Magnetic capture beads for exosome isolation

Capture beads for the isolation and analysis of exosomes by flow cytometry.

abid	Item name
ab326979	Human IgG1 Capture Beads for Flow Detection (Negative control)
ab326980	Human IgG2a Capture Beads for Flow Detection (Negative control)

For overview, typical data and additional information please visit:

www.abcam.com/ab326979

www.abcam.com/ab326980

Storage and Stability: Store in the dark, refrigerated between 2°C and 8°C. DO NOT FREEZE.

Materials Supplied

Item	Quantity	Storage Condition
ab326979 Human IgG1 Capture Beads for Flow Detection (Negative control)	25 tests	+4°C
ab326980 Human IgG2a Capture Beads for Flow Detection (Negative control)	25 tests	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Pre-enriched exosomes by ultra-centrifugation
- Magnetic Rack; MagneSphere(R) Mag. Sep. Stand 12-hole, 12x75mm
- 12x75mm Polystyrene Round Bottom Tubes (cytometer tubes)
- Sterile syringe filter with a 0.45 µm pore
- Syringe of adequate volume
- PBS with 1% BSA, pH 7.4
- ExoStep Incubation Buffer

Recommendations and Warnings

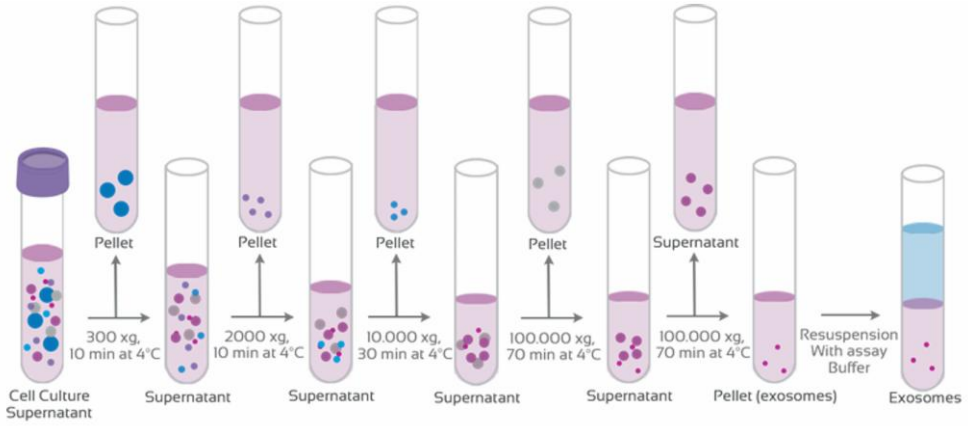
- Reagents should not be used if any evidence of deterioration is observed.
- Avoid microbial contamination of the reagent. Assay buffer can be filtered before use.
- Microspheres and reagents should be protected from prolonged exposure to light throughout this procedure.
- Microspheres are internally dyed with a fluorescent dye (fluorescent in PerCP, PerCP-Cy5, PerCP-Cy5.5 and APC). For exosome staining protocol ensure that the detector antibody does not occupy these fluorescent channels.
- The samples should be treated with appropriate handling procedures.
- Depending on the type of exosomes used, the number of exosomes may vary with respect to the concentration of the protein.
- Do not use after the expiry date indicated on the vial.
- Deviations from the recommended procedure could invalidate the analysis results. Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.
- The isolation and detection success is dependent on the quality of the sample pre-enrichment process.
- Pay attention if the kit is used in combination with annexin assays. The buffer used to work with annexin generates non-specificity with the Capture Beads.

Sample Preparation

Purification of Exosomes by Differential Ultracentrifugation

The kit has been validated for pre-enriched human exosomes from cell culture and bodily fluids, such as serum/plasma, and urine, through an ultracentrifugation protocol.

If it is planned to quantify the exosome preparation by Bradford assay to obtain the amount of total protein, do not resuspend in Assay Buffer as it contains BSA which will interfere with the measurement. For this purpose it is recommended to resuspend in PBS.



Sample Pretreatment for Direct Exosome Detection

The sample pretreatment required for direct exosome detection varies depending on the type of biological sample. Specific pretreatment protocols are available for each type of sample (e.g., plasma, urine), optimized according to their unique characteristics. Below, two sample pretreatment procedures are described: one for cell culture supernatant and another for plasma. To ensure that detected exosomes originate from your cells of interest, culture the cells with exosome depleted fetal bovine serum (FBS), because normal FBS contains extremely high levels of exosomes that will contaminate the cell derived exosomes.

Cell Culture:

Prepare the samples for the following centrifugation steps:

- 5 min at 200xg and 4°C. Collect supernatant and discard pellet.
- 10 min. at 14000xg and 4°C. Collect supernatant and discard pellet.

Plasma, serum and urine:

About 100–1000 µL of plasma/urine typically provides enough exosomes for most standard types of analysis.

- 10 min. at 500xg collect supernatant and discard pellet.
- 10 min. at 2000xg collect supernatant and discard pellet.
- 30 min. at 14000xg collect supernatant and discard pellet.

Protocol

Isolate Exosomes

1. Resuspend the capture beads by vortex for approximately 20 seconds.
2. Add 5 µL of the capture bead to each 12x75mm Polystyrene Round Bottom tube (cytometer tube). If an isotypic control needs to be added, please, do it at this point
3. Add 45 µL of ExoStep Incubation Buffer and a vortex for 20 seconds. The addition of this buffer is not mandatory, but it is recommended, as it will help to avoid unspecific binding.
4. Add between 10–15 µg of exosomes isolated by differential ultracentrifugation or up to 100 µL of direct exosomes. If protein quantification is not performed, the theoretical maximum binding capacity per bead has been estimated at 6,420 EVs per bead. This corresponds to approximately 3.85×10^7 exosomes per 5 µL bead reaction. However, experimental results indicate that saturation occurs at 3.6×10^9 EVs per test, suggesting that not all available exosomes bind during a single incubation step. Prepare the samples as described in the "Sample Preparation" section and transfer them to the appropriate tubes. Gently mix the reactions by pipetting up and down several times, followed by brief vortexing for a few seconds.
5. Incubate in the dark overnight at room temperature (RT). NO STIRRING. After incubation if only exosome isolation is needed, whithout FACS analysis, go directly to step 13.

Stain Exosomes for Flow Cytometry

6. After overnight incubation, add the suggested volume indicated of the primary detection antibody (not supplied) to the bead-bound exosomes tube. Mix gently by pipetting and/or by tapping. It is advisable to prepare an additional tube with the appropriate isotype control or without exosomes, for background determination.
7. Incubate in the dark 60 minutes at 2-8°C, without stirring.
8. Wash the sample (bead-bound exosomes) by adding 1 mL of assay buffer 1X.
9. Collect the magnetic beads by placing tubes on a magnetic rack and incubate 5 minutes or by centrifugation at 2500xg for 5 minutes. Remove supernatant from tubes by hand-decanting in the case of using the magnetic rack or by aspiration. Take care not to disturb the microspheres, and make sure not to leave more than 100 µL of supernatant in the tube.
   Go to step 13 for direct stain protocol (using direct detection antibody labeled with fluorochrome) for indirect stain protocol go to step 10.
10. Add 5 µL ofthe secondary detection reagen (not supplied) to each tube. Mix the reactions gently by pipetting up and down several times with a micropipette.
11. Incubate in the dark 30 minutes at 2-8°C, without stirring.
12. Wash the sample (bead-bound exosomes) by adding 1 mL of PBS with 1% BSA, pH 7.4.
13. Collect the magnetic beads by placing tubes on a magnetic rack and incubate 5 minutes or by centrifugation at 2500xg for 5 minutes. Remove supernatant from tubes by hand-decanting decanting in the case of using the magnetic rack or by aspiration. Take care not to disturb the microspheres, and make sure not to leave more than 100 µL of supernatant in the tube. For exosome magnetic isolation without FACS analysis, protocol ends here, on the contrary, continue with next step.
14. Resuspend the sample in 350 µL PBS with 1% BSA, pH 7.4. and acquire on a flow cytometer or store in the dark max up to 2 hours at 2-8°C, until the analysis is carried out.

Assay Acquisition

An adequate gating strategy FSC / SSC for 6 micron bead size and PerCP/APC, PerCP-Cy5/ APC or PerCP-Cy5.5/APC helps bead population identification and discrimination of doublets on flow cytometer.

1. Gate on the single population(s) on a Forward Scatter vs. Side Scatter plot in linear scale.
2. Gate on the single population(s) ona PerCP vs. APC channel (bead auto fluorescence) in logarithmic scale.
3. Using the PE channels, determine whether or not any bead populations tested "positive" for the exosome.

∆**Note:** *A positive bead will produce a fluorescent peak in the PE channel.*

Technical Support

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Version 1 | 2026-05-14

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