Code No. 131-19491（1mL）

Code No. 137-19493（5mL）

《For research use only》

MassivEV™ EV Purification Column PS

【Product information】

Code No.	Product name	Package	Storage
131-19491	MassivEV™ EV Purification Column PS	1 mL	2-10℃
137-19493		5 mL	

【Overview】

MassivEV™ EV Purification Column PS is used for a purification of extracellular vesicles (EVs) from a cell culture supernatant and provides high-purity EVs easily by PS affinity method. PS affinity enables a mild elution of intact EVs with a chelating agent because PS affinity captures phosphatidylserine (PS) on a membrane surface of the EVs in a calcium ion-dependent manner. The approximate dynamic binding capacity (DBC)* is as follows;

・1 mL: 5×10^{11} particles/mL resin

・5 mL: 2.5×10^{12} particles/mL resin

*The DBC may change depending on the conditions of the sample. The above binding capacity

is the result of evaluation using MSC-derived EVs.

【Materials and supplies required】

1. Reagent

☐ 295-96601 MassivEV™ Purification Buffer Set

☐ Cell culture medium for cell growth and EV production

e.g.) 132-19345 MSCulture™ High Growth Basal Medium

133-19331 MSCulture™ High Growth Supplement

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

298-84001 EV-Up™ MSC EV Production Supplement, AF

☐ 100% Ethanol

☐ Ultrapure water

2. Instruments

The following instruments may or may not be required depending on the operation.

☐ Centrifuge

☐ Cell strainer（e.g. FALCON #352340）

☐ 5 μm filter (e.g. GVS #1215396)

☐ 0.8 μm filter (e.g. GVS #1214568).

☐ 0.22 μm filter (e.g. Corning #431118)

☐ Incubation or water bath (which can be warmed at 37℃)

☐ Peristaltic Pump (e.g. KrosFlo Research Jr)

☐ Collection tube (e.g. Corning #430791)

☐ Ultrafiltration membrane [100kDa/PES material] (e.g. Zartrius #VS0141)

☐ Gel-filtration columns (e.g., PD SpinTrap G-25)

【Reagent preparation】

The volume of 1CV (column volume) is as follows;

・1 mL column: 1CV = 1 mL

・5 mL column: 1CV = 5 mL

1. Washing Buffer（1x）

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

e.g.) In the case of 40 mL preparation

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

2. EV Binding Enhancer/Washing Buffer

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

e.g.) In the case of 20 mL preparation

Add 200 μL of EV Binding Enhancer (100x) to 20 mL of Washing Buffer (1x).

3. EV Elution Buffer (1×)

Add 0.4CV of EV Elution Buffer to 3.6CV of ultrapure water.

e.g.) In the case of 4 mL preparation

Add 400 μL of EV Elution buffer (10x) to 3.6 mL of ultrapure water.

4. Elution/EV-Stabilizer Buffer（1×）

Add 1/100 volume of EV-Stabilizer A or B to 4CV of EV Elution Buffer (1x) depending on the purpose of buffer exchange after EV elution.

e.g.) In the case of 4 mL preparation

Add 40 µL of EV-Stabilizer A or B to 4 mL of Elution Buffer (1x).

Buffer exchange procedure	Effect	EV-Stabilizer	Administration to animal
(A)Gel filtration	Buffer exchange only	A	Possible
(B) Ultrafiltration	Buffer exchange and concentration	B	Not recommend

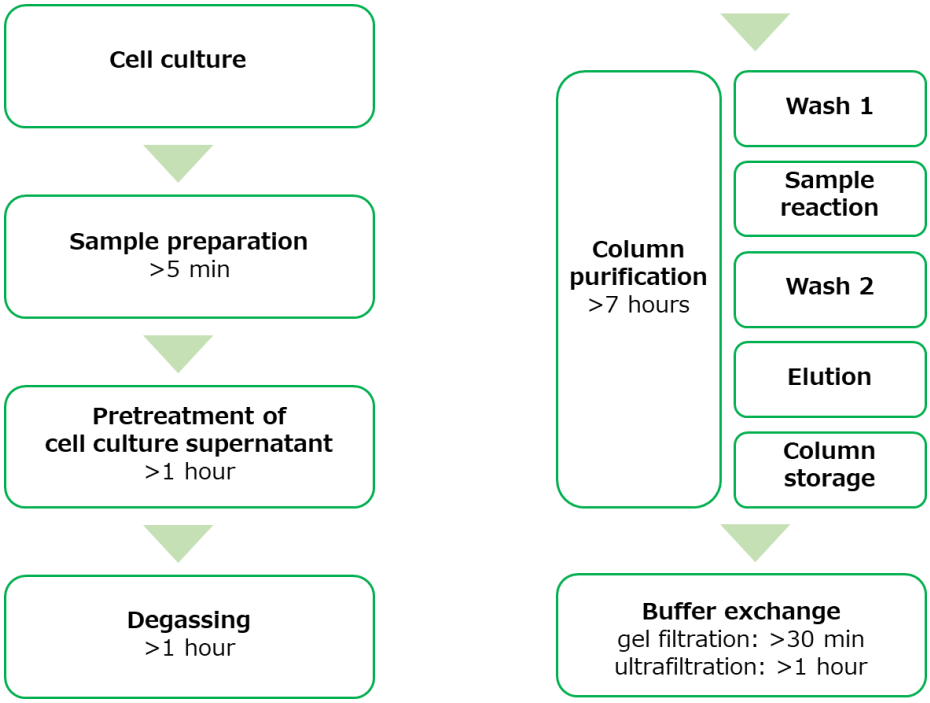
5. 20% Ethanol/Storage Buffer (1×)

Add 0.2CV of Storage Buffer (10x) and 0.4CV of 100% Ethanol to 1.4CV ultrapure water.

e.g.) In the case of 2 mL preparation

Add 200 µL of Storage Buffer (10x) and 400µL of 100% Ethanol to 1.4 mL ultrapure water.

【Flowchart】



【Procedure】

① Cell culture

(1) Perform cell expansion culture and EV production culture.

(2) Collect the cell culture supernatant.

(3) Store under refrigeration (2-10℃) or freezing (-20℃ or less) as needed.

· Recommend using serum-free medium for EV production. When using a medium supplemented with serum for EV production, impurities may be adsorbed to the resin. It may accelerate the performance deterioration of the resin.

② Addition of EV Binding Enhancer

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

· No need to add calcium if the medium contains 2 mM or more calcium. · Recommend adding calcium when its concentration in the medium is unknown. · The excessive addition of calcium may cause precipitation, but the precipitation can be removed by filter treatment in the subsequent process.

③ Pretreatment of cell culture supernatant

To remove impurities, select one of the following methods and perform pretreatment.

Option A: centrifugal pretreatment

(1) Centrifuge cell culture supernatant of ② (5,000 x g, 20 min).

(2) Collect the supernatant. (3) Process (2) with a 0.8 μm filter. · Recommend using the PES filter. · Centrifugation processing speed is based on the result of our internal study. If 0.22 μm filtering is possible in the next degassing step, other centrifugation speeds (e.g. 10,000 x g for 60 minutes) is acceptable.

Option B: filters pretreatment

(1) Process the cell culture supernatant from ② with a 40 μm cell strainer.

(2) Process (1) with a 5 μm filter.

(3) Process (2) with a 0.8 μm filter.

- Recommend using the PES filter.

④ Degassing the culture supernatant

(1) Warm the pretreated culture supernatant from procedure ③ in a 37℃ incubator or water bath to a temperature above RT (25-28℃).

(2) Process (1) with a 0.22 μm filter.

- When a refrigerated sample is returned to RT, the air in the vaporized liquid may cause air to enter the column. Therefore, make sure that the sample temperature after heating is higher than RT.
- Recommend using the PES filter.

⑤ Column purification – Wash 1

In order to avoid air contamination into the column, please bring the buffers back to RT in the procedures from ⑤ to ⑨.

(1) Stand the column for about 10 min to return it to RT after taking it from the refrigerator.

(2) Flow 10CV of Washing Buffer (1x) using a Peristaltic Pump.

- Recommended flow rate is as follows;
  1 mL column ≦0.85 mL/min 5 mL column ≦4.25 mL/min
- To prevent air from entering the column, fill the joint of the column with buffer before connecting it to the Peristaltic pump. Also, bring all buffers back to RT before use.

⑥ Column purification – Sample reaction

(1) Flow the degassed sample (④) using the Peristaltic Pump at RT and react it with resin.

- Recommended flow rate is as follows;
  1 mL column ≦0.6 mL/min 5 mL column ≦3 mL/min
- It is also possible to react at a low temperature (2-10℃). In that case, set the flow rate lower than when performing the reaction at RT and react overnight.

⑦ Column purification – Wash 2

(1) Wash the resin in the column with 20CV of EV Binding Enhancer/Washing Buffer using the Peristaltic Pump.

- Recommended flow rate is as follows;
  1 mL column ≦0.85 mL/min 5 mL column ≦4.25 mL/min

⑧ Column Purification – Elution

(1) To replace 40% of EV Binding Enhancer/Washing Buffer in the column, flow 0.4CV of EV Elution/EV-Stabilizer Buffer (1x).

(2) Set the collection tube under the column.

(3) Flow 3.6CV of EV Elution/EV-Stabilizer Buffer（1x） using the Peristaltic Pump and collect the eluate.

- Recommended flow rate is as follows;
  1 mL column ≦0.2 mL/min　5 mL column ≦1.0 mL/min
- Set the flow rate of elution slower than that of procedure ⑥.

⑨ Column purification – Column storage

(1) Flow 10CV of Washing Buffer (1x) using a Peristaltic Pump.

(2) To replace buffer in the column, flow 2CV of 20% Ethanol/Storage Buffer (1x).

(3) Wrap the parafilm on the top of the column and store it at 2-10℃.

- In the step (1), recommend setting the flow rate slower than that in procedure ⑥ Sample reaction.
- Recommended flow rate is as follows;
  1 mL column) ≦0.6 mL/min　5 mL column ≦3 mL/min
- The column can be reused up to five times.
- Wrap the parafilm around the red frame in the figure below.

⑩ Buffer exchange

(A) Buffer exchange by gel filtration

(1) Add 1/100 volume of EV Stabilizer A to the buffer to be replaced.

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

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

(4) Collect the filtrated sample.

(5) Sterilize the sample with a 0.22 μm filter.

- Refer to the manual for the gel filtration column for a gel filtration method and required amount of buffer.
- 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
- The gel filtration is not able to concentrate the eluate.
- When using a large-size gel filtration column, the sample collected in procedure ⑧ may dilute by void volume.
- Depending on the efficiency of gel filtration, it may be necessary to perform gel filtration multiple times to remove EDTA.

(B) Buffer exchange by ultrafiltration

(1) Add 1/100 volume of EV-Stabilizer B to the buffer to be replaced (27 times the volume of the eluted sample).

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

(3) Add 9-fold volume of the buffer containing EV-Stabilizer B (prepared in (1)) to (2) and perform ultrafiltration (e.g. 5,000 x g, 10-20 min).

(4) Repeat (3) two times (total of three ultrafiltration = 1,000 x buffer exchange).

(5) Collect the sample after ultrafiltration to the new tube.

(6) Sterile the sample with a 0.22 μm filter.

- Make sure to use the ultrafiltration membrane made of PES.

【Troubleshooting: In the case of air contamination into the column】

Air contamination into the column may be removed by passing 20% or 70% ethanol at the following flow rates. However, please note that the performance of the resin may deteriorate due to air contamination.

Flow rate: 1 mL column 0.85 mL/min　5 mL column 4.25 mL/min

Reference photos; before air contamination→after air contamination→air removal process→after air removal