SmartSEC™ Mini EV Isolation System

Cat # SSEC100A-1

User Manual

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

Version 1
2/1/2020

Contents

Product Description

Explore new frontiers in extracellular vesicle (EV) biology

Whether it's precious clinical samples or hard-to-collect biofluids from developmental biology model organisms, researchers are increasingly challenged by the need to isolate EVs from quite limited sample volumes. To support these researchers, SBI has configured our powerful SmartSEC™ technology into the first commercially available kit optimized for isolating EVs from 10 – 100 µL of biofluid (Table 1). Validated for samples from Drosophila, Planaria, and Arabidopsis, as well as human, mouse, and rat, the SmartSEC™ Mini EV Isolation System is ready to help you push the boundaries of EV biology.

Table 1. SmartSEC Mini recommended input volumes

ORGANISM	BIOFLUID	INPUT VOLUME (µL)
Drosophila melanogaster	Hemolymph	10
Planaria (neoblasts)	Cell culture	100
Arabidopsis thaliana	Apoplastic fluid	50
Mouse, rat, human	Serum/plasma	10 - 25

SmartSEC Mini is a proprietary chromatography-based EV isolation technology 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 fast, easy, and low sample-volume EV isolation.

- The only commercially available EV isolation kit for sample volumes as low as 10 µL
- Great for isolating EVs from a range of biofluids
- Validated in samples from human as well as model developmental biology organisms such as:
    - *Drosophila melanogaster*
    - Planaria
    - *Arabidopsis thaliana*
    - Mouse
    - Rat
- Delivers better purity and yield than ultracentrifugation
- Compatible with most downstream applications such as mass spectrometry, western blotting, nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM)

Each SmartSEC Mini kit comes with pre-loaded SmartSEC Mini columns, collection tubes, and sufficient column buffer for processing 10 samples (see Table 1 for loading volumes).

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

The SmartSEC Mini workflow is quick and easy (Figure 1). Simply pre-wash the SmartSEC Mini column, load your sample, incubate at room temperature for 30-minutes, and centrifuge for 30-seconds at 500 x g to recover isolated EVs. To maximize sample recovery, simply add more buffer to the column and collect up to two additional fractions.

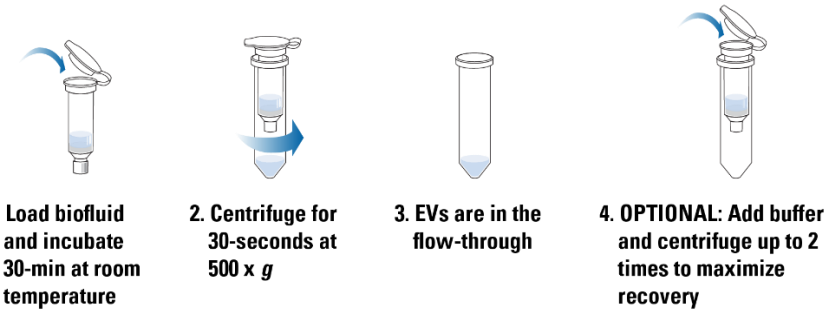

Figure 2. The simple SmartSEC Mini workflow.

List of Components

Component	Qty/Volume	Storage Temperature
SmartSEC Mini column	10 columns	+4°C - +30°C
Collection tubes	10 tubes	
SmartSEC Mini Isolation buffer	10 mL	

Additional Required and Optional Equipment Not Included in Kit

- 1.5 ml non-stick Eppendorf tube(s)
- Rotating platform/mixer
- microcentrifuge

Protocol

NOTE: This protocol has been optimized for the biofluids shown in Table 1.

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 at 10,000-12,000 × g for 15 minutes is optional.
3. Before proceeding to EV isolation step adjust sample volume with **Isolation buffer** up to 100 µl.

EVs isolation:

1. Take **SmartSEC mini column** (resins contained in storage buffer). Loosen the cap (do not remove it from the column) and snap off the bottom closure.

   ! CAUTION: save bottom closure for later steps.

2. Place the column into empty **Collection tube**.
3. Centrifuge at 500 xg for 30 sec to remove storage buffer.
4. Remove the cap and add 200 µl of **Column buffer** to the column.

   ! CAUTION: save bottom closure for later steps.

5. Centrifuge at 500 xg for 30 sec to wash the beads.
6. Discard the collection tube.
7. Place bottom closure back on the column and apply your sample on top of the resin bed.
8. Put the cap back on; place the tube on a rotating platform/mixer and allow to incubate at RT for 30 min with constant mixing.
9. Remove the cap and bottom closure, and place the column in a new 1.5 ml eppendorf tube (not provided) Centrifuge at 500 xg for 30 sec to collect first fraction of EVs (F1).
10. Add 100 µl of **Column buffer** to the column.
11. Place the column in a new 1.5 ml eppendorf tube (not provided) and centrifuge at 500 xg for 30 sec to collect second fraction of EVs (F2).
12. To maximize EV recovery, repeat steps 10 – 11 to collect third fraction of EVs (F3).
13. Analyze all the fractions separately before pooling them together, if desired. Majority of the EVs will be concentrated in the first two fractions.

Example Data and Applications

See how well SmartSEC Mini isolates EVs from low volumes in a range of species, biofluids

SmartSEC Mini provides excellent purification of EVs from as little as 10 µL of hemolymph from *Drosophila melanogaster*, as demonstrated by western blot analysis of 1 µg of equivalent protein (Figure 2). Common Drosophila exosome markers are shown next to molecular weight markers.

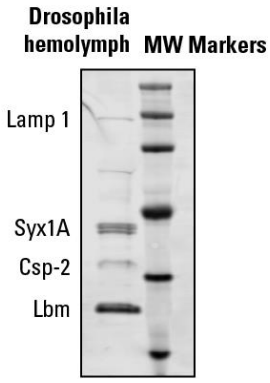

Figure 2. SmartSEC Mini provides robust isolation of EVs from Drosophila starting from 10 µL of hemolymph. *Data courtesy of Dr. Karen Linnemannstöns, University of Göttingen.*

SmartSEC Mini has also been used to isolate EVs from 100 µL Planaria neoblast culture media (Figure 3) and 50 µL of apoplastic fluid from *Arabidopsis thaliana* (Figure 4) as seen in TEM.

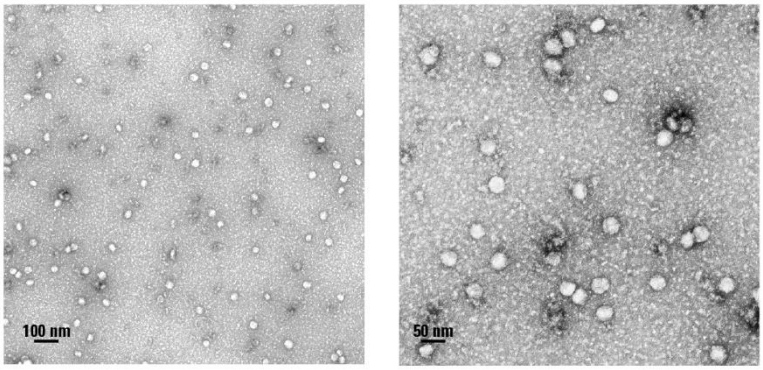

Figure 3. SmartSEC Mini provides robust isolation of EVs from 100 µL of Planaria neoblast culture media. *Data courtesy of Dr. Vidyanand Sasidharan, Stowers Institute for Medical Research.*

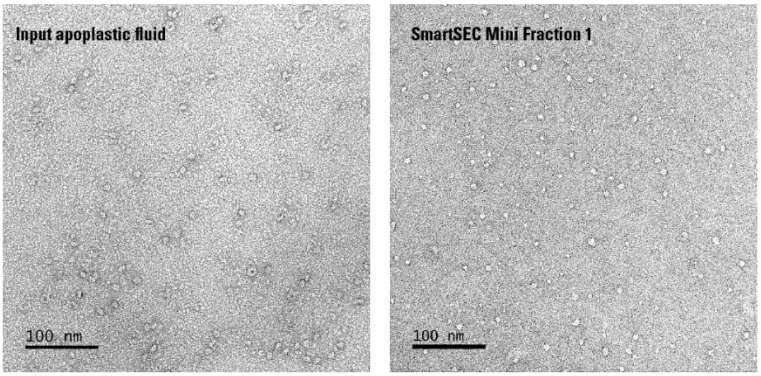

Figure 4. SmartSEC Mini provides robust isolation of EVs from 50 µL of apoplastic fluid from Arabidopsis thaliana. *Sample courtesy of Dr. Claudia Uhde-Stone, California State University, East Bay.*

SmartSEC Mini provides robust isolation of EVs from 10 µL of mouse serum (Figure 5A, Table 2) and 10 µL of human serum (Figure 5B, Table 2) as demonstrated through western blot data. In addition, SmartSEC Mini delivers more EVs than a competitor's SEC-based EV isolation product (Table 3).

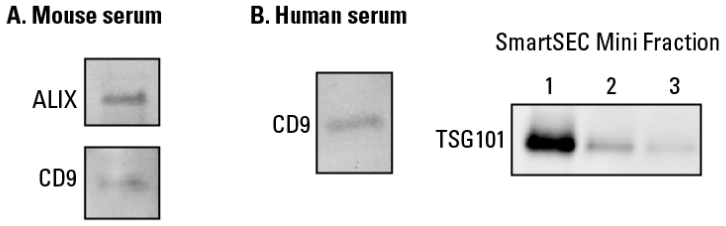

Figure 5. SmartSEC Mini delivers robust isolation of EVs from human and mouse serum.

Table 2. SmartSEC Mini delivers high yields of EVs

	Total yield (µg)	Concentration (particles/mL)	Purity (particles/µg)
Mouse serum	33.2	2.7×10^9	1.6×10^7
Human serum	25	2.95×10^9	4.7×10^7

Table 3. SmartSEC Mini delivers more EVs than a competitor's product

		Total yield (µg)
SmartSEC Mini	Fraction 1	128.0
	Fraction 2	40.8
q SEC Columns	Fraction 1	10.2
	Fraction 2	15.1
	Fraction 3	14.1
	Fraction 4	19.4
	Fraction 5	37.2

SmartSEC Mini specifically enriches for EVs versus lipoprotein particles and blood microparticles as demonstrated via mass spectrometry (Figure 6 and Table 4). We used the quick and easy SmartSEC Mini workflow to isolate EVs from 20 µL of human serum and analyzed the EV-associated proteins via mass spec. Of the ~600 proteins we identified (see Table 4 for a representative list), a functional enrichment analysis conducted using the FunRich tool

shows that over 50% of the proteins are known to be associated with exosomes whereas <30% are associated with blood microparticles and <10% are associated with lipoprotein particles (Figure 6).

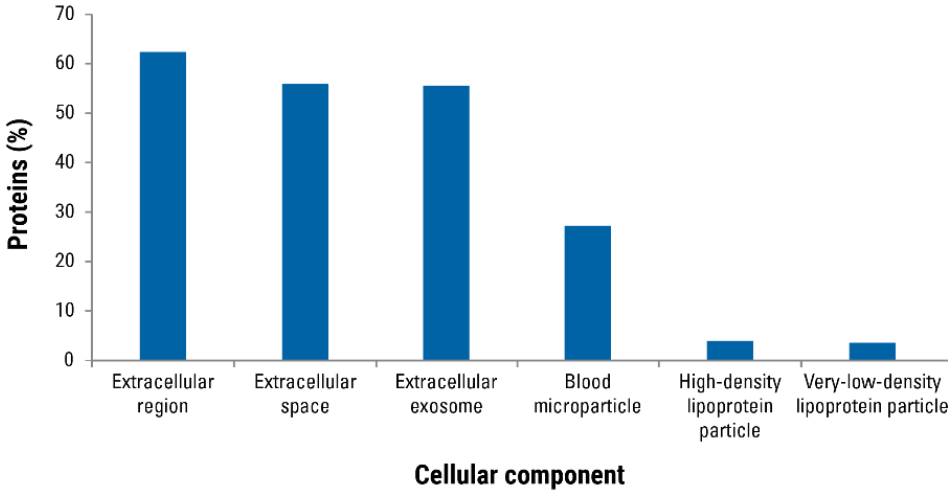

Figure 6. Mass spectrometry analysis demonstrates that SmartSEC Mini preferentially isolates EVs.

Table 4. Common EV-associated proteins identified from mass spec analysis of EVs isolated with SmartSEC Mini

UniProt accession number	Description
P60709	Actin, cytoplasmic 1; Beta-actin
P02765	Alpha-2-HS-glycoprotein
P62805	Histone H4
P26927	Hepatocyte growth factor activator
B4DY90	Tubulin beta chain
P00338	L-lactate dehydrogenase A chain
P08183	ATP-dependent translocase
P80108	Phosphatidylinositol-glycan-specific phospholipase D
P55058	Phospholipid transfer protein
P21926	CD9 antigen
P11597	Cholesteryl ester transfer protein
P07355	Annexin A2
P08238	Heat shock protein HSP 90-beta; HSP 90
P08962	CD63 antigen

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

Licensing and Warranty Statement

Limited Use License

Use of the SmartSEC Mini EV Isolation System (*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.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

- 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.

This Product is covered by licensed Patents. For information concerning licenses for commercial use, contact SBI.

Purchase of the product does not grant any rights or license for use other than those explicitly listed in this Licensing and Warranty Statement. Use of the Product for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. SBI disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Product in accordance with the terms and conditions outlined herein.

Limited Warranty

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.

SBI's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.

This page intentionally left blank.

This page intentionally left blank.

This page intentionally left blank.

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