Exosome Antibodies

EXOAB-xxx-x

User Manual

Store kit at 4°C for up to 1 month
Store kit at -20°C for up to 1 year

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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)	5 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.

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.

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Protocol for Western Blotting

This protocol begins after precipitation of the exosomes is complete.

1. Resuspend the exosomal pellet in 200 µl 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 ~20 µg protein per well.

4. Add Laemmli buffer2 (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