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ab267478 Exosome Isolation and Analysis Kit - Flow Cytometry, Cell culture

For the measurement of human exosomes in cell culture.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Exosome Isolation and Analysis Kit - Flow Cytometry, Cell culture (CD63/CD()(ab267478) is a simple immunobead assay for isolation/detection of pre-enriched CD63+ human exosomes from biofluids (plasma, urine) or cell culture media.

Using a bead-bound anti-CD63 capture antibody and a fluorochrome conjugated anti-CD9 detection antibody, the kit provides reproducible results and can be run in parallel to exosome immunophenotyping.

Incubate resuspended pre-enriched exosomes overnight with capture beads in the dark at room temperature

Add Anti-CD9 PE(Clone VJ1/20) and incubate in the dark for 60 minutes. Wash with assay buffer 1x

Collect magnetic beads and remove supernatant

Resuspend sample in 350 µL Assay Buffer 1X and store in the dark for up to 2 hours until the flow cytometry analysis is carried out

2. Materials Supplied and Storage

Store kit at 4°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted. Do not freeze.

Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Superparamagnetic capture beads (CD63+ [TEA3/18])	125 µL (5 µL/test)	4°C	4°C
Anti-CD9 antibody [VJ1/20] (PE)	125 µL (5 µL/test)	4°C	4°C
Assay Buffer 10X	10 mL	4°C	4°C
Incubation buffer	1.2 mL (45 µL/test)	4°C	4°C

3. Materials Required, Not Supplied

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

- Pre-enriched exosomes by ultra-centrifugation
- Magnetic rack; 12-hole, 12 x 75mm
- 12 x 75 mm polystyrene round bottom tubes (cytometer tubes)
- Sterile syringe filter with a 0.45 µm pore
- Syringes of appropriate volume

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

5.1 Superparamagnetic capture beads

Ready to use as supplied.

5.2 Anti-CD9 antibody [VJ1/20] (PE)

Ready to use as supplied.

5.3 Assay Buffer 10X

Dilute contents of the 10X Assay Buffer to 1X (PBS 1%BSA) in PBS, for use in this assay.

5.4 Incubation Buffer

Ready to use as supplied.

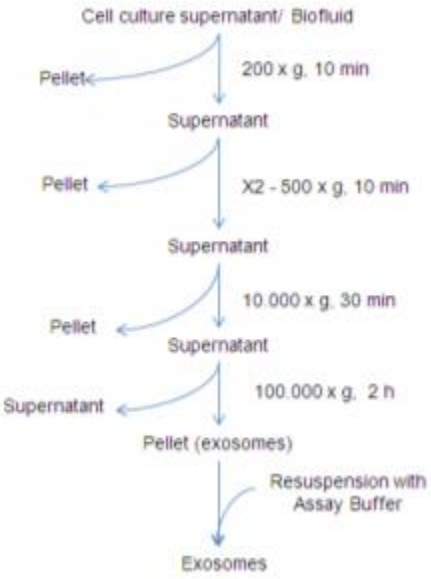
6. Sample Preparation

General sample information:

This kit allows the detection of isolated exosomes from differential ultracentrifugation as well as direct detection in the sample.

6.1 Purification of Exosomes by Differential Ultracentrifugation.

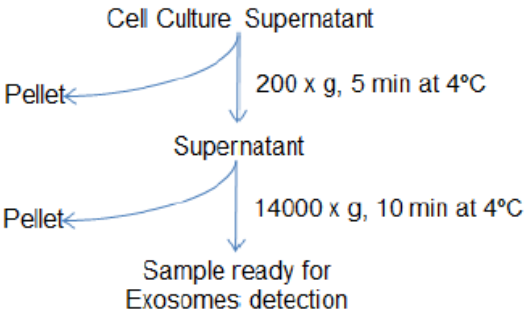
The kit has been validated for pre-enriched human exosomes from cell culture and bodily fluids, such as serum/plasma, and urine, through an ultracentrifugation protocol:



6.2 Sample pretreatment for direct exosome detection on cell culture supernatant.

The sample pretreatment for direct exosome detection from cell culture supernatant is not recommended for detection of exosomes from body fluids.

Specific sample pretreatment protocols are available for body fluids (plasma, urine) each optimized for its specific type of biological sample.
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.



7. Assay Procedure

7.1 Isolate CD63+ exosomes:

- Resuspend the capture beads by vortex for approximately 20 seconds.
- Add 5 µL of the capture bead to each 12 x 75 mm polystyrene round bottom tube (cytometer tube).
- Add 45µL of Incubation Buffer and a vortex for 20 seconds.
- Add 50µL of sample previously prepared according to "Sample Preparation" to the appropriate tubes. Mix the reactions gently by pippeting up and down several times with a pipette and vortexing for few seconds.
- Add between 10-15 µg of exosomes isolated by differential ultracentrifugation or up to 100 µl for direct exosomes, previously prepared according to "Sample Preparation", to the appropriate tubes. Mix the reactions gently by pipetting up and down several times with a pipette and vortexing for a few seconds.
- Incubate in the dark overnight at room temperature, without stirring.
- After overnight incubation wash sample (bead-bound exosomes) by adding 1 ml of Assay Buffer 1X.
- Collect the Magnetic beads by placing tubes on a magnetic rack and incubate 5 minutes or by centrifugation at 2,500 x *g* 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.

7.2 Stain exosomes for flow cytometry:

- After overnight incubation, add the suggested volume indicated of the Anti-CD9 antibody [VJ1/20] (PE) antibody (5 µl/test of the supplied antibody) 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.
- Incubate in the dark for 60 minutes at 2-8ºC, without stirring.
- Wash the sample (bead-bound exosomes) by adding 1 ml of Assay Buffer 1X.

- Collect the magnetic beads by placing tubes on a magnetic rack and incubate 5 minutes or by centrifugation at 2,500 x *g* for 5 minutes.
- Remove supernatant from tubes by hand-decanting 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.
- Resuspend the sample in 350 µL Assay Buffer 1X and analyze on a flow cytometer or store in the dark max up to 2 hours at 2-8°C, until the analysis is carried out.

7.3 Assay Acquisition.

An adequate gating strategy FSC / SSC for 6 micron bead size and PerCP/APC orPerCP-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 – see A below.
2. Gate on the single population(s) on a PerCP vs. APC channel (bead auto fluorescence) in logarithmic scale – see B below.
3. Using the FL2 channels, determine if any bead populations tested "positive" for the exosome.

Δ Note: A positive bead will produce a fluorescent peak in the FL2 channel.

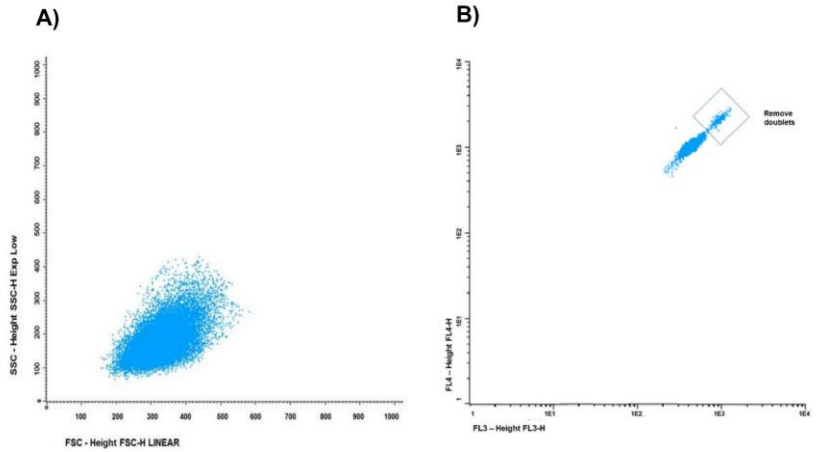


Figure 1: Dot-plot gating strategy f or acquisition and analysis. FSC v s SSC (A) and PerCP v s APC (B).

8. Recommendations and warnings

- Avoid microbial contamination of the reagent.
- Microspheres and reagents should be protected from prolonged exposure to light throughout this procedure.
- 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
- 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.

9. Typical Data

Data provided **for demonstration purposes only**.

ExoStep has been used for detection of exosome derived from different sources

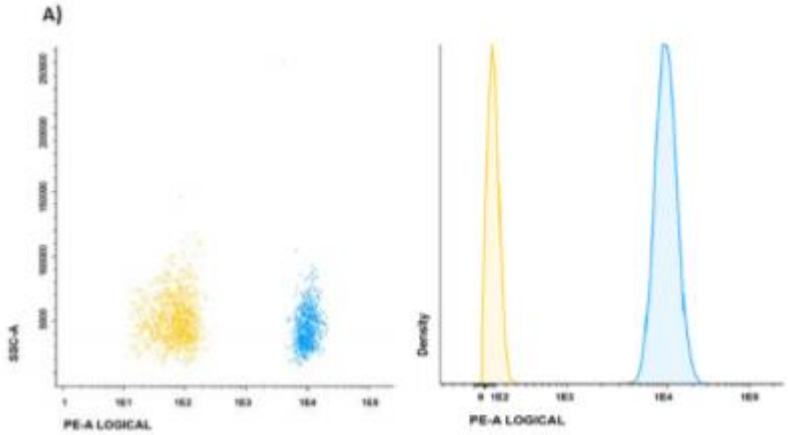


Figure 2: Flow analysis of exosomes bound to ExoStep. Cell culture exosomes, pre-enriched using Total Exosome Isolation from PC3 Cell Culture Media (A), were resuspended in PBS and bound to CD63-capture beads during an overnight incubation. The following day the bead-bound exosomes were indirect stained with primary detection antibody (CD9-PE/CD81-PE).

9.1 Performance Data

Limit of Detection (LOD), dynamic range and linearity of exosome kit was assessed.
LOD is the lowest quantity of exosomes that is distinguished from the absence of analyte (a blank value), and as a reference, was determined in >0,125 µg which corresponds with >1.5 x 10^8 vesicles. Whilst the upper limit or saturation level was established in 64 µg. For both technical specifications, exosomes from PC3 cell culture media (12 x 10^8 vesicles / µl) were used (Figure 5).

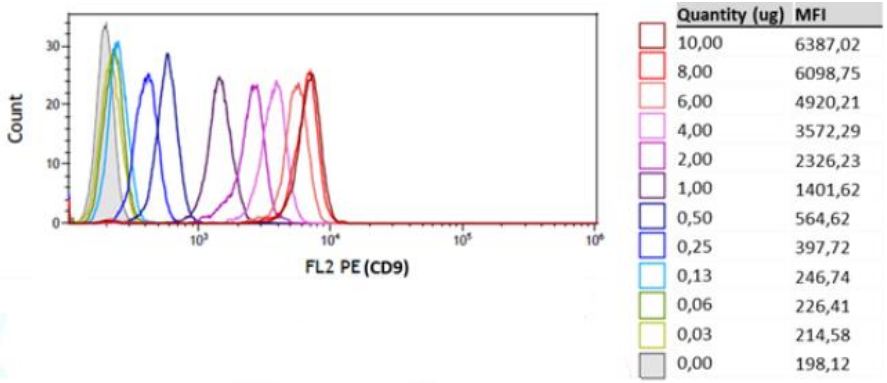


Figure 5. Dynamic range of the assay analyzed by flow cytometry. Relationship between background noise and specific signal at different exosome concentrations.

9.2 Reproducibility

Intra assay:
Was determined calculating the deviation and the CV for each of the samples by batch. Was analyzed the mean of all typical deviations and CVs of 3 days for each lot. Finally, was obtained the mean of the standard deviation and the CV of the three lots.

CV = 10%

Inter assay:
Was determined the mean of the 4 repetitions for each day and compare them between each batch taking the standard deviation and the CV. Was calculated the mean deviation thus obtained and the CV of the three days.

CV = 11%

10. Notes

Technical Support

For all technical or commercial enquiries please go to:

https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)