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ab239686 CD63 Exosome Capture Beads

For the isolation and detection of exosome.

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

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1. Overview

CD63 Exosome Capture Beads (ab239686) consists of a simple bead population, coated with a capture antibody (CD63) for isolation/detection of exosome.

The beads are intended for the immunoisolation (immunomagnetic or FACS) and Flow Cytometry analysis of pre-enriched CD63+ human exosomes from biofluids (plasma, urine) or cell culture media.

2. Protocol Summary

Prepare all reagents and samples as instructed

Add 50 µL resuspended capture beads to round bottom tubes. Add 50 µL of sample to appropriate tube. Mix well and incubate in the dark overnight at RT.

Add 5 µL Primary detection antibody. 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 100 µL of 1X assay buffer and mix gently.

Add 5 µL of secondary detection reagent. Mix well and incubate in the dark for 30 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.

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 at +4°C in the dark immediately upon receipt. Kit can be stored for 1 year from receipt, if component has not been reconstituted.
- Do not freeze.

Item	Quantity	Storage condition
CD63+ [Clone TEA3/18] capture beads	125 µL (5 µL x 25 tests) **or** 1250 µL (50 µL x 25 tests)	+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 reagent 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 CD63+ [Clone TEA3/18] capture beads:

Superparamagnetic Capture Beads. CD63+ [Clone TEA3/18] capture beads. Polystyrene micro-particles with Mean Diameter (µm) 6.5±0.2 (CV<5%), having discrete fluorescence intensity characteristics. Ready to use.

7. Sample Preparation

General sample information:

- CD63 capture beads 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 pretreatment.

7.1 Purification of Exosomes by Differential Ultracentrifugation.

- The product 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.

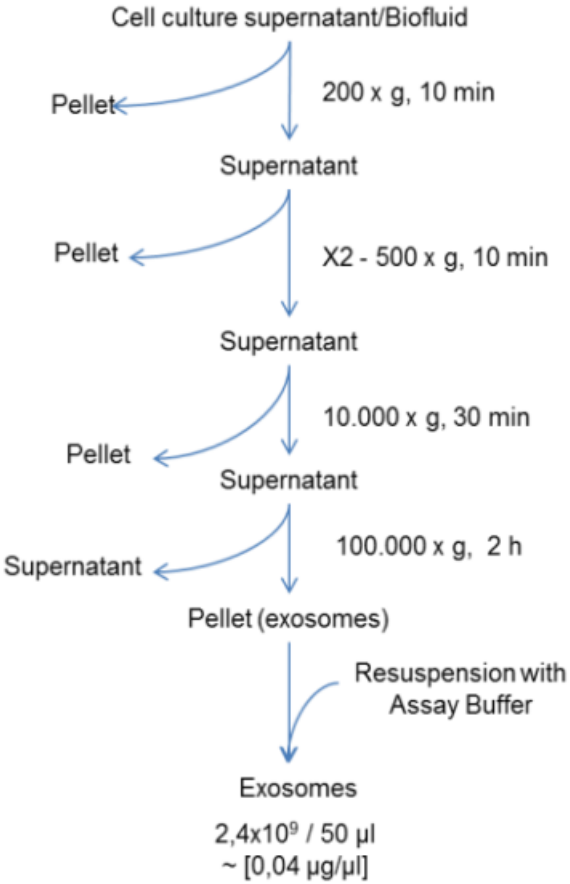

Figure 1. Workflow for the exosome pre-enrichment based on differential ultracentrifugation.

7.2 Sample pretreatment for direct exosome detection.

- 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.

7.2.1 Cell culture supernatant:

- 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.

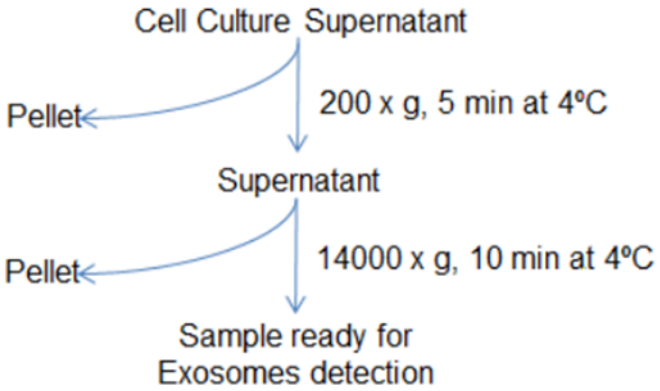

Figure 2. Cell Culture Supernatant pre-treatment workflow for direct exosome detection.

7.2.2 Plasma and serum:

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

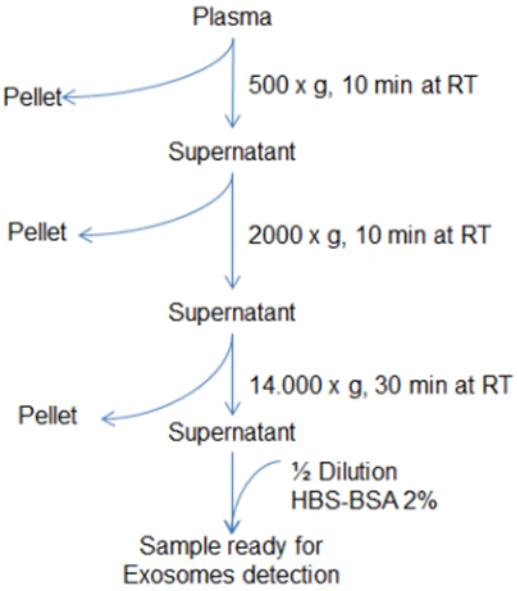

Figure 3. Plasma pre-treatment workflow for direct exosome detection.

7.2.3 **Urine:**

– 0.8 – 10 mL of urine typically provides enough exosomes for most standard types of analysis.

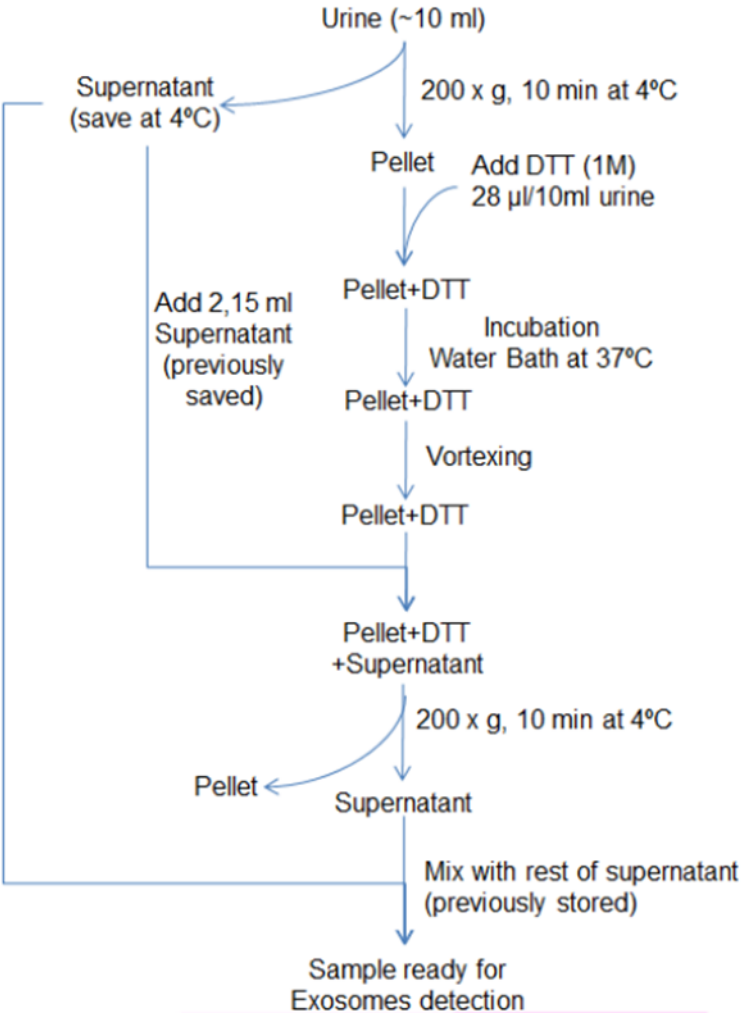

Figure 4. Urine pre-treatment workflow for direct exosome detection.

8. Assay Procedure

– Please note that to continue with the staining part of this assay you will need a primary detection antibody, secondary detection reagent and Assay buffer 10X.

8.1 Isolate exosomes:

8.1.1 Resuspend capture beads by vortex for approximately 20 s.

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

***Note: If you received 1250 uL of capture beads in your vial then use 50 uL of capture beads instead.**

8.1.3 Add 50µL of sample, 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.

8.1.4 Incubate in the dark overnight at room temperature. No stirring.

8.2 Stain exosomes for flow cytometry:

8.2.1 After overnight incubation, add the Primary detection antibody (5µL) 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.

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

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

8.2.4 Collect the Magnetic beads by placing tubes on a magnetic rack and incubate 5 min or by centrifugation at 2500 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.

8.2.5 Remove the tubes from the magnetic rack and resuspend the microspheres in the remaining 100 µL of Assay Buffer 1X. Mix gently by pipetting.

8.2.6 Add 5 µL of the Secondary detection reagent to each tube. Mix the reactions gently by pipetting up and down several times with a micropipette.

8.2.7 Incubate in the dark 30 min at 2-8°C, without stirring.

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

8.2.9 Collect the Magnetic beads by placing tubes on a magnetic rack and incubate 5 min or by centrifugation at 2500 x *g* for 5 min. Remove supernatant from tubes by Hand-decanting in the case of using the 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.

8.2.10 Resuspend the sample in 350 µL Assay Buffer 1X and acquire on a flow cytometer or store in the dark max up to 2 h at 2-8°C, until the analysis is carried out.

8.3 Assay Acquisition:

– An adequate gating strategy FSC/SSC for 6 micron bead size and FL3/FL4, helps bead population identification and discrimination of doublets on flow cytometer.

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

8.3.2 Gate on the single population(s) on a FL3 vs. FL4 channel (bead auto fluorescence) in logarithmic scale (Figure 3B).

8.3.3 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.

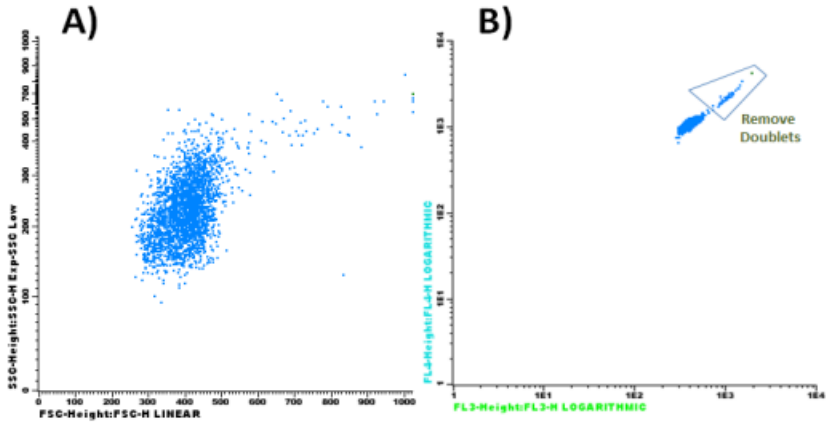

Figure 5. Dot-plot gating strategy for acquisition and analysis. FSC vs SSC (A) and FL3 vs FL4 (B).

9. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

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