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ab267479
Exosome Isolation and Analysis Kit - Flow Cytometry, Plasma (CD9/CD81)

For the isolation and analysis of exosome from plasma, urine or cell culture media.

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1. Overview

Exosome Isolation and Analysis Kit - Flow Cytometry, Plasma (CD9/CD81) (ab267479) is a simple immunobead assay for isolation/detection of exosome, using a bead-bound anti-CD9 capture antibody and a PE conjugated anti-CD81 detection antibody. The kit provides reproducible results and can be run in parallel to exosome immunophenotyping.

The kit is intended for the immunoisolation (immunomagnetic or FACS) and Flow Cytometry analysis of pre-enriched CD9/CD81 human exosomes from biofluids (plasma, serum, urine) or cell culture media.

2. Protocol Summary

Prepare all reagents and samples as instructed

Add 5 µL resuspended capture beads to round bottom tubes. Add 10-15 µg (up to 100 µL) of exosomes isolated by differential centrifugation. Mix well and incubate in the dark overnight at RT.

Wash with 1 mL of 1X Assay Buffer.

Collect the beads either on magnetic rack or by centrifugation. Remove supernatant, taking care to not disturb the microspheres.

Add 5 µL of Primary DetectionAntibody (CD81-PE). Mix well and incubate in the dark for 60 mins at 4°C. Wash with 1X Assay Buffer.

Collect the beads either on magnetic rack or by centrifugation. Remove supernatant, taking care to not disturb the microspheres.

Resuspend in 350 µL 1X assay buffer and acquire on a flow cytometer (store for a maximum of 2 hours in the dark).

3. 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.

4. Materials Supplied, and Storage and Stability

- Store kit at +4°C in the dark immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Do not freeze.

Item	Quantity	Storage condition
CD9+ Capture beads	6000 beads/test (5 µL/test)	+4°C
Primary detection antibody: Anti-CD81-PE [Clone M38]	5 µL/test	+4°C
Assay Buffer 10X	10 mL	+4°C
Incubation Buffer	1.2 mL	+4°C

5. 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, 12x75mm.
- 12x75 mm Polystyrene Round Bottom Tubes (cytometer tubes).
- Sterile syringe filter with a 0.45 µm pore.
- Syringe of adequate volume

6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 CD9+ Capture beads:

Polystyrene micro-particles with Mean Diameter (µm) 6.5±0.2 (CV<5%), having dicrete fluorescence intensity characteristics. Ready to use.

6.2 Primary detection antibody: Anti-CD81-PE:

Ready to use.

6.3 Assay Buffer 10X:

Dilute contents of the 10X Assay Buffer to 1X (PBS 1% BSA) in PBS, for use in this assay. Assay buffer 1X can be filtered before use to avoid microbial contamination of the reagent.

6.4 Incubation Buffer:

Ready to use.

7. Sample Preparation

General sample information:

- The kit allows the detection of isolated exosomes from differential ultracentrifugation as well as direct detection in the sample without the need for ultracentrifugation, just with simple pre-treatment.

7.1 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.
- For reference, after pre-enrichment protocol, exosomes resuspension at $2.4x10^9$ /50µL concentration will be suitable.
- The principle for exosome purification is the same for cell culture and bodily fluids, but due to the viscosity of some fluids it is necessary to dilute them with an equal volume of PBS, before centrifugations.

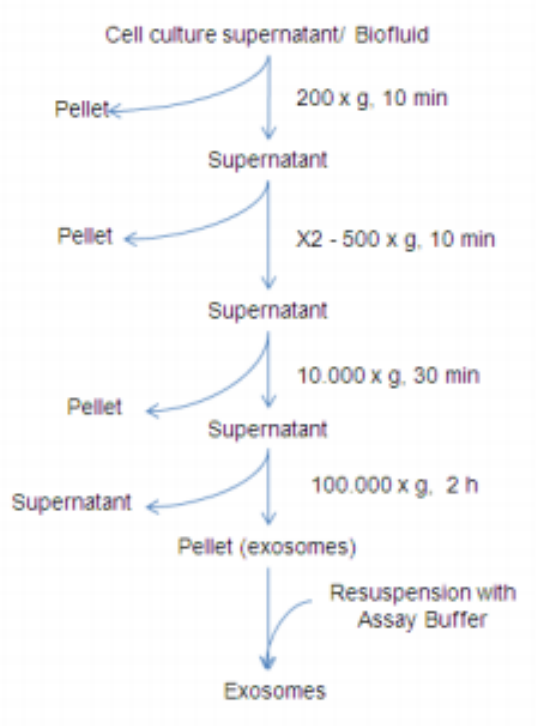

Figure 1. Workflow for the exosome pre-enrichment based on differential ultracentrifugation.

7.2 Sample pretreatment for direct exosome detection on human plasma.

- The sample pretreatment for direct exosome detection from plasma is not recommended for detection of exosomes from any otherbody fluids or cell culture media.
- 100 -1000 µL of plasma typically provides enough exosomes for most standard types of analysis.

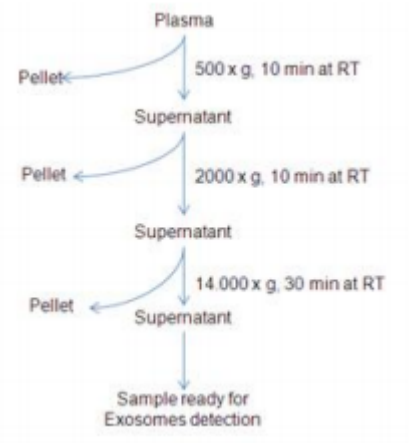

Figure 2. Plasma pre-treatment workflow for direct exosome detection.

8. Recommendations and Warnings

8.1 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. Assay Procedure

9.1 Isolate CD9$^+$ exosomes:

9.1.1 Resuspend capture beads by vortex for approximately 20 s.

9.1.2 Add 5 µL of the capture bead to each 12x75 mm Polystyrene Round Bottom tube (cytometer tube).

9.1.3 Add 45µL of Incubation Buffer and a vortex for 20 seconds

9.1.4 Add between 10-15 µg of exosomes isolated by differential ultracentrifugation or 100 µl for direct exosomes, previously prepared according to Section 7, to the appropriate tubes. Mix the reactions gently by pipetting up and down several times with a pipette and vortexing for few seconds.

9.1.5 Incubate in the dark overnight at room temperature. DO NOT STIR.

9.1.6 After overnight incubation wash the sample (bead-bound exosomes) by adding 1 ml of Assay Buffer 1X.

9.1.7 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 (Fig. 4) 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.

9.2 Stain exosomes for flow cytometry:

9.2.1 After overnight incubation, add the suggested volume indicated of the Primary detection antibody (5µL/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.

9.2.2 Incubate in the dark 60 min at 2-8°C, without stirring.

9.2.3 Wash the sample (bead-bound exosomes) by adding 1 mL of Assay Buffer 1X.

9.2.4 Collect the Magnetic beads by placing tubes on a magnetic rack and incubate 5 min or by centrifugation at 2,500 x *g* for 5 min. Remove supernatant from tubes by hand-decanting in the case of using a magnetic rack or by aspiration.

Δ **Note**: Take care not to disturb the microspheres, and make sure not to leave more than 100 µL of supernatant in the tube.

9.2.5 Resuspend the sample in 350 µL Assay Buffer 1X 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.

9.3 Assay Acquisition:

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

9.3.1 Gate on the single population(s) on a Forward Scatter vs. Side Scatter plot in linear scale (Figure 3A).

9.3.2 Gate on the single population(s) on a PerCP vs. APC channel (bead auto fluorescence) in logarithmic scale (Figure 3B).

9.3.3 Using the FL2 channels, determine whether or not any bead populations tested "positive" for the exosome.

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

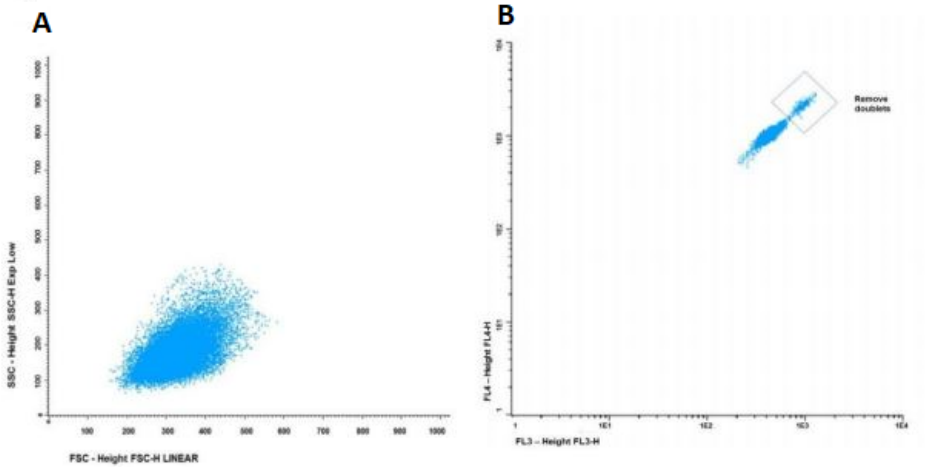

Figure 3. Dot-plot gating strategy for acquisition and analysis. FSC vs SSC (A) and PerCP vs APC (B).

10. Typical Data

10.1 Flow cytometry analysis

Flow cytometry analysis - data provided **for demonstration purposes only**.

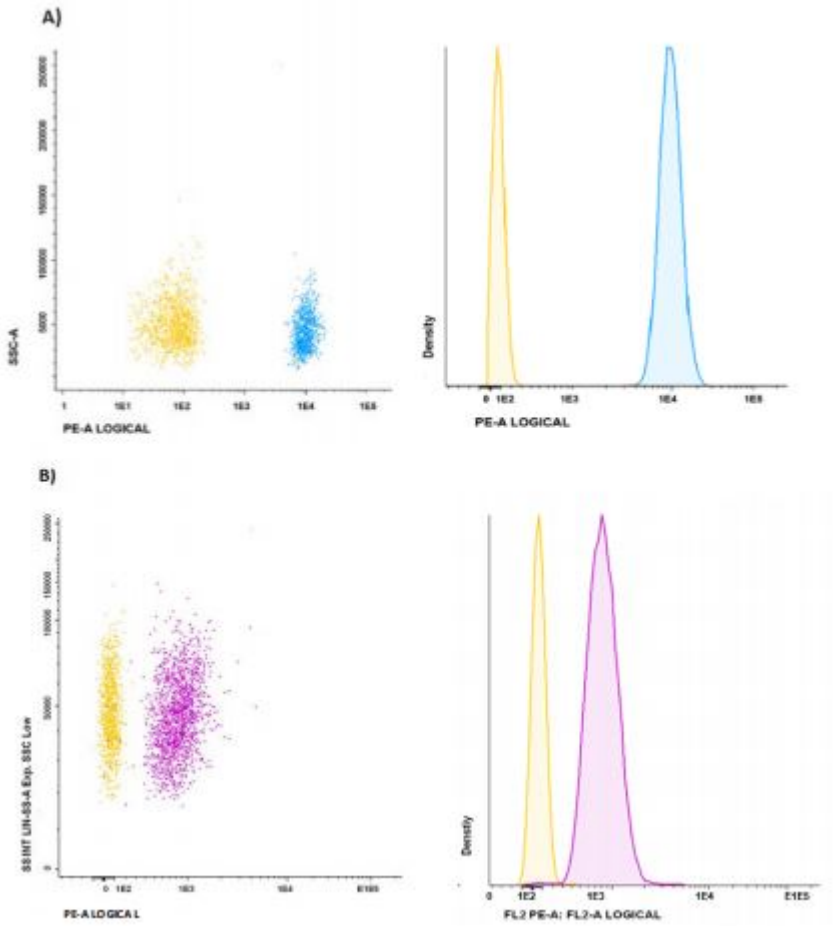

Figure 4. Flow analysis of exosomes. Cell culture exosomes, pre-enriched using Total Exosome Isolation from PC3 Cell Culture Media (A) and human plasma (B), were resuspended in PBS and bound to CD9-capture beads during an overnight incubation. The following day the bead-bound exosomes were direct stained with primary antibody detection (CD9/CD81-PE) and analyzed by flow cytometry.

10.2 Performance Data

Limit of Detection (LOD), dynamic range and linearity of the kit was assessed.

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

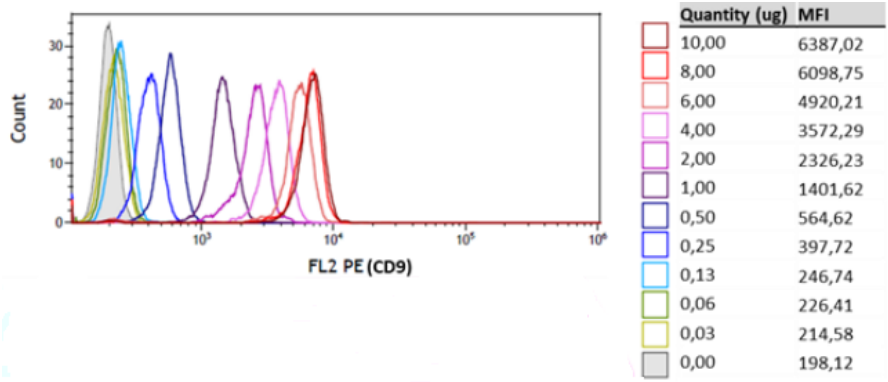

Figure 5. Dynamic range of the assay analyzed by flow cytometry. Relationship between background noise and specific signal at different exosome concentrations.

Several measurements of multiple concentrations of lyophilized exosomes were analyzed across the reportable range of the kit, finding the linearity of the kit in a broad range of concentrations, allowing fluorescence interpolation in the estimation of concentrations.

10.3 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%

11. Notes

Technical Support

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