System Biosciences

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SmartSEC-DeLipo™ Advanced sEV Isolation Kit

Cat # SSEC-DLP-200A, DLP20A

User Manual

Storage:
The kit should be stored at +4°C - +30°C.

Version 2
5/9/2025

Contents

Product Description

Purer EVs with Less Lipoproteins

Elevate your small extracellular vesicle (sEV) isolation game with our cutting-edge SmartSEC-DeLipo™ kit, engineered to provide unmatched purity and quality while efficiently depleting lipoprotein contaminants. Harnessing the power of SmartSEC™ coupled with innovative lipoprotein removal technology, this kit streamlines the sEV isolation process, ensuring pure sEVs samples for downstream analyses.

- New generation of mixed-mode chromatography
- Advanced Lipoprotein Depletion
- Fast and Easy Isolation of High Yield and Purity sEV
- Versatile Compatibility with Various Applications

Importance of Removing Lipoproteins from sEV

Extracellular vesicles (EVs) from blood are of great importance to understand the biological role of circulating EVs and to develop EVs as biomarkers of disease. However, isolating EVs from blood poses challenges due to the simultaneous presence of lipoprotein particles. Lipoproteins may interfere with downstream analyses or functional assays performed on EVs. For instance, lipoproteins contain microRNAs as their cargos. In studies investigating the biological roles of EVs or their potential as biomarkers, the presence of lipoproteins could confound the results or mask the true effects of EVs. Recently, several studies showed that ultracentrifugation or size exclusion chromatography (SEC) alone cannot completely separate blood EVs from lipoprotein particles. Therefore, improved separation of EVs from lipoproteins is crucial for a detailed functional analysis of circulating EVs, thus making blood a viable source for EV biomarker discovery.

Advanced sEV Isolation with SmartSEC-DeLipo

Our cutting-edge SmartSEC-DeLipo™ kit was engineered to provide unmatched purity and quality while efficiently depleting lipoprotein contaminants for sEV isolation. Harnessing the power of SmartSEC coupled with innovative lipoprotein removal technology, this kit streamlines the sEV isolation process, ensuring much purer sEVs samples for downstream analyses.

New generation of mixed-mode chromatography: Our SmartSEC-Delipo™ Kit utilizes SmartSEC™ columns, a new generation of mixed-mode chromatography, which provides the dual functionality of size exclusion and affinity interaction modes. Combining the two modes within a single resin media eliminates the time consuming and labor-intensive processes of classical SEC, delivering highly purified sEV samples.

Advanced Lipoprotein Depletion: Selectively removes lipoproteins from the sample, eliminating interference and ensuring the isolation of pure sEV populations, experiencing a new standard of purity.

Efficient Workflow: With a user-friendly protocol designed for efficiency and reproducibility, researchers can easily integrate SmartSEC-DeLipo™ into their workflows, saving time and resources.

High Yield and Purity: Achieve consistently high yields of pure sEVs, free from lipoprotein contaminants, enabling robust downstream applications such as RNA sequencing, proteomics, and functional studies.

Versatile Compatibility: Compatible with various sample types containing lipoprotein including plasma, serum, breast milk et al, ensuring versatility and flexibility in experimental design.

Superior Performance: SmartSEC-DeLipo™ ensures consistent and reliable performance, empowering researchers with confidence in their results and facilitating reproducible experiments.

Various Applications: Ideal for a wide range of research applications including biomarker discovery, disease diagnostics, therapeutic development, and elucidating the role of sEVs in intercellular communication.

Experience the future of sEV isolation with SmartSEC-DeLipo™ Advanced sEV Isolation Kit. Unlock unparalleled purity, efficiency, and reliability, and accelerate your journey towards groundbreaking discoveries in the dynamic field of extracellular vesicle biology.

List of Components

Table 1. Components of SSEC-DLP-200A

Component Name	Quantity/Volume	Storage temperature
SmartSEC Single column	10 columns	+4°C - +30°C
DeLipo column	10 columns	+4°C - +30°C
Collection tubes	10 tubes	+4°C - +30°C
2 ml Eppendorf tubes	10 tubes	+4°C - +30°C
Column buffer	50 ml	+4°C - +30°C

Table 2. Components of DLP-20A

Component Name	Quantity/Volume	Storage temperature
DeLipo column	20 columns	+4°C - +30°C
Collection tubes	20 tubes	+4°C - +30°C
2 ml Eppendorf tubes	20 tubes	+4°C - +30°C
Column buffer	50 ml	+4°C - +30°C

The kits are shipped at +4°C - +30°C and should be stored at +4°C - +30°C. Properly stored kits are stable for 9 months from the date received.

How it works

The SmartSEC-DeLipo™ workflow is fast and easy. Simply apply 250 µL of cleared serum or plasma with additional column buffer or up to 0.5 mL of other biofluids directly to the pre-washed column, incubate, and centrifuge to elute the Evs then go through DeLipo column to remove lipoprotein from the isolated sEVs.

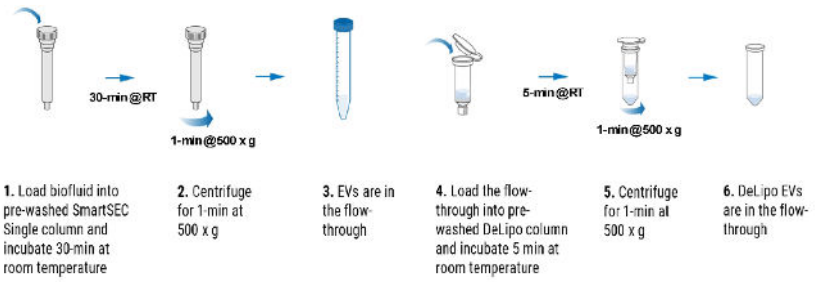

Figure 1: Workflow of SmartSEC-DeLipo™.

Protocol

A. General Comments

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).

B. 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.

Please Note:

For plasma sample, we recommend de-fibrinating plasma before sEV isolation for efficient recovery and high yields using Thrombin Plasma Prep (TMEXO-1)

For breast milk sample, centrifuge the sample at 2,000 x g for 10 min to remove fat globules, transfer supernatant to a new tube and centrifuge at 12,000 x g for 30 min, then filter the supernatant with 0.2um PVDF filter. Filtered sample is ready for sEV isolation.

C. sEV Isolation

1. Take SmartSEC Single column (resin contained in storage buffer) and remove the cap and the bottom closure.

   ! CAUTION: save the cap for later steps.

2. Place the column in an empty 15 ml centrifuge tube (not provided).
3. Centrifuge at 500 x g for 30 sec to remove storage buffer.
4. Add 1 mL of Column buffer to the column.
5. Centrifuge at 500 x g for 30 sec to wash the beads.
6. Discard the 15 ml centrifuge tube.
7. The column is ready to your sample.
8. Place bigger white bottom closure on the bottom of SmartSEC single 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 new 15 ml centrifuge tube as sample collection tube ready for sEVs collection (not provided).
12. Remove the bottom closure and immediately place the column in the sample collection tube (step 11) and loosen the cap half way.

! CAUTION: Once the bottom closure is removed, the sample will start leaking out from the column. Please place column in the sample collection tube immediately after removing the bottom closure. 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. Centrifuge at 500 x g for 30 sec to collect sEVs.
14. Isolated sEV is ready for DeLipo treatment

D. DeLipo Treatment

Protocol for DLP-20A starts from here

15. Take DeLipo column (resin contained in storage buffer) and remove the cap and the bottom closure.

! CAUTION: save the cap for later steps.

16. Place the column in an empty Collection tube (provided).
17. Centrifuge at 500 x g (or 2000 rpm) for 30 sec to remove storage buffer using benchtop centrifuge (Eppendorf).
18. Discard the flow-through and place the column back into the collection tube.
19. Add 0.5 mL of Column buffer to the column and centrifuge at 500 x g (or 2000 rpm) for 30 sec to wash the column.
20. Repeat steps 18 – 19 one more time to wash the column.
21. The DeLipo column is ready for your sample.
22. Plug the bottom of the DeLipo column with the smaller white bottom closure and apply isolated sEV sample (from step 14) on top of the resin bed and close the DeLipo column with the cap.
23. Incubate at RT for 5 min with rotation.
24. Have 2 ml Eppendorf tube as sample collection tube (provided) ready for sEVs collection.
25. Remove the bottom closure and immediately place the DeLipo column in the sample collection tube (step 24) and loosen the cap half way.

! CAUTION: Once the bottom closure is removed, the sample will start leaking out from the column. Please place column in the sample collection tube immediately after removing the bottom closure. 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.

26. Centrifuge at 500 x g (or 2000 rpm) for 30 sec to collect sEVs.
27. Store your sEVs for immediate. For long term storage store isolated sEV at -80°C. EV-GuardTM EV Storage Buffer (EXSBA-10) is recommend for store isolated sEV at different temperature.

Supporting Data

SmartSEC-DeLipo™ isolates much purer sEV

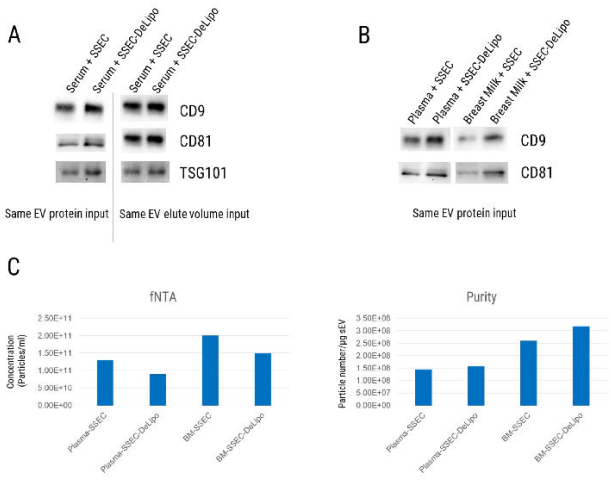

Figure 2. sEV isolated with SmartSEC-DeLipo™ is much purer. *(A) sEV isolated from 250ul of serum with SmartSEC-DeLipo™ shows higher expression of EV markers, such as CD9, CD81 and TSG101 than those isolated with SmartSEC™ single when same amount of EV protein were applied for western blot. When same volume of eluted sEV samples were applied for western blot, EV markers' expression is about the same for sEV isolated from 250ul of serum with SmartSEC-DeLipo™ or SmartSEC™ single. (B) sEV isolated from 250ul of plasma or breast milk with SmartSEC-DeLipo™ shows higher expression of EV markers, such as CD9, CD81 and TSG101 than those isolated with SmartSEC™ single when same amount of EV proteins was applied for western blot. (C) fNTA data shows that sEV particle number were decreased in plasma or breast milk sample when isolated with SmartSEC-DeLipo™ in comparison with that isolated with SmartSEC™ single. However, when purity was evaluated with EV particle number per same amount of EV protein, sEV isolated with SmartSEC-DeLipo™ demonstrates higher purity than those isolated with SmartSEC™ single.*

SmartSEC-DeLipo™ removes most of the lipoproteins

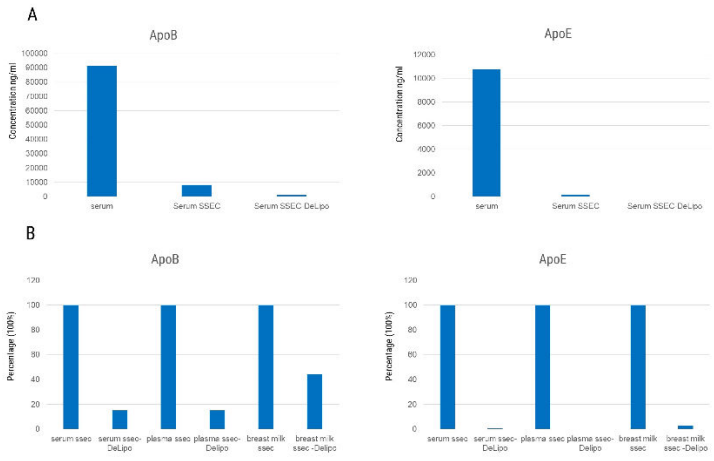

Figure 3. Most of the Lipoproteins are removed in sEV isolated with SmartSEC-DeLipo™. *(A) ApoB and ApoE lipoproteins' concentrations were evaluated in serum sample, sEV isolated from serum with SmartSEC™ single and sEV isolated from serum with SmartSEC-DeLipo™ by ELISA. SmarSEC™ single dramatically reduced Lipoproteins in isolated sEV and SmartSEC-DeLipo further deplete the remaining Lipoproteins in yield sEV. (B) Percentage of ApoB and ApoE depletion was calculated based on ApoB and ApoE concentration in sEV isolated with SmartSEC-DeLipo™ vs. SmartSEC™ single from serum, plasma and breast milk samples.*

SmartSEC-DeLipo™ isolated sEV possess typical sEV morphology

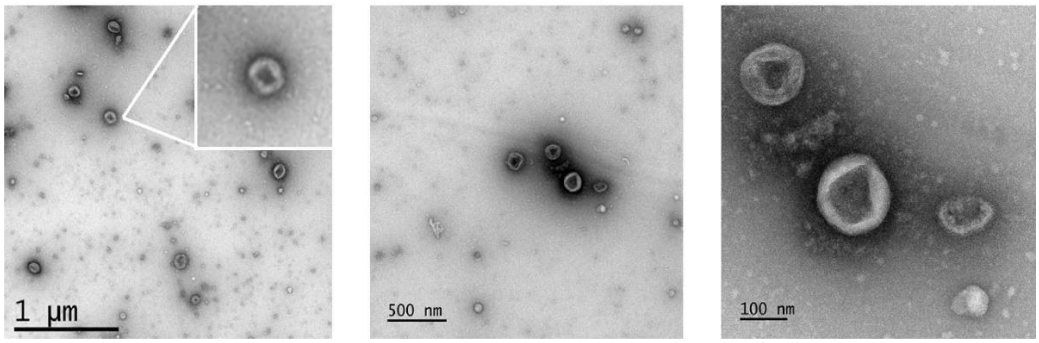

Figure 4. sEV isolated with SmartSEC-DeLipo™ shows typical EV morphology. *Transmission electron microscopy (TEM) of sEVs isolated from serum using SmartSEC-DeLipo™ possess typical EV morphology—intact vesicles with a double layer of membranes.*

SmartSEC-DeLipo™ isolate sEV is biological functional

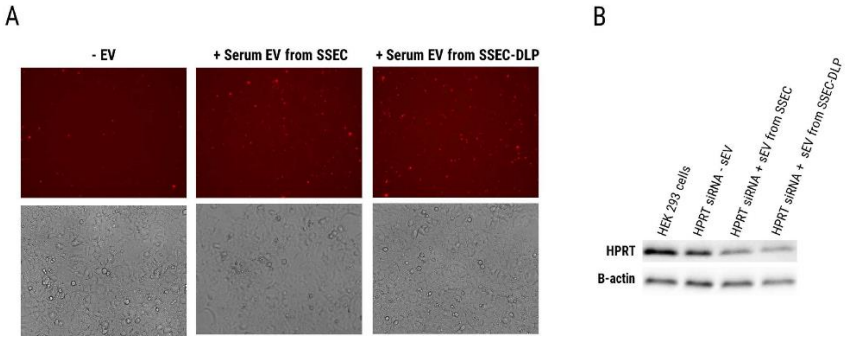

Figure 5. sEV isolated with SmartSEC-DeLipo™ is biologically functional to delivery small RNA cargo to the recipient cells. *(A) Cy3-labeled control siRNA were loaded without or with sEV isolated from serum with SmartSEC™ single or SmartSEC-DeLipo™ using Exo-Fect siRNA/miRNA reagent (EXFT200A-1) and delivered to HEK 293 cells. Cells imaged 36-48 hours post transfection. Shown are the bright-field images (bottom row), and fluorescence images (top row). Both sEV isolated with SmartSEC single or SmartSEC-DeLipo™ can efficiently transfer Cy3-labeled siRNA to most of the imaged cells. (B) HPRT siRNA were loaded without or with sEV isolated from serum with SmartSEC™ single or SmartSEC-DeLipo™ using Exo-Fect siRNA/miRNA reagent (EXFT200A-1) and delivered to HEK 293 cells. Cells were lysed and applied for western blot 48 hours post transfection. Both sEV isolated with SmartSEC™ single or SmartSEC-DeLipo™ can efficiently transfer HPRT siRNA to HEK 293 cells and achieve knockdown of the endogenous HPRT expression.*

SmartSEC-DeLipo™ isolate sEV can be used for biomarker discovery

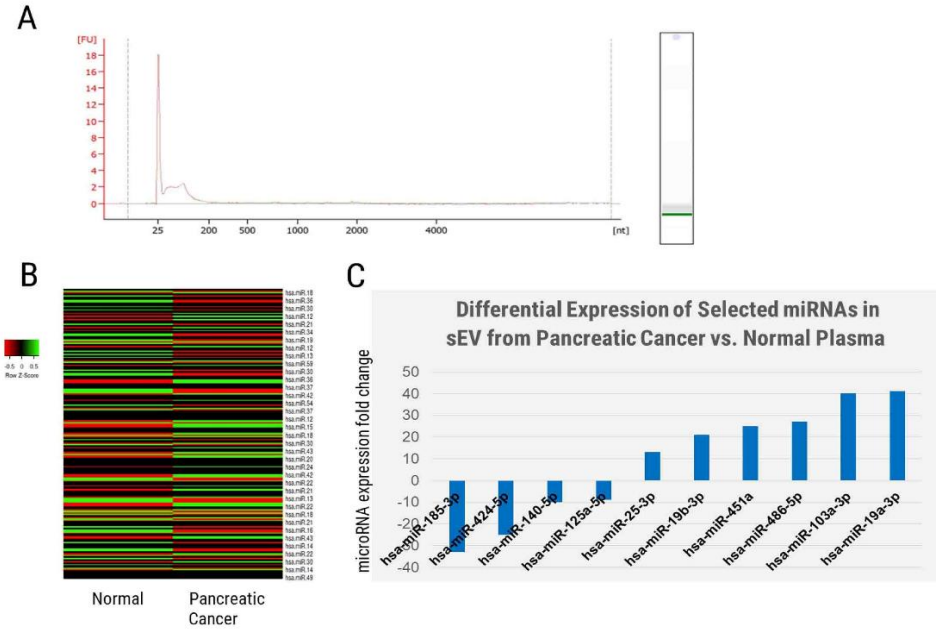

Figure 6. sEV isolated with SmartSEC-DeLipo™ can be used for small RNA profiling and biomarker discovery. *sEV isolated from normal vs. pancreatic cancer patient plasma with SmartSEC-DeLipo™ were used for sEV RNA isolated using EVery100B-1. Then RNA samples were analyzed by Agilent Bioanalyzer. (A) Electrophoretic spectrums and gel images of the sEV RNA derived from the plasma of pooled normal plasma. Then sEV RNA samples from normal and pancreatic patient sample were reverse transcribed into cDNA using Every 200B-1, followed with Every miRNome Profiler (EVery600B-1) (B) Heat map of normal vs. pancreatic cancer plasma miRNA expression profiler. Representative data normalized to global mean of each sample. Pancreatic cancer patient plasma showed distinct miRNA expression profiles compared to normal plasma. (C) Differential expression of selected miRNAs in normal vs. pancreatic cancer plasma. Data normalized with global mean. Expression of miR-25-3p, miR-19b-3p, miR-451a, miR-486-5p, miR-103a-3p and miR-19a-3p are significantly upregulated, while expression of miR-185-3p, miR-424-5p, miR-140-5p and miR-125a-5p is significantly downregulated in pancreatic cancer plasma compared to normal one*.

SmartSEC-DeLipo™ isolate sEV can be used for proteomic study

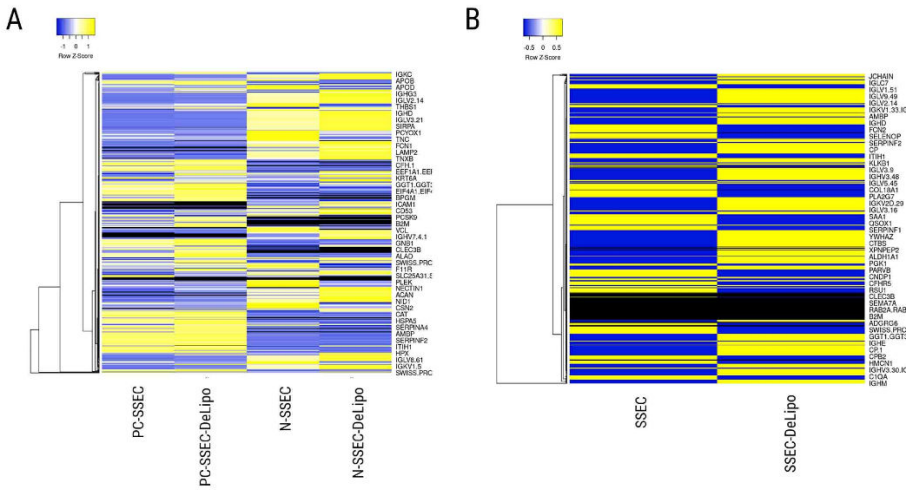

Figure 7. sEV isolated with SmartSEC-DeLipo™ can be used for proteomic studies*. sEV isolated from normal vs. pancreatic cancer patient plasma with SmartSEC-DeLipo™ or SmartSEC™ single were analyzed by mass spectrometry. (A) Heatmap of normal vs. pancreatic cancer plasma sEV proteomic profiler using either SmartSEC™ single or SmartSEC-DeLipo™. Pancreatic cancer patient plasma sEV showed distinct proteome compared to normal plasma. (B) sEV from normal pooled plasma isolated with either SmartSEC™ or SmartSEC-DeLipo™ also showed differentiated proteome.*

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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Licensing and Warranty Statement

Limited Use License

Use of the SmartSEC-DeLipo™ Single 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.

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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.

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