SmartSEC® HT EV Isolation System for Serum & Plasma

Cat # SSEC096A-1, SSEC008A-SAM

User Manual

Storage: Store at +4°C to +30°C

Version 1
7/16/2019

Contents

Product Description

Researchers interested in developing extracellular vesicles (EVs) for therapeutic and diagnostic use have been hampered in their efforts by the lack of a ready-to-use EV isolation method that is truly high throughput. SBI is proud to be the first company to help researchers overcome this challenge with the SmartSEC™ HT EV Isolation System for Serum & Plasma (Cat.# SSEC096A-1), a proprietary chromatography-based EV isolation method that comes in a 96-well plate format.

SmartSEC HT delivers EV isolation that combines all the benefits of size exclusion chromatography (SEC)—purity, yield, reproducibility, and preservation of EV integrity—with a contaminant trapping feature that overcomes the limitations of conventional SEC for a fast, easy, and high-throughput workflow (Figure 1). Simply prep the filter plate, apply 250 – 500 µL of cleared serum or plasma directly to each well, incubate, and centrifuge to elute the first fraction. Add an equal volume of SmartSEC Isolation Buffer and centrifuge again into a second collection plate to elute the second fraction. Depending on the volume of sample loaded, the majority of EVs will be collected in either the first or second elution (we recommend collecting the two fractions separately for analysis and choosing to pool or not depending on your needs).

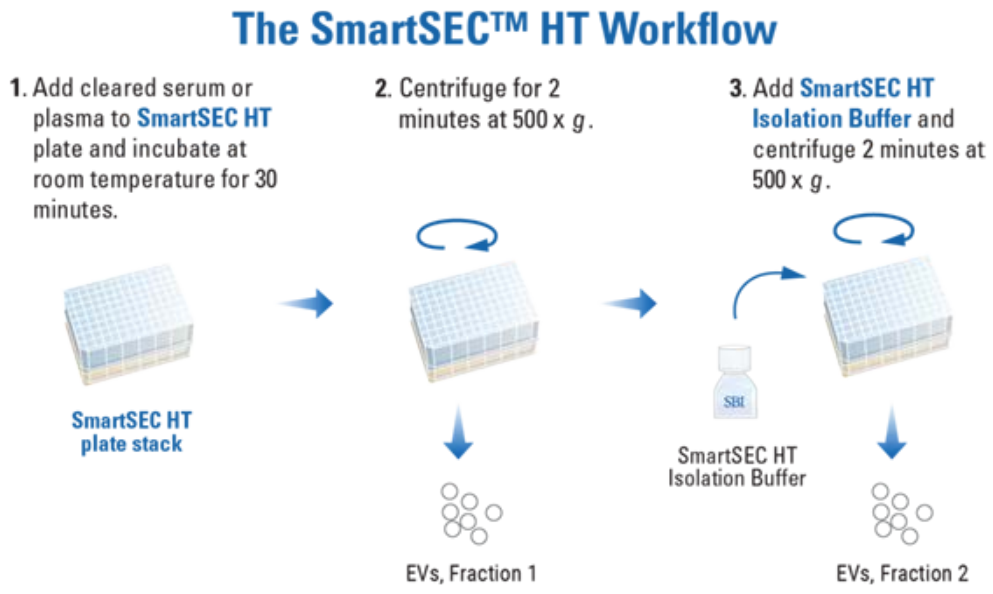


Figure 1. The SmartSEC HT workflow is quick and easy.

Each SmartSEC HT kit comes pre-filled with optimized amounts of SmartSEC resin in a 96-well filter plate, SmartSEC Isolation Buffer, and 2 collection plates. Each well of the filter plate can be loaded with 250 – 500 µL of serum or plasma, and the entire SmartSEC HT System is compatible with manual and automated liquid handling systems (please see requirements below).

List of Components

Component	Qty/Volume	Storage Temperature
SmartSEC HT Isolation plate* (127 x 85 x 45 mm)	1 x 96-well plate	4°C to 30°C
SmartSEC Isolation Buffer	250 mL	4°C to 30°C
SmartSEC HT Collection plate* (127 x 85 x 31 mm)	2 x 96-well plates	4°C to 30°C

*Our filter and collection plates are designed for both manual and automated liquid handling instruments within the ANSI/SBS standard.

Additional required and optional equipment not included in kit

- Centrifuge: use a swing-out rotor with microplate carriers capable of handling the SmartSEC HT plate stack (at least 75 mm high clearance).
- Multichannel pipette: use an 8 or 12 multi-channel pipette for quick and easy pipetting of liquids into the SmartSEC plate. **Tip**: it is recommended to use dispensing liquid function.
- Automated liquid handler system: any system within the ANSI/SBS standard can be used [optional]

SmartSEC HT Isolation Plate Specifications

Feature	Specification
Isolation plate	127 x 85 x 45 mm
Number of wells	96
Well capacity	2 mL
Working sample volume/well	250-500 µL
Volume of resin slurry/well	1.8 mL
Storage buffer	20% Ethanol
Recommended storage temperature	4°C - 30°C
Working temperature	Room temperature
Centrifugation force (max)	300-500 x g (max 700 x *g)*

Storage

The SmartSEC HT System is shipped at room temperature and should be stored between 4°C to 30°C. Properly stored kits are stable for 12 months from the date received.

General Considerations

Important considerations when using the SmartSEC HT System:

- **Droplets on the bottom sealing mat do not impair plate performance**
  After shipping and storage, some of the storage solution may be seen as droplets on the surface of the bottom seal mat when removed. This is not leakage and plate can be used.

- **Open the SmartSEC HT plate using the instructions below**
  Failure to open the SmartSEC HT plate using the instructions below may result in resin remaining attached to the top sealing mat.

- **Not all wells of the SmartSEC HT plate need to be used at once**
  See the instructions on how to open the SmartSEC HT plate below for more details on preserving unused wells for future studies.

- **Avoid leaks**
  Always keep the SmartSEC HT plate over a collection plate when in use and/or seal the bottom of the plate under any unused wells. Make sure the bottom of the SmartSEC HT plate is flush with the top of the collection plate—if necessary, use the provided stickers to fasten the two plates together.

- **Avoid contamination**
  Keeping the SmartSEC HT plate over a collection plate and/or sealing the bottom of the plate under any unused wells will help keep your samples free from contamination. Never place the SmartSEC HT plate directly on your lab bench or other surface.

- **Reduce evaporation**
  The SmartSEC HT protocol includes a 30-minute incubation. To reduce evaporation, be sure to keep the top of your plate sealed during the incubation step.

Protocol

! NOTE: When processing fewer than 96 samples, the un-used wells on the plate can be preserved for future experiments by doing either one of the following:

1- Cut both seal mats (top and bottom) with a razor blade in between the used and un-used section, remove the mats only for the area intended for samples, and keep the un-used section properly sealed at all times during processing of the samples. The un-used section can be saved for future sample run(s).

2- Process the entire plate according to the protocol below; fill "empty" (un-used) wells with buffer for all steps described. At the end: seal the bottom and add enough buffer to the un-used wells to keep the entire resin bed submerged, and put the top seal mat back on to prevent evaporation. The un-used section can be saved for future sample run(s).

A. Sample preparation

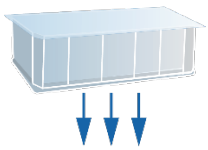
1. For each well to be used, thaw 250-500 µl of serum/plasma on ice.
2. Centrifuge at 3,000 × g for 15 minutes to remove cellular debris. Transfer the supernatant to a new tube.
3. To remove very large vesicles (such as apoptotic bodies) centrifuge the supernatant for 10-15 additional minutes at >=12,000 x g and transfer the supernatant to a new tube.

B. Opening and preparing the SmartSEC HT plate

! CAUTION: remove the plastic wrap and make sure the top and bottom mats are securely placed and the sample plate is properly sealed.

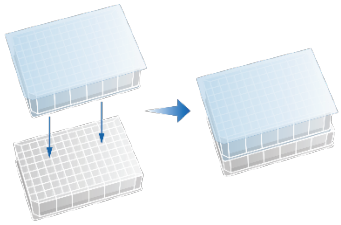
1. Mix the resins in the wells by inverting the SmartSEC HT plate a few times as showed in the picture below:

2. Next, tap the bottom of the SmartSEC HT plate on a flat surface gently to return resins to the bottom:

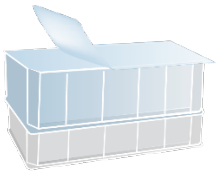


3. Hold the SmartSEC HT plate horizontally and remove the bottom mat:

4. Make the plate stack by placing the sample plate over a collection plate.



5. Remove the top mat from the sample plate.



6. Centrifuge the plate stack for 1 minute at 500 x g to remove storage buffer. Discard the storage buffer and re-assemble the plate stack.

   ! NOTE: If desired, use the provided stickers to tape the plate stack together to minimize movement during centrifugation.

7. Add 500 µl of SmartSEC Isolation Buffer/well to wash the resin.
8. Centrifuge the plate stack for 1 minute at 500 x g to remove the wash buffer

   ! NOTE: If any visible buffer remains in the wells, spin at 700 x *g* for 1 minute.

9. Discard the wash buffer from the collection plate and re-assemble the plate stack. Your SmartSEC HT plate is now ready for samples.

C. Isolating EVs from serum or plasma

1. Apply 250 - 500 µL of serum or plasma per well. Place the top mat on the SmartSEC HT plate to seal it and then incubate for 30 minutes at room temperature.
2. Collect the first fraction of purified EVs by centrifuging the plate stack for 2 minutes at 500 x g.
3. Collect the second fraction of purified EVs by removing and placing the sample plate over a new collection plate, add equal volume of SmartSEC buffer as the starting serum/plasma to each well, followed by centrifuging the stack for 2 min at 500 x g.
4. Disassemble the stack and discard the sample plate (top); the EVs collected in both fractions are now ready to use for your downstream applications.

! NOTE: Distribution of EV concentration and purity may vary in each fraction depending on the initial volume of starting biofluid. We recommend analyzing the two fractions separately before pooling them.

D. Sample storage

1. For short term storage: seal EV containing collection plates with the provided self-adhesive plate sealers and store at 4°C.

   *We recommend using the purified EVs within one week of isolation.

2. For long term storage: seal EV containing collection plates with the provided self-adhesive plate sealers and store at -20°C.

Example Data and Applications

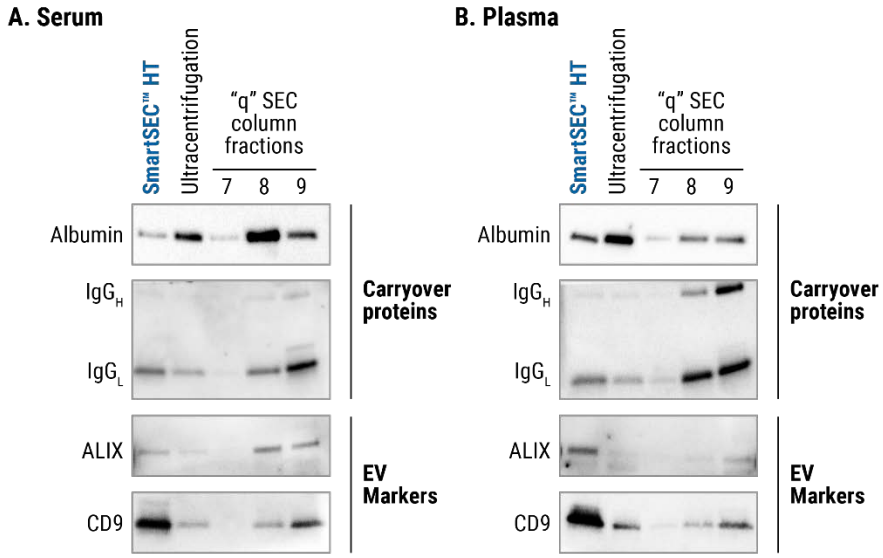


Figure 2. SmartSEC™ HT delivers high yields of EVs with low amounts of contaminating protein*. EVs were prepared from 500 µL of serum (A) or plasma (B) using the indicated methods. For Western blot analysis, 1 µg protein equivalent from the first fraction was loaded into each lane. SmartSEC™ HT performs better than ultracentrifugation and a competitor's "q" SEC column.*

The yield and purity of EVs isolated from serum and plasma using SmartSEC™ HT, ultracentrifugation, and a commercial "q" SEC column are compared in Figure 2, example elution profiles shown in Figure 3, and additional information on yield shown in Table 1. SmartSEC™ HT delivers high yields of clean EVs, performing better than both ultracentrifugation and a competitor's "q" SEC column.

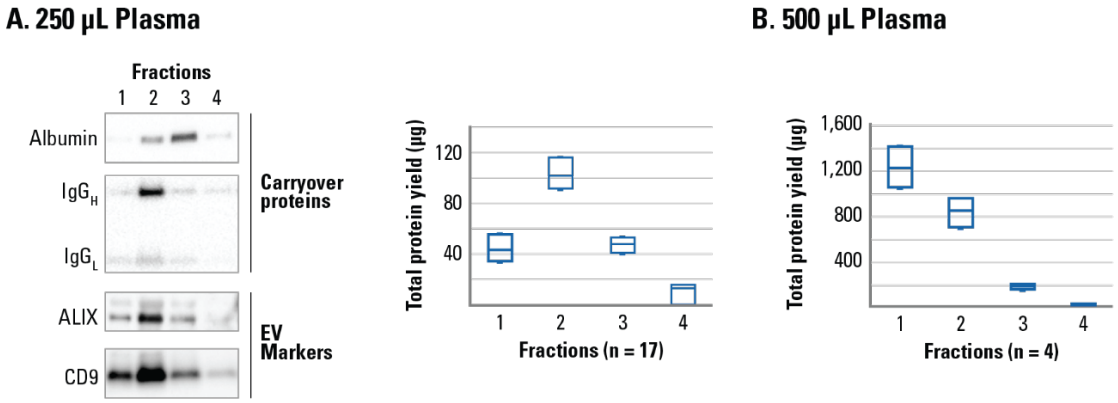


Figure 3. The majority of EVs elute in the first two fractions. *As is typical for SEC, the elution profile is dependent on the input sample and eluent volumes. For 250 µL of input plasma (A), fraction 2 contains the most EVs whereas for 500 µL of input plasma (B), fraction 1 contains the most EVs. When first using SmartSEC HT, we recommend isolating fractions 1 and 2 separately, analyzing, and then choosing to pool or not depending on your needs.*

Table 1. Comparison of EV yields when using SmartSEC HT, ultracentrifugation, and a competitor's "q" SEC column

Sample	Sample volume (µL)	EV isolation method	EV yield (µg protein)*	EV yield (# of particles)**
Serum	250 µL	SmartSEC HT	239	1.48×10^{11}
		Ultracentrifugation	4.34	0.08×10^{11}
		Competitor's "q" SEC column***	101.6	0.44×10^{11}
	500 µL	SmartSEC HT	1,220	2.50×10^{11}
		Ultracentrifugation	5.4	0.05×10^{11}
		Competitor's "q" SEC column***	152.5	0.72×10^{11}
Plasma	250 µL	SmartSEC HT	159	2.62×10^{11}
		Ultracentrifugation	3.77	0.014×10^{11}
		Competitor's "q" SEC column***	76	0.80×10^{11}
	500 µL	SmartSEC HT	2,026	3.40×10^{11}
		Ultracentrifugation	4.4	0.03×10^{11}
		Competitor's "q" SEC column***	112	0.277×10^{11}

*EV yield determined using a Qubit protein assay
**EV yield determined using fNTA
***Values provided for Competitor's "q" SEC column are for pooled fractions 7, 8, and 9.

Technical Support

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

For additional information or technical assistance, please call or email us at:

System Biosciences (SBI)
2438 Embarcadero Way
Palo Alto, CA 94303

Phone: (650) 968-2200
Toll-Free: (888) 266-5066
Fax (650) 968-2277

E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

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Use of SmartSEC HT EV Isolation System for Serum & Plasma (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

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- The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.
- The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.
- This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

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SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This

limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

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System Biosciences (SBI)
2438 Embarcadero Way
Palo Alto, CA 94303

Phone: (650) 968-2200
Toll-Free: (888) 266-5066
Fax: (650) 968-2277

E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com