Exo-Check Exosome Antibody Arrays

Cat # EXORAY-4

User Manual

Please see individual components for storage conditions

Version 3
8/21/2018

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Product Description

The Exo-Check antibody array (4 and 8 array formats) has 12 pre-printed spots including 8 antibodies for known exosome markers (CD63, CD81, ALIX, FLOT1, ICAM1, EpCam, ANXA5 and TSG101) 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 GM130 cis-Golgi marker monitors any cellular contamination in your exosome isolations. 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 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)	1 ml	2 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)	5 ml	10 ml	4°C
Detection Buffer	20 ml	40 ml	4°C
Membranes	4	8	4°C

Not supplied: HRP developer reagent. AMSBIO 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^{0}C**) 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 upper left notched corner can be seen in the left-hand side on top.
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.

Example Data and Applications

	1	2	3	4	5	6
A	Positive	GM130	FLOT-1	ICAM	ALIX	CD81
B	CD63	EpCAM	ANXA5	TSG101	Blank	Positive

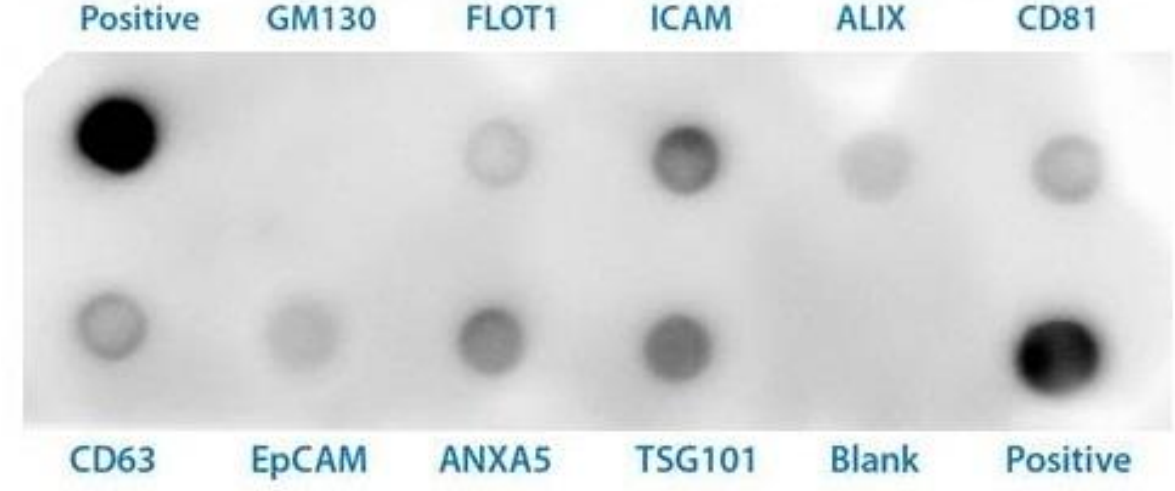

Figure 1. Data for array spots obtained from 50 µg of normal pooled human serum exosome (isolated using ExoQuick) lysate.

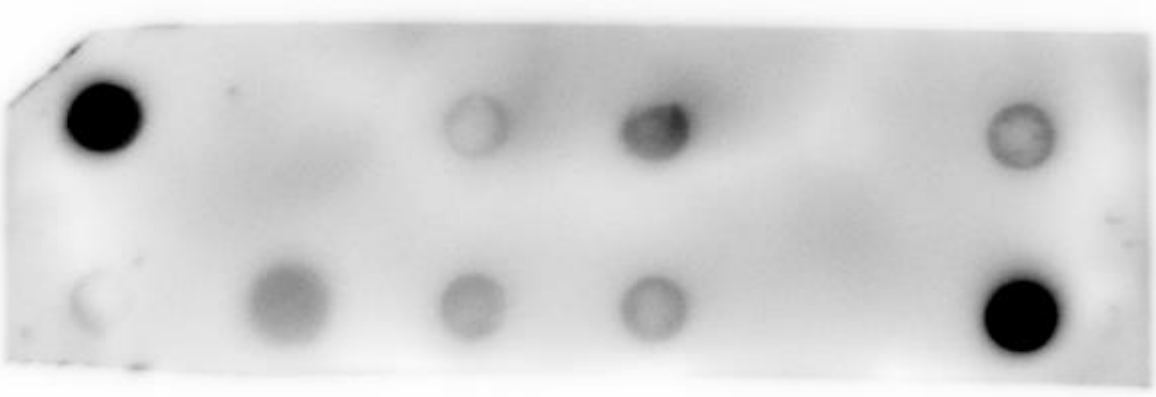

Figure 2. Data for array spots obtained from 100 µg of HEK293T exosome (isolated using ExoQuick-TC) lysate. Key for individual spots is same as in Figure 1.

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