated by the red box in the figure below.

Wrap the parafilm around the area indicated by the red box.

10. Buffer Exchange of the Eluate

(A) Buffer Exchange by Gel Filtration

(1) Add 1/100 volume of EV-stabilizer A to the buffer you plan to use for replacement.

(2) Load the eluate (purified EV) onto the gel filtration column.

(3) Perform gel filtration using the buffer prepared in (1).

(4) Collect the gel-filtered sample.

(5) Sterilize the sample using a 0.22-μm filter.

- For the gel filtration method and required buffer volume, please refer to the manual of the gel filtration column being used.
- Recommended gel filtration columns:
  PD SpinTrap G-25: Cytiva #28-9180-04
  PD MidiTrap G-25: Cytiva #28-9180-08
  PD Desalting columns: Cytiva #17-0851-01
- Gel filtration cannot be used to concentrate the eluate.
- When using large gel filtration columns, the sample collected in step 8 may become diluted due to the void volume.
- Depending on the efficiency of the gel filtration, multiple rounds may be necessary to remove EDTA.

B. Buffer Exchange by Ultrafiltration

(1) Add 1/100 volume of EV-Stabilizer B to the buffer you plan to use for replacement (27 times the volume of the eluate).

(2) Add the eluate (purified EV) to a 100 kDa ultrafiltration membrane.

(3) Add 9 times the volume of the buffer containing EV-Stabilizer B (prepared in (1)) and perform ultrafiltration (centrifugation guideline: 5,000xg for 10-20 minutes).

(4) Repeat (3) twice more (for a total of 3 rounds of ultrafiltration, achieving 1,000-fold buffer exchange).

(5) Collect the ultrafiltered sample into a new tube.

(6) Sterilize the sample using a 0.22-μm filter.

- Be sure to use ultrafiltration membranes made of PES material.
- Recommended ultrafiltration columns:
  VIVASPIN 500, MWCO 100,000, PES: SARTORIUS #VS0141/VS0142
  VIVASPIN 6, MWCO 100,000, PES: SARTORIUS #VS0641/VS0642

[Troubleshooting: If Air Enters the Column]

It may be possible to remove air that has entered the column by passing 20% ethanol through it at the specified flow rate. However, please note that air entry may degrade the resin's performance.

Flow rates: For a 1-mL column: 0.85 mL/min; for a 5-mL column: 4.25 mL/min

Reference photos: before air entry → after air entry → air removal process → after air removal