



ExoMS™ Total Protein Capture Kit

Cat# EXOMS120A-8

User Manual

See manual for storage conditions

Version 3
1/13/2019

A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the License and Warranty Statement contained in this user manual.

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Product Description

The ExoMS™ Total Protein Capture Kit (for all EVs) represent the latest innovation from SBI, an established leader in exosome research tools. By providing a validated, robust method to capture total EV proteins, these kits offer researchers an opportunity to discover novel EV-associated 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 an 8 reaction format. It can process EVs isolated using the following methods from any biofluid/media:

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

Our proprietary affinity-based resin in the traps many common protein precipitates present in EV preps such as albumin and IgG, ensuring minimal presence of these contaminants during sample prep and loading into LC/MS.

List of Components

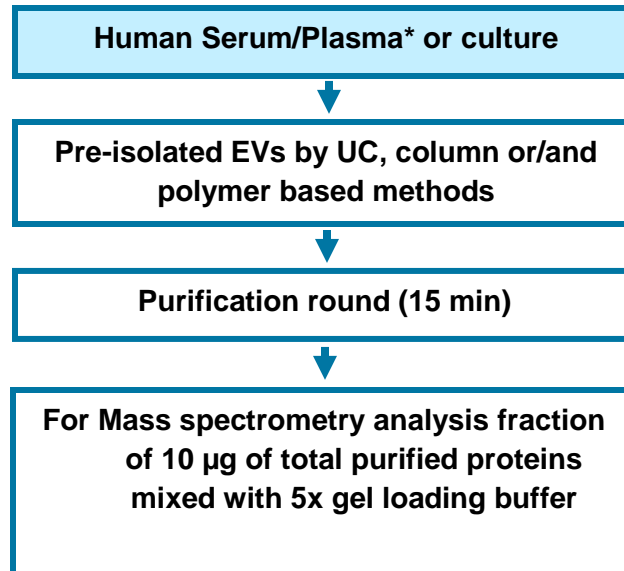
Item	8 reaction kit	Storage Temperature
Buffer A	2 ml	4°C
Buffer B	10 ml	4°C
1M Tris pH 8.0	150 µl	4°C
Purification columns	8 columns	4°C
2ml Eppendorf tubes	8 tubes	4°C
Collection tubes	8 tubes	4°C
Mass spectrometry gel loading buffer (5X)	50 µl	-20°C

Storage

The ExoMS™ kit is shipped on +4°C and should be **stored** at +4°C except 5X gel loading buffer which should be stored at -20°C. Properly stored kits are stable for 12 months from the date received.

General Information

Schematic Workflow



*To isolate exosomes from **plasma**, we recommend using the **Thrombin Plasma Prep for Exosome Precipitation Reagent** (Cat# TMEXO-1, not included). Plasma contains fibrin which will precipitate along with ExoQuick causing an insoluble pellet to form. The thrombin reagent will help to dissolve the fibrin, thus increasing the yield of exosomes precipitated.

Protocol for ExoMS for total proteomics

For EVs isolated by UC, column or/and polymer based methods

Purification step for serum/plasma:

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

1. Isolate EVs by the method of choice.
2. Add equal volume of **Buffer A** to isolated EVs (v/v). For example: If you have 200 μ l of EVs add 200 μ l of Buffer A.
! **NOTE:** Do not exceed 400 μ l 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 μ l 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 μ l 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. Add 10 μ l of **1M Tris pH 8.0** to purified EVs to ensure pH is 8-9.
14. Discard the column.

B. Sample preparation to Mass spectrometry:

1. Measure protein concentration.
2. Add gel loading buffer x5 to 10 μ g of the total exosomal proteins.

We **highly recommend** in-gel digestion protocol for mass spectrometry of the proteins. For details on the protocol please consult your mass spectrometry facility.

Example Data and Applications

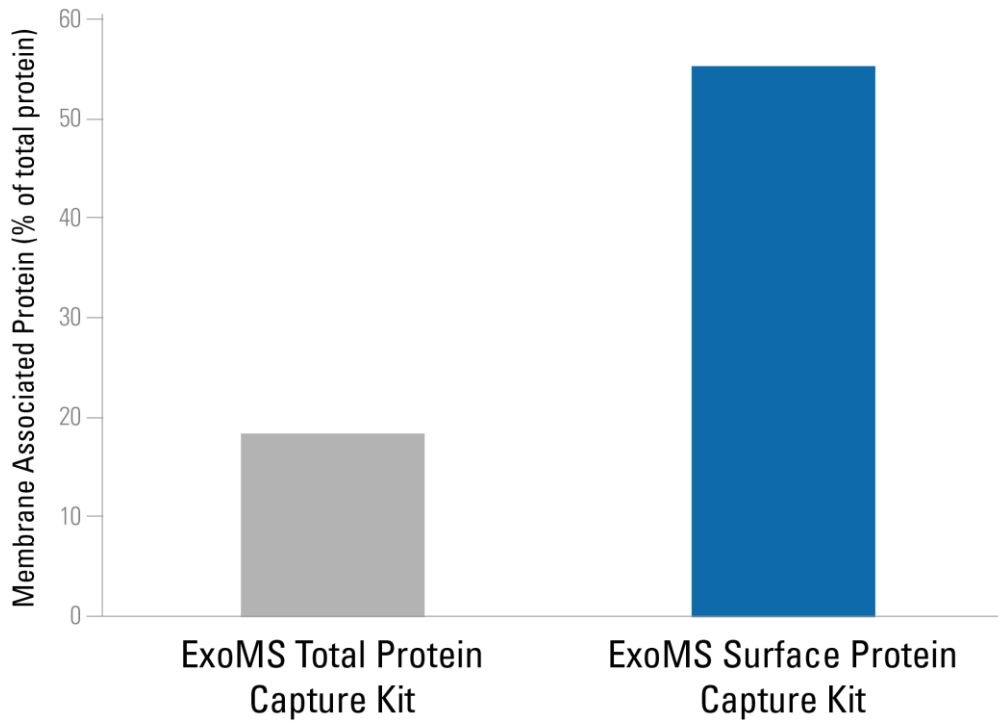


Figure 1. Membrane-associated proteins are enriched in human serum EV samples processed with the ExoMS Surface Protein Capture Kit compared to samples processed with the ExoMS Total Protein Capture Kit.

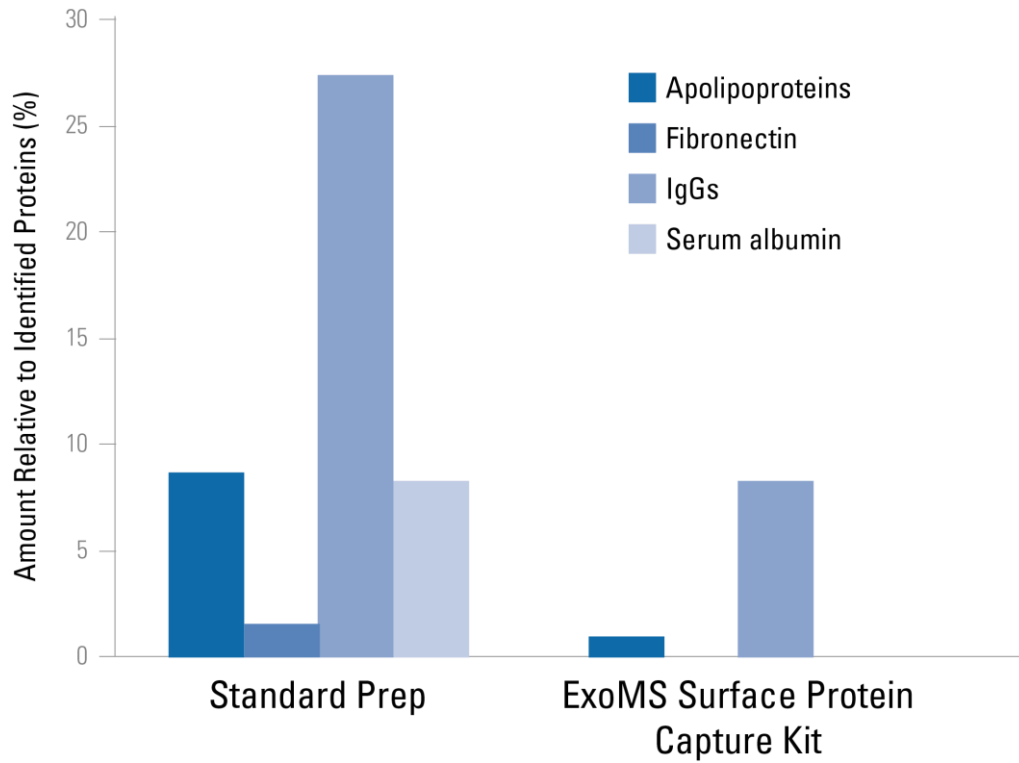


Figure 2. Common carryover proteins are reduced in human serum EV samples processed with the ExoMS Surface Protein Capture Kit compared to samples processed using a standard protocol. Common carryover proteins are reduced in human serum EV samples processed with the ExoMS Surface Protein Capture Kit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

Limited Use License

Use of the ExoMS Total Protein 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:

- 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.

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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