ab314688 – 640/660 nm Extracellular Vesicle Membrane Staining Kit

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab314688

Storage and Stability:

Store at -20°C upon arrival and protect from light. Product is stable for at least 6 months from date of receipt when stored as recommended.

Reconstitution:

To prepare 500X stain solution, dissolve one vial of EV Membrane Stain in 100 uL of Reconstitution Solution. Pipette gently up and down to mix. The 500X stain solution can be stored protected from light for up to 6 months at 4°C.

Δ Note: *Reconstitution Solution contains 0.05% sodium azide.*

Materials Supplied

Item	100 Tests	500 Tests	Storage Condition
640/660 EV Membrane Stain	1 vial	5 vials	-20°C
Reconstitution Solution	1 mL	1 mL	-20°C

Considerations for Detecting Exosomes by Flow Cytometry

- Exosomes are extremely small vesicles (~30-150 nm in diameter), a size which is near or below the size detection limit of some flow cytometers. We recommend determining the size detection limit of your instrument by running sizing beads (for example, ranging from 0.02-2 um) in SSC before attempting to detect purified exosomes. We also recommend running sizing beads before each exosome detection experiment and using them to set the SSC threshold. Exosomes that are bound to affinity beads are large enough to detect on any instrument.
- Consider using a 405 nm laser for the SSC instead of a 488 nm laser, for improved sensitivity for small particles.
- Use a low flow rate to keep the event rate and abort rate low. This will result in reduced instrument noise. Dilute the stained samples in filtered PBS if necessary.
- For best results, buffers used for suspending and staining exosomes should be filtered through a 0.2 um filter to remove particulates.

Considerations for Staining with EV Membrane Stains

The following are general considerations for using EV Membrane Staining Kits. See Experimental Protocols for step-by-step instructions for use.

- EV Membrane Stains have been validated using flow cytometry. Results on other instruments may vary based on the instruments size detection limit and other parameters.
- Individual exosomes and EVs are too small to be imaged by conventional fluorescence or confocal microscopy, but clusters of EVs taken up by cells may be visualized. EV Membrane Stains have not been validated for labelling exosomes for cellular uptake. It may be necessary to remove free stain (by ultrafiltration, for example) before attempting to apply labelled exosomes to cells.
- Exosomes can be imaged by super-resolution microscopy. The 410/450 fluorophore is compatible with SIM and STED.
- EV Membrane Stains have been validated for staining exosomes isolated using several different methods, including PEG precipitation, size exclusion chromatography, and affinity bead isolation. Staining results may vary depending on the exosome isolation method used.
- Staining with EV Membrane Stain gives a bright signal and low background under typical staining conditions, concentrations between 1X and 100X has also produced excellent results. The dye concentration may require optimization for different samples and detection systems.
- EV Membrane Stains can be used for co-staining with fluorescently labelled primary antibodies. Co-staining can be performed concurrently or sequentially (see "Antibody Co-Staining of Purified Exosomes" under Experimental Protocols)

Experimental Protocols

Δ Note: *Before beginning, please read "Considerations for Staining EVs with EV Membrane Stains".*

Staining purified exosomes

This protocol was developed for staining purified exosomes with EV Membrane Stains for detection by flow cytometry.

1. Isolate or purify EVs or exosomes using the procedure of your choice.
2. Aliquot 100 uL of exosomes into FACS tubes or microcentrifuge tubes.
3. Prepare 1X EV Membrane staining solution by diluting the 500X stock solution 1:500 in 1X PBS (ab285410) (e.g., add 2 uL EV Membrane stain to 1 mL PBS).

Δ Note: *The concentration of EV Membrane stain can be optimized by the user; concentrations ranging from 1X to 100X give good signal.*

4. In addition to the EV Membrane-stained exosome samples, it is helpful to include the following controls (the buffer should be an appropriate negative control for the exosomes, such as a mock purification or the buffer used to suspend the exosomes):
    a. Buffer alone (no exosomes, no stain)
    b. Buffer plus EV Membrane stain
    c. Exosomes alone (no stain)
5. Add 900 uL of 1X EV Membrane staining solution to each tube containing 100 uL sample. Remember to also add the staining solution to the "buffer plus EV Membrane stain" control.
6. Incubate at room temperature for 30 minutes, protected from light.
7. Run the samples on a flow cytometer. For tips for flow cytometry detection of purified exosomes read "Considerations for Detecting Exosomes by Flow Cytometry ".

Antibody co-staining of purified exosomes

This protocol was developed for staining purified exosomes with both EV Membrane Stains and fluorescent antibodies and detecting them by flow cytometry.

Δ Note: *Use labelled primary antibodies at the manufacturer's recommended concentration or try staining in the range of 0.1-5 ug/mL. Either co-incubation or sequential incubations can be performed as described below.*

1. Follow steps 1-3 in the "Staining Purified Exosomes" protocol above. In addition to the antibody and EV Membrane co-stained exosome samples, it is helpful to include the following controls (if using multiple antibodies, include "buffer plus antibody" and single-stain controls for each antibody).

   Buffer controls
   a. Buffer alone (no exosomes, no stain)
   b. Buffer plus EV Membrane stain
   c. Buffer plus antibody

   Exosome controls
   a. Unstained exosomes
   b. Single-stain EV Membrane stain
   c. Single-stain antibody

2. Choose whether to co-stain by co-incubation (proceed to step 3) or sequential incubation (proceed to step 4).

3. Co-incubation of antibodies and EV Membrane stain:
   a. Add 900 uL of 1X EV Membrane staining solution to each tube containing 100 uL of exosomes. Remember to also add the staining solution to the "buffer plus EV Membrane stain" control and the EV Membrane single-stain control tubes.
   b. Add fluorescent antibody conjugate to the samples at the desired concentration. For example, to the 1 mL staining reaction, add 1 ug antibody for 1 ug/mL. Remember to also add the antibody to the "buffer plus antibody" control and the antibody single-stain control tubes.
   c. Continue to steps 6-7 in the "Staining Purified Exosomes" protocol.

4. Sequential incubation of antibodies and EV Membrane stain:
   a. Add fluorescent antibody conjugate to the samples at the desired concentration. For example, to the 100 uL exosome sample, add 0.1 ug antibody for 1 ug/mL. Remember to also add the antibody to the "buffer plus antibody" control and the antibody single-stain control tubes.
   b. Incubate at room temperature for 30 minutes, protected from light.
   c. Add 900 uL of 1X EV Membrane staining solution to each sample tube. Remember to also add the staining solution to the "buffer plus EV Membrane stain" control and the EV Membrane single-stain control tubes.
   d. Continue to steps 6-7 in the "Staining Purified Exosomes" protocol.

Staining bead-bound exosomes

This protocol was developed for exosomes bound to magnetic antibody capture beads, stained with EV Membrane Stains, and detected by flow cytometry.

1. Prepare exosomes bound to the magnetic capture beads of your choice, according to the manufacturer's recommended procedure.
2. Prepare the following control tubes:
   a. a. Beads alone (no exosomes or stain)
   b. b. Beads plus EV Membrane stain (no exosomes)
3. Prepare 10X EV Membrane staining solution by diluting the 500X stock solution 1:50 in 1X PBS (e.g., add 2 uL EV Membrane stain to 100 uL PBS).
4. Place the tubes with beads on a magnet for 1 minute, remove and discard the supernatant.
5. Remove the tubes with beads from the magnet and suspend in 100 uL of 10X EV Membrane staining solution. Remember to also add the staining solution to the "beads plus EV Membrane stain" control.
6. Incubate at room temperature for 30 minutes, protected from light.
7. Place the tubes on a magnet for 1 minute, remove and discard the supernatant.
8. Remove the beads from the magnet, add 100 uL of sterile-filtered PBS and gently pipet up and down to resuspend.
9. Place the tubes on a magnet for 1 minute, remove and discard the supernatant.
10. Remove the tubes from the magnet, add 500 uL of sterile-filtered PBS and gently pipet up and down to resuspend.
11. Run the samples on a flow cytometer.

Spectral properties of 640/660 nm Extracellular Vesicle Membrane Staining Kit

Item	Laser Line (nm)	Detection Channel	Ex/Em (nm)
640/660 EV Membrane Stain	633-640	APC	642/663

For technical support contact information, visit: www.abcam.com/contactus

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