



Mouse Exo-Check Exosome Antibody Arrays

Cat # EXORAY400A-4, Cat #EXORAY400A-8

User Manual

Please see individual components for storage conditions

Version 1
3/1/2023

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Product Description

The Mouse Exo-Check Exosome Antibody Arrays have been specifically optimized for detection of proteins derived from mouse samples. It has 12 pre-printed spots including 8 antibodies for known exosome markers (Cd63, Cd81, Alix, Flot1, Icam1, Epcam, Anxa5 and Tsg101), one Cis-Golgi matrix protein marker (Gm130) and 4 controls. The two positive control signals provide a bright signal that indicates that the detection reagents are working properly. The Blank spot serves as a background control. The array can be used with protein lysate from exosomes obtained from a wide variety of methods, including ultracentrifugation, precipitation-based, and size exclusion chromatography. The kits come complete with a secondary detection mixture conjugated to HRP. The Exo-Check arrays are semi-quantitative and can be used to evaluate the relative abundance of certain exosome protein markers from a given set of samples.

List of Components

Components	4 array kit	8 array kit	Storage Temperature
Lysis Buffer (10X)	0.5 ml	1 ml	Room temp
Labeling Reagent	4 x 20 µl	8 x 20 µl	-20°C
Columns	4	8	4°C
Column Buffer	20 ml	40 ml	4°C
Blocking Buffer	20 ml	40 ml	4°C
Wash Buffer (20X)	6 ml	12 ml	4°C
Detection Reagent A	20 ml	40 ml	4°C
Detection Reagent B	15 µl	25 µl	4°C
Membranes	4	8	4°C

Not supplied: HRP developer reagent. SBI recommends the use of **Advansta Western Bright Sirius HRP substrate**, catalog# K12043-C20.

Storage

The kits are shipped on blue ice and should be stored at +4°C (**except for Labeling Reagent, which must be stored at -20°C and Lysis buffer that must be stored at room temperature**) upon receipt. Properly stored kits are stable for 3 months from the date received.

General Information

The various exosome antibody spots will provide signals of varying degree, depending upon the source of the isolated exosomes. Note that for a given sample, signals from each spot on the array cannot be directly compared since the antibody concentrations are different across the array. However, it can be compared across samples to provide a qualitative assessment of a given spot's signal intensity.

Protocol for Exo-Check Arrays

Sample preparation

1. Start with isolated EVs resuspended in 1X PBS.
2. Perform a protein quantitation assay (e.g. BCA or Qubit assay) to determine the amount of protein in your sample. Transfer 50 µg of sample to a new tube; save the rest for future use.
3. Add 10X Lysis Buffer to the 50 µg of sample (from step 2) to a final concentration of 1X. Vortex for 15-30 seconds.

Note: No centrifugation is needed during the lysate preparation.

4. Equilibrate the Labeling Reagent to room temperature.
5. Add 1 µl of Labeling Reagent* to the entire content from step 3. Vortex to mix the sample.
*Use one amber tube of Labeling Reagent per reaction/membrane. Do not save the reagent. Always use a fresh amber tube for preparing a new sample.
6. Incubate at room temperature for 30 mins with constant mixing.
7. Remove excess Labeling Reagent using the provided columns:
 - a. Vortex the column to resuspend the medium.
 - b. Loosen the screw cap and twist off the bottom closure.
 - c. Place the column in a collection tube.
 - d. Remove the storage buffer by centrifuging at 800 x g for 1 min. Discard the storage buffer.
 - e. Equilibrate the column by adding 400 µl Column Buffer.
 - f. Centrifuge for 1 min at 800 x g. Discard the flow-through and place the column into the collection tube.
 - g. Repeat steps e-f 4 times for a total of 5 times.
 - h. Apply the sample from step 6 (100-180 µl) slowly in the middle of the packed bed.
Note: If the sample was less than 140 µl, add a stacker volume of Column Buffer to reach 140 µl.
 - i. Elute the sample (i.e., labeled exosome lysate) by centrifuging at 800 x g for 2 mins.
8. In a new 15 ml conical tube, combine the 50 µg of labeled exosome lysate with 5 ml Blocking Buffer and mix by inverting the tube 3 times.

Antibody array capture

1. Remove the membrane from the white envelope using forceps, grabbing the edge of the membrane.
2. In a small, clean tray, briefly wet the membrane in 5 ml distilled water for 2 mins at room temperature.
3. Carefully, decant the water from the membrane. Pipette the 5 ml labeled exosome lysate/blocking buffer mixture to one membrane. Place the membrane "face-up" by positioning the membrane such that the notch is at the top right corner when the membrane is vertical (longer side vertical as in Fig 1 on page 5).
4. Incubate the tray/membrane mixture shaking at 2-8°C overnight on a shaker or rocker.

Membrane washing and signal detection

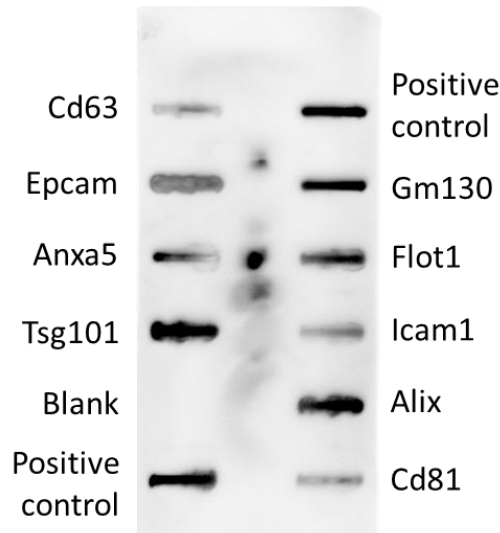
1. Next day, decant the lysate/blocking mixture from the membrane carefully.
2. Add 5 ml of 1X Wash Buffer (diluted from 20X stock with DiH₂O) and rock gently for 5 min at room temperature.

*Prepare the Detection Buffer by mixing 5 ml of Detection Reagent A + 2.5 μ l of Detection Reagent B per blot. Double the respective amounts if developing 2 blots.

3. Decant 1X Wash Buffer. Repeat step 2 once more. Decant after the last wash.
4. Add 5 ml Detection Buffer to the membrane and incubate for 30 - 45 min at room temperature on a shaker or rocker.
5. Prepare the Developer mixture (not included) about 5 mins before the end of step 4 according to the manufacturer's recommendation.
6. Decant the Detection Buffer and add 5 ml 1X Wash Buffer and shake for 5 min at room temperature.
7. Decant the 1X Wash Buffer, repeat wash twice for a total of three washes. Decant after the last wash.
8. Develop and image the membrane according to instrument manufacturer's recommendations.

Example Data and Applications

A. Serum



B. Cell Culture

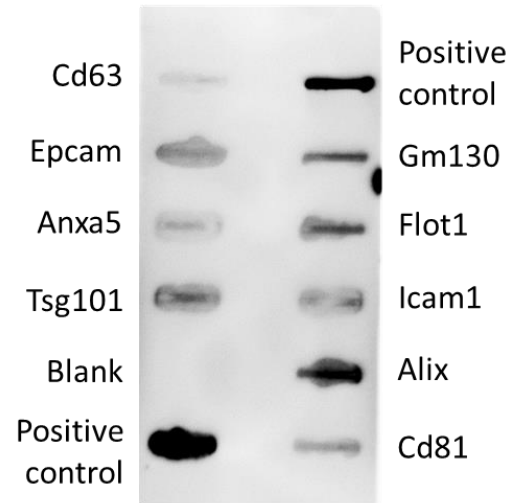


Figure 1. Data for array spots obtained from 50 µg of wildtype mouse serum exosome (isolated using SmartSEC) lysate (A) or RAW5 264.7 mouse macrophage cell exosome (isolated using ExoQuick-TC) lysate (B).

Information about the exosome protein targets

POSITION	PROTEIN ID	NOTES	LINK TO EXOCARTA ENTRY FOR THE MOUSE PROTEINS
A1	Cd63	Tetraspanin	ExoCarta 12512
A2	Epcam	Epithelial cell adhesion molecule; often found in cancer-derived exosomes	ExoCarta 4072
A3	Anxa5	Annexin A5	ExoCarta 11747
A4	Tsg101	Tumor susceptibility gene 101	ExoCarta 22088
A5	Blank	Background control	N/A
A6	Positive Control	Positive control for HRP detection	N/A
B1	Positive Control	Positive control for HRP detection	N/A
B2	Gm130	Cis-Golgi matrix protein	N/A
B3	Flot1	Flotillin-1	ExoCarta 14251
B4	Icam1	Intercellular adhesion molecule 1	ExoCarta 15894
B5	Alix	Programmed cell death 6 interacting protein (Pcd6ip)	ExoCarta 18571
B6	Cd81	Tetraspanin	ExoCarta 12520

Mouse Array Chart

	[A]	[B]
1	Cd63	PC
2	Epcam	Gm130
3	Anxa5	Flot1
4	Tsg101	Icam1
5	Blank	Alix
6	PC	Cd81

Table 1. Information for each spot on the array

Next Steps and Related Products

Exosome/EV Isolation		
High-purity, high-yield SEC-based isolation from a range of biofluids		
High-throughput SEC-based isolation from serum and plasma, 96-well format	SmartSEC™ HT	SSEC096A-1
Single format SEC-based isolation, validated for human serum, plasma, and CSF	SmartSEC™ Single	SSEC200A-1
High purity, polymer-based EV isolation		
Isolation from serum and plasma	ExoQuick® ULTRA	EQUltra-20A-1
Isolation from tissue culture media and other fluids	ExoQuick-TC® ULTRA	EQUltra-20TC-1
General purpose, polymer-based EV isolation		
Isolation from serum and plasma	ExoQuick®	EXOQ20A-1
Isolation from tissue culture media and other fluids	ExoQuick-TC®	EXOTC50A-1
Protein Characterization of Exosomes		
Antibody Arrays	Exo-Check Exosome Antibody Array	EXORAY200B-4 EXORAY210B-8
ELISA	ExoELISA-ULTRA Complete Kits	EXEL-ULTRA-CD63-1 EXEL-ULTRA-CD81-1 EXEL-ULTRA-CD9-1
RNA extraction from Exosomes		
Obtain high yields of total exosome/EV RNA, including small RNAs	EVery EV RNA Isolation Kit	EVery100B-1
Flexible & efficient RNA extraction from exosomes	SeraMir Exosome RNA Column Purification Kit	RA808A-1
cDNA synthesis from EV RNA		
Quick and easy cDNA synthesis optimized for use with the EVery EV RNA Purification System	EVery cDNA Synthesis Kit	EVery200B-1
EV miRNA profiling		
qPCR-based exosomal miRNA profiling plate for human serum and plasma samples	EVery miRNome Profiler for Human Serum and Plasma	EVery500B-1
Synthetic small RNA controls for your RNA isolation and cDNA synthesis steps	EVery miRNA Spike-in Kit	EVery600B-1

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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