SmartSEC™ Single for EV Isolation

Cat # SSEC200A-1

User Manual

Store Kits at +4°C - +30°C upon receipt

Version 1
5/8/2020

Contents

Product Description

Get outstanding SEC-based EV isolation with SmartSEC™ Single

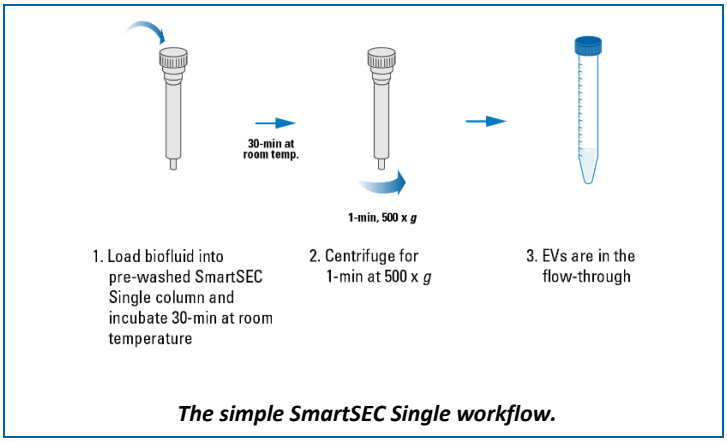
SmartSEC Single uses the same proprietary chromatography-based extracellular vesicle (EV) isolation technology as our popular SmartSEC HT plate and SmartSEC Mini kit but in a single tube format. SmartSEC technology combines all the benefits of size exclusion chromatography (SEC)—purity, yield, reproducibility, and preservation of EV integrity—with a contaminant trapping feature that enhances the capabilities of conventional SEC. The result is best-in-class EV isolation that is fast, easy, and clean.

- Quick and easy isolation
- Better purity and yield than ultracentrifugation
- Higher EV concentration per fraction than competitors' conventional size exclusion chromatography kits
- Validated for human serum, plasma, and CSF as well as *Aplysia californica* hemolymph
- Compatible with most downstream applications such as mass spectrometry, western blotting, nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM) and RNA-sequencing (NGS)

Each SmartSEC Single Kit comes with sufficient individual SmartSEC columns and buffer to process 10 samples.

The SmartSEC Single workflow is complete in as little as 30 minutes

Simply apply 100 – 250 µL of cleared serum or plasma with additional column buffer or up to 4 mL of other biofluids directly to the pre-washed column, incubate, and centrifuge to elute the EVs.



The simple SmartSEC Single workflow.

List of Components

Component	Qty/Volume	Storage Temperature
SmartSEC Single column	10 columns	+4°C - +30°C
Column buffer	50 mL	

The kit is shipped at +4^{0}C - +30^{0}C and should be stored at +4^{0}C - +30^{0}C. Properly stored kits are stable for 9 months from the date received.

Protocol

The protocol is optimized for 100 µl – 250 µl of serum/plasma samples.

Since serum/plasma samples contain large amounts of blood related proteins (Albumin, IgG, globulins and etc.), we don't recommend to apply more than 250 µl of the sample, particular if your downstream application is mass spectrometry analysis or TEM imaging.

If your biofluid is not serum or plasma you can consider increasing the volume of the starting material up to 4 mL (for example CSF, Urine).

Sample preparation:

1. Collect the biofluid and centrifuge at 3,000 × g for 15 minutes to remove cellular debris.
2. To remove large vesicles differential centrifugation step at 10,000-12,000 × g for 15 minutes is optional.

EVs isolation:

1. Take **SmartSEC Single column** (resin contained in storage buffer) and remove the cap and the bottom closure.
   ! **CAUTION:** save the cap and the bottom closure for later steps.
2. Place the column in an empty **Collection tube**.
3. Centrifuge at 500 xg for 30 sec to remove storage buffer.
4. Add 1 mL of **Column buffer** to the column.
5. Centrifuge at 500 xg for 30 sec to wash the beads.
6. Discard the collection tube.
7. The column is ready to your sample.
8. Place **bottom closure** back on the column and apply your sample on top of the resin bed.

9. Add additional amount of PBS to the column to bring the total sample volume up to 500 ul (for example: for 250 µl of starting sample applied, add 250 µl of 1xPBS or column buffer. For 500 ul of starting sample applied, no additional 1xPBS or column buffer needed) and close the column with the cap.
10. Incubate at RT for 20 -30 min with rotation.
11. Have a sample collection tube of your choice ready for EVs collection (not provided).
12. Loosen the cap half way and remove the bottom closure.

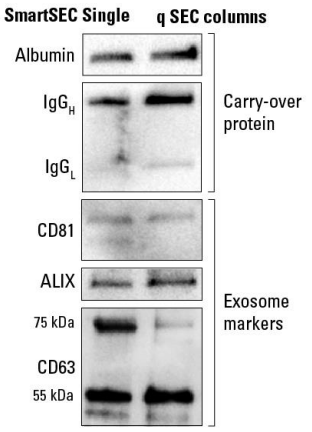
    ! CAUTION: if the bottom closure is removed first, the sample will start leaking out from the column. In addition, we strongly recommend keeping the cap half way inserted into the column due to the residual liquid that might still be present in the cap.
13. Place the column in the sample collection tube (step 11) and centrifuge at 500 xg for 30 sec to collect EVs.
14. Store your EVs for immediate use at +4°C up to one week. For long term storage store isolated EVs at -20°C or -80°C.

Example Data and Applications

SmartSEC Single performs better than competitor's q SEC technology

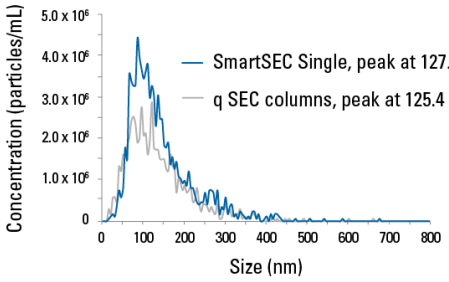
To understand how well SmartSEC Single performs compared to a competitor's q SEC columns, we isolated EVs from 250 µL of human serum using both SmartSEC Single and q SEC columns and analyzed 1 µg of protein equivalent from each isolation method on a western blot (Figure 1) and using fluorescent nanoparticle tracking analysis (fNTA, Figure 2). EVs isolated using SmartSEC Single show lower levels of carry-over proteins such as albumin and IgGs (Figure 1) and a higher number of particles per mg of protein (Figure 2) than EVs isolated using the q SEC columns, demonstrating the higher purity delivered by SmartSEC Single.

In addition, SmartSEC Single delivered EVs at ~6-fold higher concentration than the q SEC columns (Figure 1), for overall superior performance.



	Serum	
Isolation method	SmartSEC Single	q SEC columns
Concentration (µg/µL)	0.40	0.06
Volume (µL)	750	1,500
Total yield (µg)	300	90

Figure 1. Western blot analysis shows that SmartSEC Single delivers higher yields of cleaner EVs than a competitor's q SEC columns.

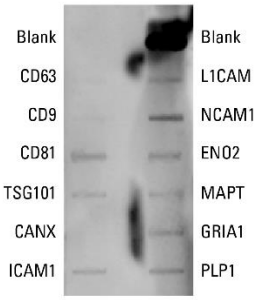


	Serum	
Isolation method	SmartSEC Single	q SEC columns
Concentration (particles/mL)	9.3 x 10^{10}	6.7 x 10^9
Yield (particles)	6.9 x 10^{10}	1.0 x 10^{10}
Purity (particles/mg)	23.3 x 10^{10}	11.0 x 10^9

Figure 2. fNTA show that SmartSEC Single delivers higher yields of cleaner EVs than a competitor's q SEC column.

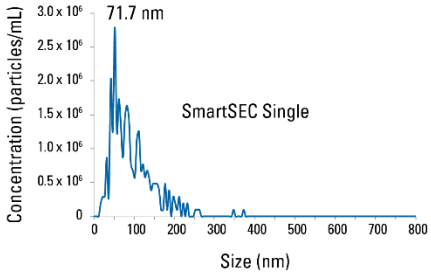
SmartSEC Single cleanly isolates EVs from CSF

SmartSEC Single can isolate EVs from cerebrospinal fluid (CSF) with no detectable cellular contamination, as shown in the ExoCheck Neuro Array in Figure 3. The isolated EVs possess both neural and exosomal markers, suggesting that the EV prep is enriched for vesicles of neural origin. Interestingly, EVs isolated from CSF tend to be smaller in size than EVs isolated from serum (Figure 4).



	CSF
Isolation method	SmartSEC Single
Concentration (µg/µL)	0.08
Volume (µL)	750
Total yield (µg)	60

Figure 3. SmartSEC Single isolates neuronal EVs from CSF.



	CSF
Isolation method	SmartSEC Single
Concentration (particles/mL)	9.3×10^{10}
Yield (particles)	6.9×10^{10}
Purity (particles/mg)	23.3×10^{10}

Figure 4. EVs isolated from CSF by SmartSEC Singles are smaller than EVs isolated from serum.

EVs isolated using SmartSEC Single possess typical EV morphology

Transmission electron microscopy (TEM) of EVs isolated from serum using SmartSEC Single possess typical EV morphology—intact vesicles with lipid bi-layer membranes—with little visible background debris (Figure 5).

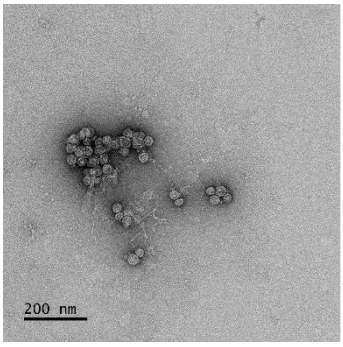


Figure 5. EVs isolated using SmartSEC Single possess typical EV morphology.

EVs isolated with SmartSEC Single and a competitor's q SEC columns have very similar proteomic profiles

We analyzed EVs isolated from human serum using SmartSEC Single and a competitor's q SEC columns using mass spectrometry. Both methods yielded very similar proteomes, with 86% overlap (Figure 6).

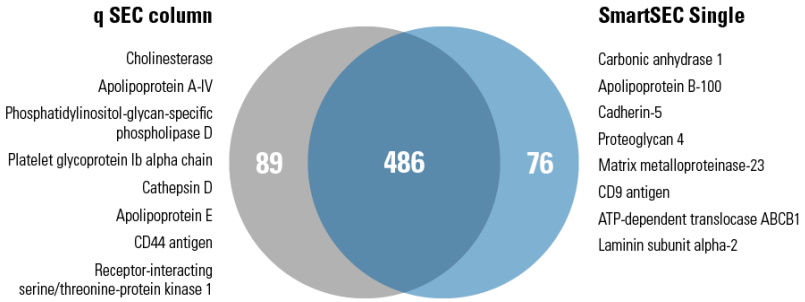


Figure 6. EVs isolated with SmartSEC Single and a competitor's q SEC columns have very similar proteomic profiles.

SmartSEC Single can be used for downstream proteomic studies

To demonstrate the versatility and power of SmartSEC Single for enabling EV research, we used SmartSEC Single technology to isolate EVs from normal serum and serum from prostate cancer patients and used mass spectrometry to analyze the respective proteomes. A selection of identified proteins is shown in the table below, and the full list can be downloaded from **https://www.systembio.com/wp-content/uploads/SmartSEC-Single-mass-spec-data.xlsx**.

	Accession number	Protein Description	Peptide Count	
			Serum from healthy donors	Serum from donors with prostate cancer
1	P04275	von Willebrand factor; vWF; Contains: von Willebrand antigen 2; von Willebrand antigen II; Flags: Precursor;	1	259
2	A2NUT2	Lambda-chain (AA -20 to 215) {ECO:0000313|EMBL:CAA32725.1}; Flags: ...	209	110
3	B2RMS9	Inter-alpha (Globulin) inhibitor H4 (Plasma Kallikrein-sensitive ...	25	206
4	Q08380	Galectin-3-binding protein; Basement membrane autoantigen p105; ...	144	135
5	O43866	CD5 antigen-like; Apoptosis inhibitor expressed by macrophages ...	183	73
6	P07225	Vitamin K-dependent protein S; Flags: Precursor;	3	258
7	P00450	Ceruloplasmin; EC 1.16.3.1; Ferroxidase; Flags: Precursor;	91	110
8	Q1HP67	Lipoprotein, Lp(A) {ECO:0000313|EMBL:ABF47086.1};	1	124
9	P02790	Hemopexin; Beta-1B-glycoprotein; Flags: Precursor;	63	23
10	P07996	Thrombospondin-1; Glycoprotein G {ECO:0000303|PubMed:6777381}; ...		61

Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site: **http://www.systembio.com**

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