



Exo-Check Exosome Antibody Array (Neuro) Standard Kit

Cat # EXORAY500A-4, EXORAY500A-8

User Manual

Please see individual components for storage conditions

Version 1
8/21/2018

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Product Description

The Exo-Check Exosome Antibody Array (Neuro) Standard kit (4 and 8 array formats) has 14 pre-printed spots including 5 antibodies for well-known exosomal-associated markers (CD9, CD63, CD81, ICAM1, and TSG101), 6 neuronal-associated markers (L1CAM, NCAM1, ENO2, MAPT, GRIA1, and PLP1) and 3 controls (1 – Positive assay control, 1 – negative control, and 1 – cellular contamination control). The combination of targets on the array provides a general survey of relative abundance of markers from EV preparations with the simplicity of a dot blot assay. The array uses 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 exosome and neuronal-associated markers from a given set of samples.

List of Components

Components	4 array kit	8 array kit	Storage Temperature
Lysis Buffer (10X)	0.4 ml	0.8 ml	4°C
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 Buffer	20 ml	40 ml	4°C
Membranes	4	8	4°C

Not supplied: HRP developer reagent. SBI recommends the use of **Advansta WesternBright 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**) upon receipt. Properly stored kits are stable for 3 months from the date received.

General Information

The sample size is based on using 10-50 µg of total protein input (as measured by Qubit or BCA assay). Neuronal-associated and exosome target bands will provide signals of varying degree, depending upon the source of the isolated EVs. Note that for a given sample, signals from each band 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 band's signal intensity. Arrays should be imaged at different exposures (e.g. short and long) to compare signal intensities for individual bands, which will help to better determine presence/absence of given band(s) across samples.

Protocol:

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 10-50 µg of sample to a new tube; save the rest for future use.
3. Add 10X Lysis Buffer to the 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 10-50 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 according to diagram below – slotted side on upper right hand corner.

NC	PC
CD 63	L1CAM
CD 9	NCAM1
CD 81	ENO2
TSG101	MAPT
CANX	GRIA1
ICAM1	PLP1

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.
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 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.

Next Steps and Related Products

Application	Related Products	Website links
Precipitation of Exosomes from other biological fluids		
Exosome Isolation from Tissue Culture Media	ExoQuickTC	https://www.systembio.com/micrna-research/exoquick-exosomes/ordering
Exosome Isolation from Plasma	ExoQuick Plasma prep and Exosome precipitation kit	https://www.systembio.com/micrna-research/exoquick-exosomes/ordering
Protein Characterization of Exosomes		

Western blotting	Exosome antibodies	https://www.systembio.com/micrna-research/exosome-antibody/exoab
ELISA	ExoELISA Kits	https://www.systembio.com/micrna-research/exosome-antibody/elisas
Quantification of Exosomes		
Quantification of exosomes	EXOCET Assays	https://www.systembio.com/micrna-research/exosome-antibody/exocet-assay
RNA extraction from Exosomes		
RNA extraction and profiling	SeraMir kits	https://www.systembio.com/micrna-research/seramir-exosome-rna-profiling/overview

Example Data and Applications

Standard Array

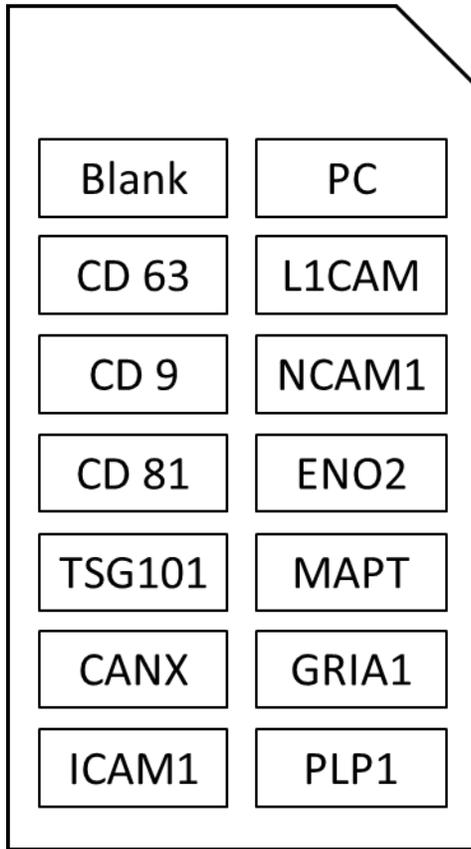


Figure 1. Schematic of array targets on the Standard array

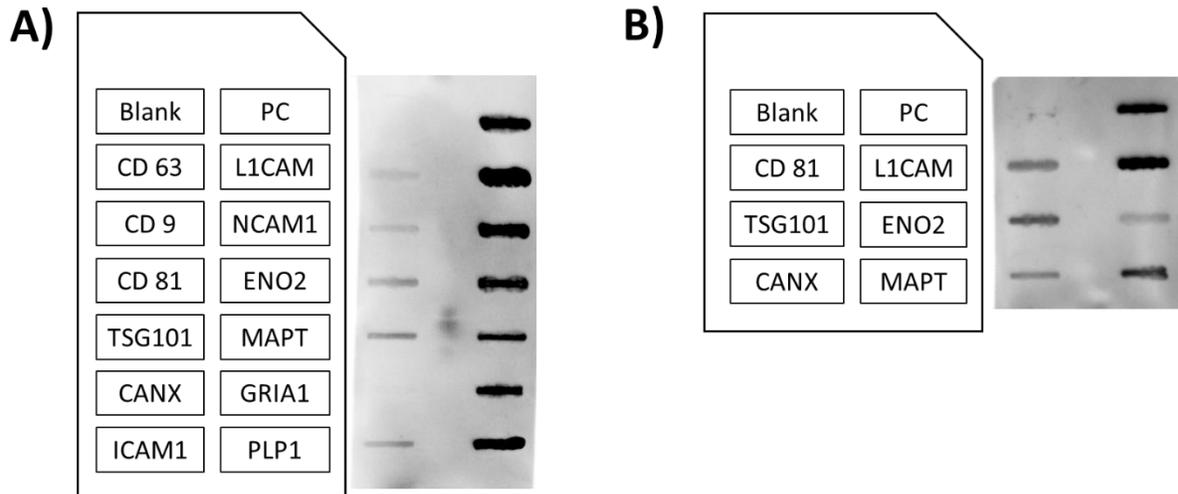


Figure 2: Representative array results for (A) standard format and (B) mini-format. EVs were isolated from 250 μ l of human serum from two different serum pools (Pool 1 and Pool 2) using ExoQuick and 10 μ g of EV-associated protein (as measured by Qubit protein assay) were loaded on the standard array using EVs from Pool 1 and 50 μ g of EV-associated protein from Pool 2 were loaded on the mini-array. Blots were imaged on the BioRad ChemiDoc XRS system.

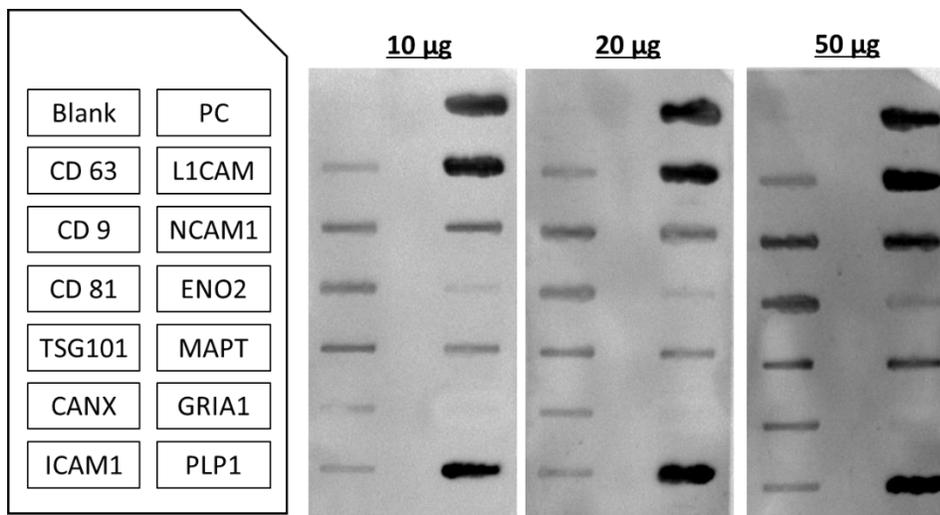


Figure 3: Assay sensitivity of Exo-Check array as a function of EV protein input. EVs were isolated from 250 μ l of human serum using ExoQuick and 10-50 μ g of EV-associated protein (as measured by Qubit protein assay) were loaded and imaged on the BioRad ChemiDoc XRS system. Signals from each spot on the array show good reproducibility across different protein inputs

Licensing and Warranty Statement

Limited Use License

Use of the Exo-Check Exosome Antibody (Neuro) Arrays (*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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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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