2 mL Extracellular Vesicle SEC Columns

Size exclusion chromatography (SEC) columns for extracellular vesicle isolation

Catalog # 100-0415 5 columns

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

Extracellular Vesicle Size Exclusion Chromatography (SEC) Columns are ideal for the isolation and purification of extracellular vesicles (EVs) from numerous types of biological samples, including plasma, serum, and cell culture media (Baranyai et al.). SEC columns provide an efficient method for separation of EVs from circulating proteins (Baranyai et al.). Compared to other methods of EV isolation, SEC causes minimal alteration in vesicle characteristics such as functionality and shape (Gámez-Valero et al.). The isolated EVs are suitable for downstream analysis including flow cytometry, western blot, nucleic acid extraction, and/or functional assays.

The EV isolation protocol using the SEC columns is easy and takes approximately 15 minutes. The columns can be used up to five times, and are available in two additional sizes (0.5 mL [Catalog #100-0414] and 20 mL [Catalog #100-0416]) for different sample volume ranges.

Storage and Stability

Store at 2 - 8°C. Product stable until expiry date on label. Do not use if columns are damaged.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
D-PBS (Without Ca++ and Mg++)	37350
Costar® Microcentrifuge Tubes, 1.7 mL	38089

Sample Preparation

PLASMA AND SERUM

1. Centrifuge plasma or serum at 300 x *g* for 10 minutes. Remove the supernatant and transfer to a new microcentrifuge tube.
2. Centrifuge the plasma or serum layer (from step 1) at 1200 x *g* for 20 minutes. Remove the supernatant and transfer to a new microcentrifuge tube.
3. OPTIONAL: Centrifuge supernatant at 10,000 x *g* for 30 minutes to remove cellular debris and large vesicles. Remove the supernatant and transfer to a new microcentrifuge tube.

URINE

1. Transfer urine into a 50 mL conical tube (e.g. Catalog #38010).
2. Centrifuge urine at 300 x *g* for 10 minutes. Remove the supernatant and transfer to a 100 kDa molecular weight cut-off (MWCO) centrifuge tube (e.g. PALL catalog #MAP100C37) and centrifuge to concentrate 10-fold according to manufacturer's instructions.

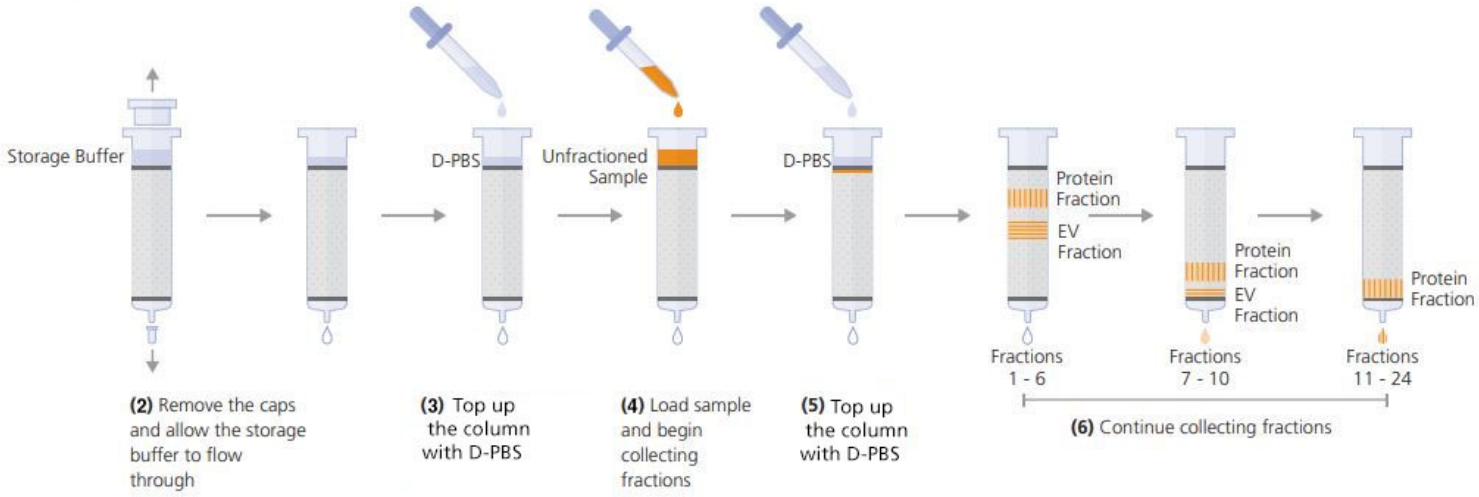
CONDITIONED MEDIUM

1. Harvest conditioned medium and transfer to a 50 mL conical tube (e.g. Catalog #38010).
2. Centrifuge the conditioned medium at 300 x *g* for 10 minutes. Remove the supernatant and transfer to a new 50 mL conical tube.
3. Centrifuge supernatant (from step 2) at 1200 x *g* for 20 minutes. Remove the supernatant and transfer to a new 50 mL conical tube.
4. OPTIONAL: Centrifuge supernatant (from step 3) at 10,000 x *g* for 30 minutes to remove cellular debris and large vesicles.
5. Remove the supernatant (from step 3 or step 4) and transfer to a 100 kDa MWCO centrifuge tube (e.g. PALL catalog #MAP100C37) and centrifuge to concentrate 10-fold according to manufacturer's instructions.

Extracellular Vesicle Isolation

EXTRACELLULAR VESICLE SEC COLUMN PROTOCOL DIAGRAM

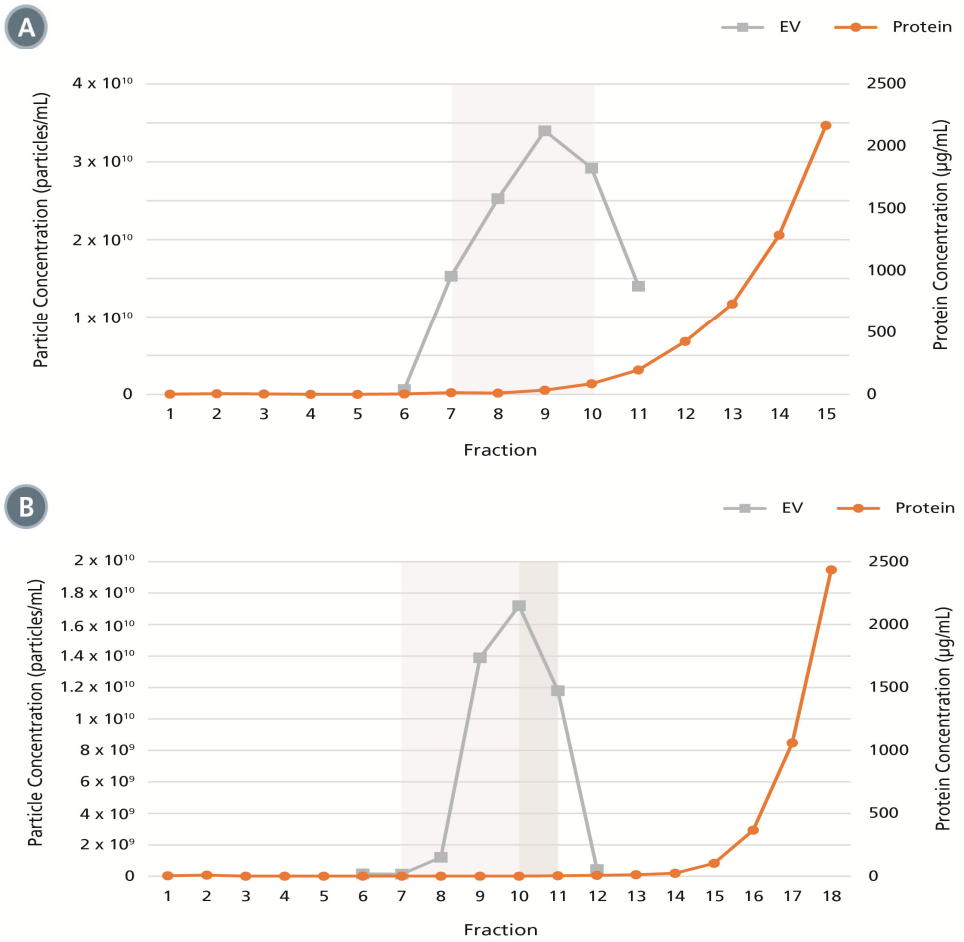
Numbers in brackets refer to steps in Directions for Use.



DIRECTIONS FOR USE

1. Stabilize the SEC column in an upright position.
2. Remove the cap from the top and bottom of the column and allow the storage buffer to flow through. Discard flowthrough. Do not allow the column to run dry.
3. Top up the column with D-PBS (Without Ca++ and Mg++; D-PBS). Allow a total of 20 mL of D-PBS to flow through. Discard flowthrough. Immediately proceed to step 4.
   NOTE: Keep the column gel matrix wet at all times.
4. Load 0.5 - 2 mL of sample to the top of the column and start collecting 500 µL fractions immediately.
5. When all of the sample has been loaded onto the gel matrix, top up the column with D-PBS. Continue to top up the column with D-PBS and do not allow the column to run dry.
6. Continue to collect 500 µL fractions until desired fractions have been collected.
   NOTE: EVs typically elute from fractions 7 - 10. Proteins elute from fraction 11 onwards. Elution profile may vary with sample types. Retain all fractions for content analysis.
7. To wash the column, top up the column with D-PBS and allow a total of 20 mL of D-PBS to flow through. Discard flowthrough.
8. Add 1 mL of D-PBS to the column and close the cap on the top and bottom of the column. This will ensure that the gel matrix remains wet.
   NOTE: Store column at 2 - 8°C; each column can be used up to 5 times. SEC columns are not considered sterile.

Data



(A) The 2 mL Extracellular Vesicle SEC Column was loaded with 2 mL of human plasma. 500 µL fractions were collected and analyzed for particle and protein content by nanoparticle tracking analysis (NTA) and bicinchoninic acid (BCA) assays, respectively. EVs were detected in fractions 7 - 10, while proteins were detected in fractions 11 onwards.

(B) The 2 mL Extracellular Vesicle SEC Column was loaded with 2 mL of 10-fold concentrated serum-free medium conditioned by mesenchymal stem cell culture. 500 µL fractions were collected and analyzed for particle and protein content by NTA and BCA assays, respectively. EVs were detected in fractions 7 - 10, while proteins were detected in fractions 14 onwards.
NOTE: Fractions 11 - 12, which are outside of the typical EV elution range, may still contain EVs with low protein contamination for serum-free media samples.

Related Products

For related products, including EasySep™ Human Extracellular Vesicle Positive Selection Kits and antibodies, visit www.stemcell.com or contact us at techsupport@stemcell.com.

References

1. Baranyai T et al. (2015) Isolation of exosomes from blood plasma: qualitative and quantitative comparison of ultracentrifugation and size exclusion chromatography methods. PLoS One 10(12): e0145686.
2. Gámez-Valero A et al. (2016) Size-exclusion chromatography-based isolation minimally alters extracellular vesicles' characteristics compared to precipitating agents. Sci Rep 6(1): 33641.
3. Vallabhajosyula P et al. (2017) Tissue-specific exosome biomarkers for noninvasively monitoring immunologic rejection of transplanted tissue. J Clin Invest 127(4): 1375–91.