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ab267480 Exosome Isolation and Analysis Kit - Flow Cytometry, Urine (CD63 / CD9)

For the measurement of exosomes in human urine.

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

Table of Contents

1. Overview

Exosome Isolation and Analysis Kit - Flow Cytometry, Urine (ab267480) 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.

2. Protocol Summary

Incubate resuspended pre-enriched exosomes overnight with capture beads in the dark at room temperature, with no stirring.

Add Anti-CD9 antibody [VJ1/20] (PE) and incubate in the dark for 60 minutes at 4 °C, without stirring

Wash sample by adding 1 mL Assay Buffer 1X

Collect magnetic beads using magnetic rack or by centrifugation, remove supernatant.

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

Store kit at 4°C in the dark immediately on receipt and check below for storage for individual components. **Do not freeze.** 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.

Item	Quantity	Storage temp (before prep)	Storage temp (after prep)
Superparamagnetic Capture Beads (CD63[TEA3/18])	3000 beads/test (50 µL/test) *	4°C	4°C
Anti-CD9 antibody [VJ1/20] (PE)	5 µL/test *	4°C	4°C
Assay Buffer 10X	10 mL	4°C	4°C

*Enough provided for 25 tests.

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
- 12x75 mm polystyrene round bottom tubes (cytometer tubes)
- 0.45 µm sterile syringe filter
- 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 Superparamagnetic Capture Beads (CD63[TEA3/18])

Ready to use as supplied.

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

Ready to use as supplied.

6.3 Assay Buffer 10X

Dilute to 1X for use in this assay.

7. Sample Preparation

General sample information:

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

7.1 Purification of exosomes by differential ultracentrifugation:

This 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.

The principal 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.

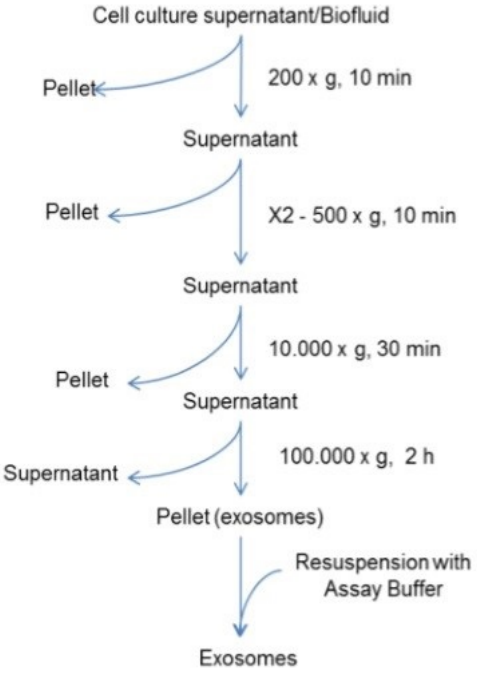

Figure 1. Workflow for the exosome pre-enrichment based on differential ultracentrifugation.

7.2 Sample pre-treatment for direct exosome detection on human urine:

The sample pre-treatment for direct exosome detection from urine is not recommended for detection of exosomes from any other bodily fluids or cell culture media.
0.8 – 10 mL of urine typically provides enough exosomes for most standard types of analysis.

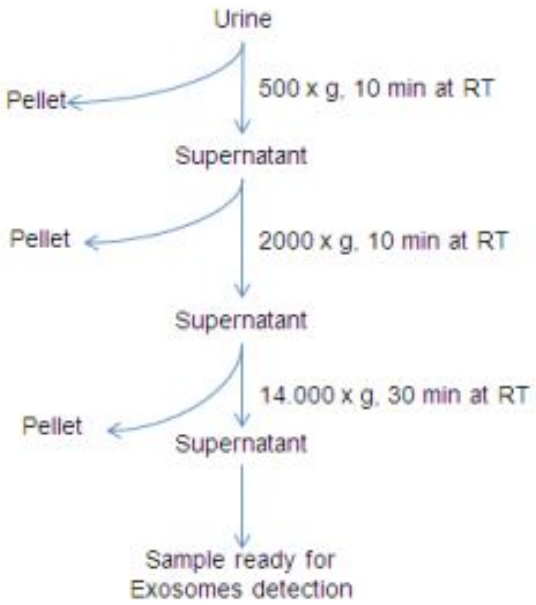

Figure 2. Urine pre-treatment workflow for direct exosome detection.

Add assay buffer 10x to the sample up to final concentration of 1% (block the sample).

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 $CD63^+$ exosomes:

1. Resuspend the capture beads by vortex for approximately 20 seconds.
2. Add 100 µL of the capture bead to each 12 x 75 mm polystyrene round bottom tube (cytometer tube).
3. Add 50 µL of sample previously prepared according to Section 6 Sample Preparation to the appropriate tubes. Mix the reactions gently by pipetting up and down several times with a pipette and vortexing for few seconds.
4. Incubate in the dark overnight at room temperature, without stirring.
5. After overnight incubation wash the sample (bead-bound exosomes) by adding 1 ml of Assay Buffer 1X.
6. 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.

9.2 Stain exosomes for flow cytometry:

1. After overnight incubation, Add the suggested volume indicated of the Anti-CD9 antibody [VJ1/20] (PE) (5 µL 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.
2. Incubate in the dark 60 minutes at 2-8°C, without stirring.
3. Wash the sample (bead-bound exosomes) by adding 1 mL of Assay Buffer 1X.

4. 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.
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.

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.

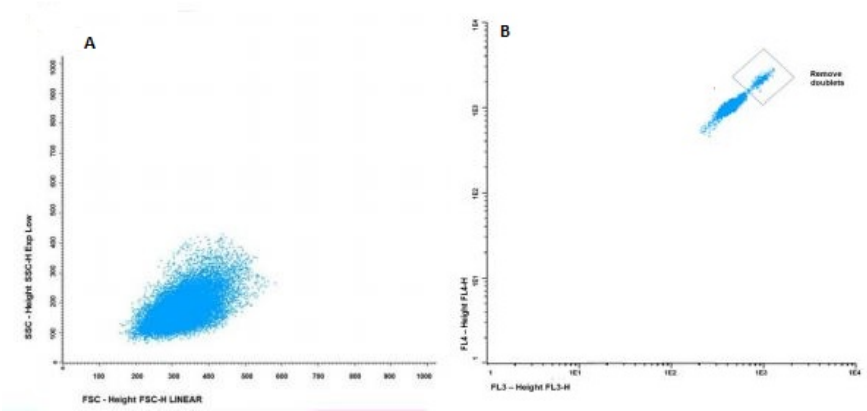

Figure 3. Dot-plot gating strategy for acquisition and analysis. FSC vs SSC (A) and PerCP v s APC (B).

10. Typical Data

10.1 Flow Cytometry Analysis:

Exosome Isolation and Analysis Kit - Flow Cytometry, Urine (CD63 / CD9) (ab267480) has been used for detection of exosome derived from different sources.

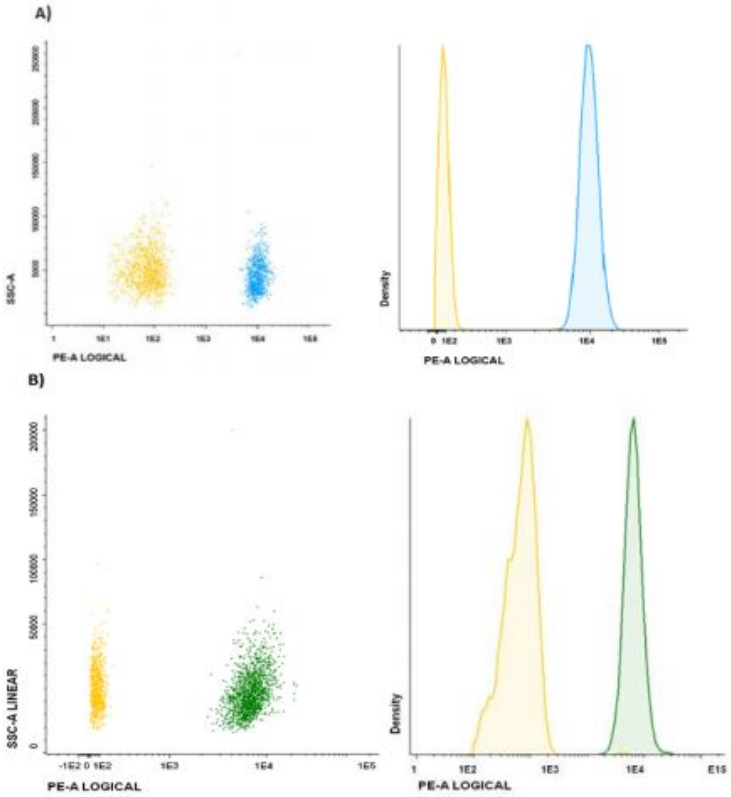

Figure 4. Flow analysis of exosomes. Cell culture exosomes, pre-enriched using Total Exosome Isolation from PC3 Cell Culture Media (A), were

resuspended in PBS and bound to CD63-capture beads during an overnight incubation. The following day the bead-bound exosomes were direct stained with primary detection antibody (CD9-PE) and analyzed by flow cytometry.

10.2 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 4).

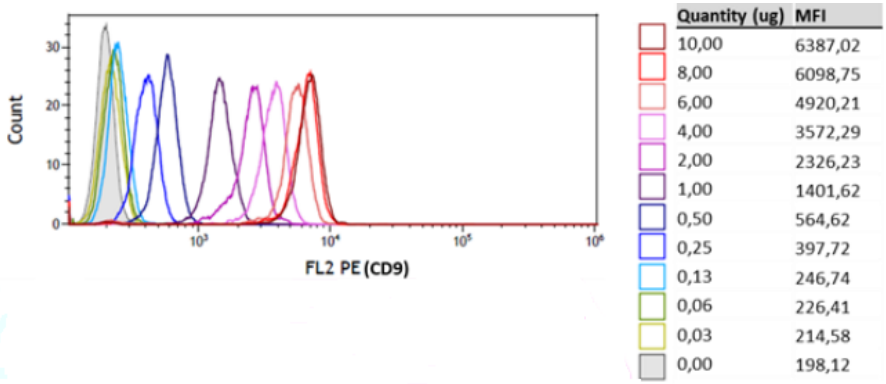

Figure 5. Dynamic range of the assay analyzed by flow cytometry. Relationship between background noise and specific signal at different exosome concentrations.

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

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)