ExoMS™ Surface Protein Capture Kit (Tissue Culture EVs)

Cat# EXOMS110A-4, EXOMS111A-8

User Manual

Store kit at +4^{0}C and -20^{0}C

Version 3
1/13/2019

Contents

Product Description

The ExoMS™ Surface Protein Capture Kits (for tissue culture EVs) represent the latest innovation from SBI, an established leader in exosome research tools. By providing a validated, robust method to selectively capture surface and membrane-associated proteins from extracellular vesicles (EVs), these kits offer researchers an opportunity to discover surface-associated EV proteins using powerful LC/MS approaches. With low residual protein carryover, the kits increase detection of low-abundance biomarkers that are often missed using traditional approaches.

The kit comes in two reaction formats, processing 4 (Cat #EXOMS110A-4) or 8 (Cat #EXOMS111A-8) different samples. Both kits can process exosomes isolated using the following methods:

- Polymer-based precipitation (e.g. ExoQuick™-TC, ExoQuick-TC ULTRA)
- Column-based
- Ultracentrifugation

Our proprietary affinity-based resin in our kits traps many common protein precipitates present in tissue culture media EV preps such as albumin, ensuring minimal presence of these contaminants during sample prep and loading into LC/MS. In addition, our robust surface and membrane protein capture strategy, based on a two-step biotinylation and bead capture method, captures biotinylated proteins away from total EV protein for highly selective capture of surface and membrane associated proteins of interest.

List of Components

Item	Volume	Storage Temperature
Purification columns	4 columns	4^0C
Buffer A	1 ml	4^0C
Buffer B	5 ml	4^0C
Buffer Tris pH 8.0	50 µl	4^0C
Modification reagent	4 tubes	-20^0C
Stop Buffer	400 µl	4^0C
Lysis buffer	300 µl	4^0C
Capture buffer	25 ml	4^0C
Capture magnetic beads	400 µl	4^0C
Wash buffer	6 ml	4^0C

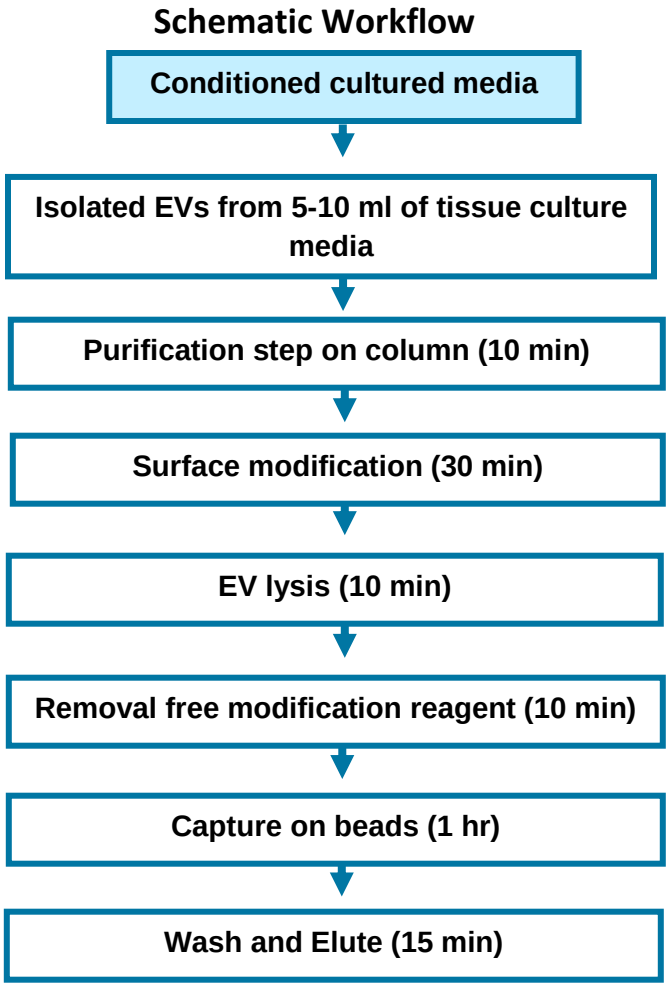
Elution buffer	300 µl	-20^{0}C
Free modification reagent removal columns	4 columns	4^{0}C

***Note: Above table is for the 4 reaction kit. For 8 reaction kit, the volumes of reagents and number of columns will be doubled**

Storage

The ExoMS™ kit is shipped at +4°C and should be **stored** at +4°C except Modification reagent and Elution buffer, both of which should be **stored at -20°C**. Properly stored kits are stable for 6 months from the date received.

General Information



Materials provided by users:

- 15 ml conical tubes
- Magnetic apparatus for capture of magnetic beads
- Microcentrifuge tubes

Protocol for ExoMS

For EVs isolated by UC, column or polymer based methods (from 5-10 ml of media). If you have isolated EVs using ExoQuick-TC ULTRA, proceed to step B.:

A. Purification step (column capacity is ~4 mg of contaminants):

1. Isolate EVs by the method of choice. **If you have isolated EVs using ExoQuick-ULTRA or ExoQuick-ULTRA-TC proceed to step B.**
2. Add equal volume of **Buffer A** to isolated EVs (v/v). For example: If you have 200 µl of EVs add 200 µl of Buffer A.
   ! **NOTE:** Do not exceed 400 µl of total volume.
3. Take out **Purification column**, loosen screw cap and snap off the bottom closure. Place the column into a collection tube.
   ! **NOTE:** Save the bottom closure for steps 8-12.
4. Centrifuge at 1, 000 x g for 30 seconds to remove storage buffer.
5. Discard the flow-through and place the column back into the collection tube.
6. To wash the column, remove the cap and apply 500 µl of **Buffer B** on top of the resin and centrifuge at 1,000 x g for 30 seconds. Discard the flow through.
   ! **NOTE:** Save the cap for steps 9-12.
7. Repeat step 6 one more time to wash the column.
8. Plug the bottom of the column with the bottom closure. Apply 100 µl of **Buffer B** on top of the resin to get it ready for sample loading.
9. Add entire content of isolated EVs from step 2 (or up to volume equivalent of 4 mg of total protein) to the resin. Securely, place the top cap on the column.
10. Mix at room temperature (RT) on a rotating shaker for no more than 5 minutes.
    ! **CAUTION:** Sample will start to elute as soon as the bottom closure is removed.
11. Transfer the column to a 2 ml tube, loosen the screw cap and remove the bottom closure.
12. Centrifuge at 1,000 x g for 30 seconds to obtain purified EVs.
13. Discard the column.

B. Modification reaction:

1. Add 10 µl of Buffer **Tris pH 8.0** to 500 µl of purified EVs to ensure pH 8-9.
2. Add 510 µl of purified EVs to the tube with Modification reagent powder. Mix well.
3. Incubate for 30 min **on ice**.
4. Add 85 µl of **Stop buffer** to stop the reaction.

C. EVs lysis:

1. Add 60 µl of **Lysis buffer** to ~600 µl of modified EVs. Mix well by vortexing for 10 sec.
2. Incubate on ice for 10 min.

D. Free modification reagent removal column:

1. Take out **Free reagent removal column** and mix the resin in the column by vortexing.
2. Remove the column's bottom closure and loosen (do not remove) the cap.
3. Place column into a collection 15 ml tube (not provided) and centrifuge at 1,000 x g for 2 min to remove the storage solution.

4. Discard flow-through and place the column back into the collection tube. Add 1 ml of **Capture buffer** and centrifuge at 1,000 x g for 2 min.
5. Repeat step 4 additional times.
6. The column is ready for buffer exchange.
7. Place the column in a new collection 15 ml tube.
8. Apply lysed sample ~700 µl of the sample from Step C on top of the resin.
9. Centrifuge at 1,000 x g for 3 min to recover proteins in Capture buffer.

E. Capture of modified proteins by magnetic beads:

1. Mix magnetic beads well by vortexing.
2. Transfer 100 µl of the beads into a microcentrifuge tube (not provided).
3. Add 0.5 ml of Capture buffer and vortex 5 sec.
4. Place the tube on the magnet for 1 min and discard the supernatant.
5. Add ~700 µl of the sample to Capture magnetic beads with the proteins from Step D.
6. Incubate for 1 hr at room temperature with continuous gentle rotation.

F. Washes and elution from the beads (for in-gel digestion):

1. Place the tube on the magnet for 1 min and collect the supernatant (unbound, non-modified proteins). If you are not interested in this fraction you can discard it.
2. Add 0.5 ml of **Wash buffer** and incubate for 5 min with continuous rotation.
3. Place the tube on the magnet for 1 min and discard the supernatant.
4. Repeat washing 2X more times.
5. Add 75 µl of **Elution buffer** to the beads and incubate at 95°C for 5 min.
6. Place the tube on the magnet for 1 min and **collect the supernatant** into a new microcentrifuge tube (not provided).
7. The samples are ready for loading on protein gel and/or in-gel digestion for proteomics study.

! **NOTE:** Alternative elution buffer (0.1% SDS) should be used with the same elution protocol if you choose to use in-solution digestion protocol.

G. Mass spectrometry analysis

We **highly recommend** in-gel digestion of eluted proteins separated by SDS-PAGE. In case you would like to use in-solution digestion protocol please use alternative elution buffer (not provided). For details on the protocol/s please consult your sample prep technician at your mass spectrometry facility.

Example Data and Applications

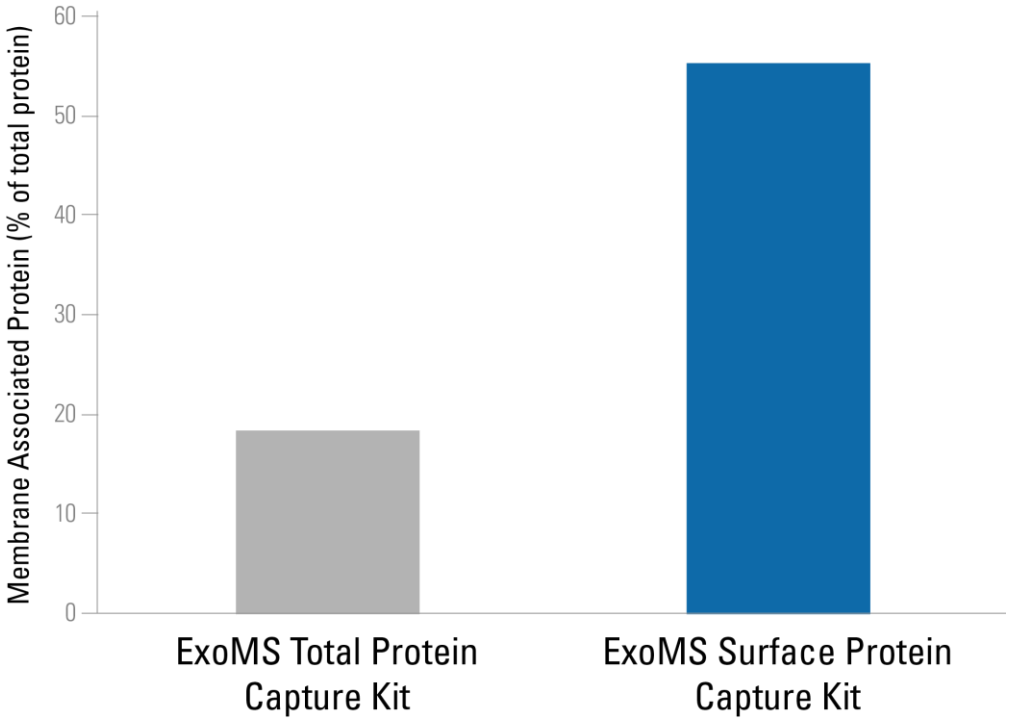


Figure 1. Membrane-associated proteins are enriched in human serum EV samples processed with the ExoMS Surface Protein Capture Kit compared to samples processed with the ExoMS Total Protein Capture Kit.

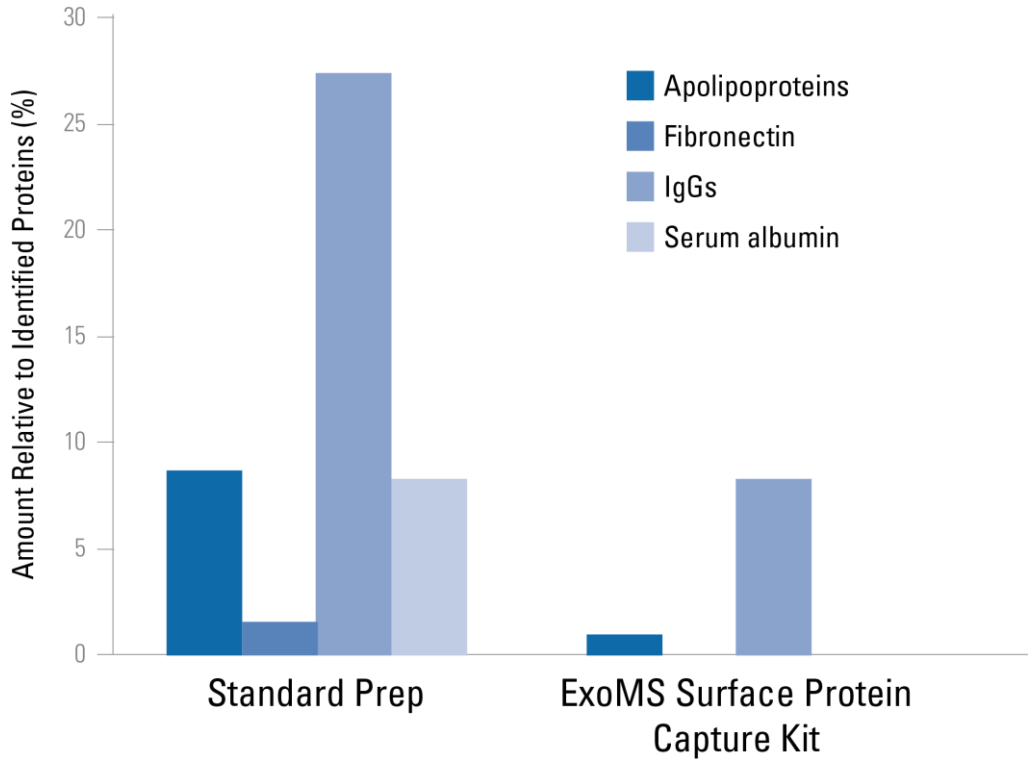


Figure 2. Common carryover proteins are reduced in human serum EV samples processed with the ExoMS Surface Protein Capture Kit compared to samples processed using a standard protocol.

Identified Proteins	Accession Number	Molecular Weight	ExoMS
Alpha-2-macroglobulin	sp|P01023|A2MG_HUMAN	163 kDa	846
Haptoglobin	sp|P00738|HPT_HUMAN	45 kDa	190
Desmoplakin	sp|P15924|DESP_HUMAN	332 kDa	170
Pregnancy zone protein	sp|P20742|PZP_HUMAN	164 kDa	165
Ceruloplasmin	sp|P00450|CERU_HUMAN	122 kDa	149
Serpin B3	sp|P29508|SPB3_HUMAN	45 kDa	111
Serpin B4	sp|P48594|SPB4_HUMAN	45 kDa	104
Haptoglobin-related protein	sp|P00739|HPTR_HUMAN	39 kDa	101
Actin, cytoplasmic 2	sp|P63261|ACTG_HUMAN	42 kDa	75
Hemopexin	sp|P02790|HEMO_HUMAN	52 kDa	72
14-3-3 protein sigma	sp|P31947|1433S_HUMAN	28 kDa	65
Complement C4-B	sp|P0C0L5|CO4B_HUMAN	193 kDa	63
Apolipoprotein B-100	sp|P04114|APOB_HUMAN	516 kDa	55
Alpha-2-HS-glycoprotein	sp|P02765|FETUA_HUMAN	39 kDa	50
Protein S100-A9	sp|P06702|S10A9_HUMAN	13 kDa	46
Epiplakin	sp|P58107|EPIPL_HUMAN	556 kDa	45
Vitamin D-binding protein	sp|P02774|VTDB_HUMAN	53 kDa	44
Annexin A2	sp|P07355|ANXA2_HUMAN	39 kDa	43
Galectin-7	sp|P47929|LEG7_HUMAN	15 kDa	42
Glyceraldehyde-3-phosphate dehydrogenase	sp|P04406|G3P_HUMAN	36 kDa	39
Junction plakoglobin	sp|P14923|PLAK_HUMAN	82 kDa	38
Fatty acid-binding protein, epidermal	sp|Q01469|FABP5_HUMAN	15 kDa	37
Pyruvate kinase PKM	sp|P14618|KPYM_HUMAN	58 kDa	37
Alpha-enolase	sp|P06733|ENOA_HUMAN	47 kDa	36

Denotes surface or membrane-associated proteins not found in total exosome preps

Figure 3. Top 25 surface and membrane-associated proteins captured using ExoMS Surface Protein Capture Kit from human serum EV sample. Proteins highlighted indicate those not found in total exosome preparations

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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Limited Use License

Use of the ExoMS Surface Capture Kit (*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:

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- 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 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.

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