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# Exosome Antibodies

EXOAB-xxx-x

## User Manual

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**Store kit at 4°C for up to 1 month**

**Store kit at -20°C for up to 1 year**

Version 13  
3.27.2024

**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 EXOAB products are antibodies used for Western blotting of proteins from precipitated exosomes. Please refer to the Product Analysis Certificate for each antibody for a full description of each individual antibody.

## List of Components

Item	Amount	Suggested dilution
Exosome specific primary antibody	25 µl	1:1000 in 5% non-fat dry milk dissolved in TBST
Exosome validated secondary antibody (Goat anti-Rabbit HRP)	10 µl	1:20,000 in 5% non-fat dry milk dissolved in TBST

## Other required reagents

We recommend using a protease inhibitor cocktail when preparing the protein samples. This can be purchased from any number of vendors.

For chemiluminescent detection, we recommend SuperSignal West Femto Chemi-luminescent Substrate, THERMO catalog# 34095.

## Storage

The kits are shipped at on blue ice and should be stored at +4°C for up to 1 month, or -20°C for up to 1 year. Properly stored kits are stable for 1 year from the date received.

## General Information

The Exosome Antibodies have been designed to detect exosome marker proteins from approximately 20 µg of exosomal protein isolated from serum exosomes. The exact exosome marker proteins vary among exosomes and are often glycosylated. These glycosylations can affect the size at which the marker proteins appear on a Western blot and bands may appear at several different sizes.

The secondary antibody that is included in this kit has been optimized to enhance the signal-to-noise ratio. We do not recommend using other secondary antibodies with the EXOAB antibodies at this time.

## Protocol for Western Blotting

This protocol begins after precipitation of the exosomes is complete.

1. Resuspend the exosomal pellet in 200  $\mu$ l RIPA buffer (with appropriate protease inhibitor cocktail added) to exosome pellet and vortex 15 seconds.
2. Place at room temperature for 5 minutes (to allow complete lysis).
3. Perform standard Bradford protein assay to determine yield. We recommend running  $\sim$ 20  $\mu$ g protein per well.
4. Add Laemmli buffer<sup>2</sup> (with Beta-mercaptoethanol) and heat at 95°C for 5 minutes.
5. Chilled on ice for 5 minutes before loading onto gel.
6. Perform standard SDS-PAGE electrophoresis and Western transfer onto PVDF or nitrocellulose membrane.
7. Block with 5% dry milk in Tris Buffered Saline + 0.05% Tween (TBS-T) for 1 hour.
8. Incubate blot overnight at 4°C with Exosome specific primary antibody (i.e. EXOAB) at 1:1000 dilution (5% dry milk in TBS-T).
9. Wash 3X with TBS-T.
10. Incubate one hour at room temperature with the included secondary antibody (Goat-Rabbit-HRP) antibody at 1:20,000 dilution (5% dry milk in TBS-T).
11. Wash 3X with TBS-T.
12. Incubate blot with chemi-luminescence substrate and visualize on film or other imaging equipment. We recommend the SuperSignal West Femto Chemi-luminescent Substrate, THERMO catalog# 34095.

## Recipes

### 1X RIPA buffer

25mM Tris-HCl pH 7.6  
150mM NaCl  
1% NP-40  
1% sodium deoxycholate  
0.1% SDS

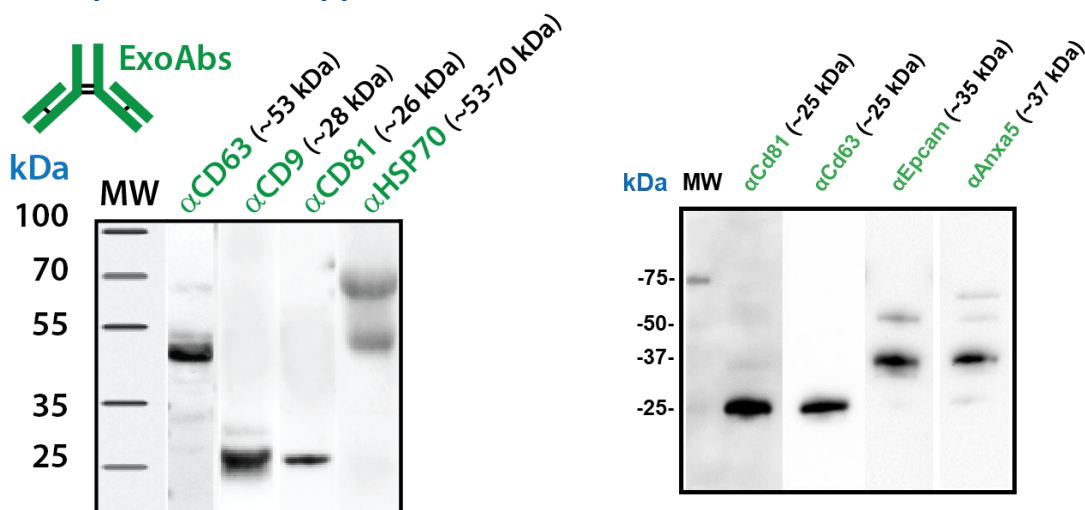
### 2X Laemmli buffer

4% SDS  
20% glycerol  
10% 2-mercaptoethanol  
0.004% bromophenol blue  
0.125 M Tris-HCl pH 6.8

## Next Steps and Related Products

Application	Related Products	Website links
<b>Precipitation of Exosomes from other biological fluids</b>		
Exosome Isolation from Tissue Culture Media	ExoQuickTC	<a href="https://www.systembio.com/exoquick-tc">https://www.systembio.com/exoquick-tc</a>
Exosome Isolation from Plasma	ExoQuick Plasma prep and Exosome precipitation kit	<a href="https://www.systembio.com/exoquick-plasma-prep-with-thrombin">https://www.systembio.com/exoquick-plasma-prep-with-thrombin</a>
<b>Protein Characterization of Exosomes</b>		
Antibody Arrays (human)	ExoCheck Assays	<a href="https://www.systembio.com/exo-check-exosome-antibody-array">https://www.systembio.com/exo-check-exosome-antibody-array</a>
Antibody Arrays (mouse)	ExoCheck Assays	<a href="https://www.systembio.com/mouse-exo-check-exosomes-antibody-arrays">https://www.systembio.com/mouse-exo-check-exosomes-antibody-arrays</a>
<b>Quantification of Exosomes</b>		
Quantification of exosomes	EXOCET Assays	<a href="https://www.systembio.com/products/exosome-research/exosome-quantitation/exocet-exosome-quantitation-kit">https://www.systembio.com/products/exosome-research/exosome-quantitation/exocet-exosome-quantitation-kit</a>
<b>RNA extraction from Exosomes</b>		
EV RNA extraction	EVERyRNA Isolation	<a href="https://www.systembio.com/every-ev-rna-isolation-kit">https://www.systembio.com/every-ev-rna-isolation-kit</a>

## Example Data and Applications



**ExoQuick Exosome Serum Western Analysis**

**Mouse serum EVs isolated with SmartSEC-Single (6ug/lane)**

Western blot analysis of serum exosomes using the recommended protocol. 20 µg of human serum exosome proteins were loaded into each lane. All Exosome specific primary antibodies were used at 1:1,000 dilution and Exosome validated secondary antibody was used at 1:20,000 dilution. We recommend the SuperSignal West Femto Chemi-luminescent Substrate, THERMO catalog# 34095.

## 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:

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## Licensing and Warranty Statement

### Limited Use License

Use of the Exosome Antibodies (*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.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

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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.

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