ab326320 – Mouse Exosome Detection Kit

For research use only - not intended for diagnostic use.

The kit is a simple immunobead assay for isolation/detection of exosome, 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.

Storage and Stability:

The entire kit may be stored in the dark at 4°C. DO NOT FREEZE. The kit is stable until the expiry date stated on the box label if kept at 4°C. Do not use after the date indicated. For prepared reagent storage, see table below.

Materials Supplied

Item	Quantity	Storage Condition
Incubation Buffer	25 Test	4°C
Assay Buffer 10X	10 mL	4°C
Secondary detection reagent	25 Test	4°C
Superparamagnetic Capture Beads	25 Test	4°C
Primary detection antibody	25 Test	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 (Stand 12-hole, 12x75mm).
- 12x75 mm Polystyrene Round Bottom Tubes (cytometer tubes).
- Sterile syringe filter with a 0.45 µm pore
- Syringe of adequate volume.

Recommendations

a. Avoid microbial contamination of the reagent.
b. Assay buffer 10X can be filtered before use.
c. Microspheres and reagents should be protected from prolonged exposure to light throughout this procedure.
d. 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.
e. The samples should be treated with appropriate handling procedures.
f. 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.
g. Deviations from the recommended procedure could invalidate the analysis results.
h. Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.
i. The isolation and detection success is dependent on the quality of the sample pre-enrichment process.
j. Pay attention if the kit is used in combination with annexin assays. The buffer used to work with annexin generates non-specificity with the Mouse Exosome Detection kit.

Sample Preparation

This kit allows the detection of isolated exosomes from differential ultracentrifugation, precipitation or size exclusion purification as well as direct detection in the sample without previous isolation and just with simple pretreatment.

a) <u>Purification of Exosomes by Differential Ultracentrifugation</u>
The kit has been validated for pre-enriched human exosomes from cell culture and body fluids, such as serum/plasma, and urine, through an ultracentrifugation protocol. The principle for exosome purification is the same for cell culture and body fluids, but due to the viscosity of some fluids it is necessary to dilute them with an equal volume of PBS, before centrifugations.

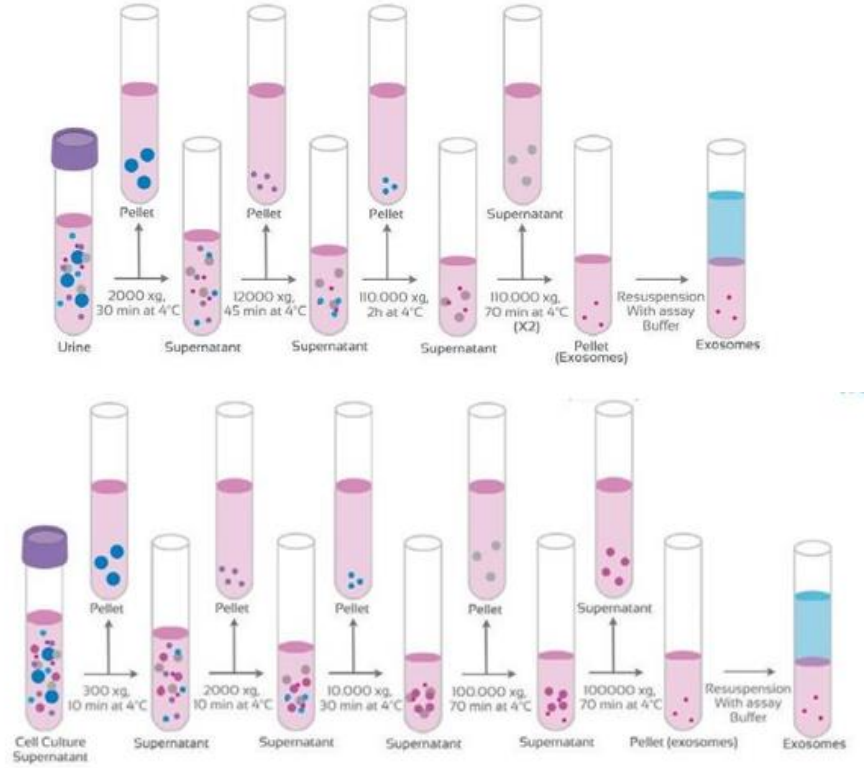

Figure 1: Workflow for the exosome pre-enrichment based on differential ultracentrifugation for Cell culture supernatant and Urine samples.

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.

b) 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 cells derived exosomes.

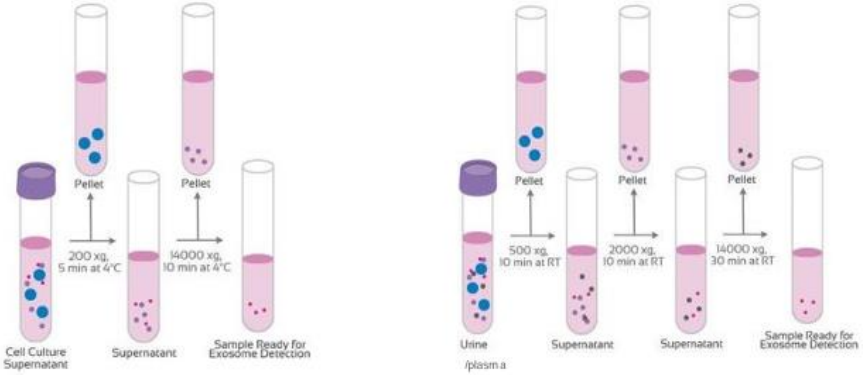

Figure 2: Cell culture, Human plasma and Urine pretreatment workflow for direct exosome detection

Protocol

Isolate CD63+ exosomes

1. Resuspend the capture beads by vortex for approximately 20 seconds.
2. Add 5µL of the capture bead to each 12x75 mm Polystyrene Round Bottom tube (cytometer tube).
3. Add 45µL of Incubation Buffer and a vortex for 20 seconds.
4. Add between 10-15ug of exosomes isolated by differential ultracentrifugation or until 100ul 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 few seconds.
5. Incubate in the dark overnight at room temperature (RT). NO STIRRING.
6. After overnight incubation wash the sample (bead-bound exosomes) by adding 1ml of Assay Buffer 10X.
7. 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 (Fig. 4) or by aspiration. Take care not to disturb the microspheres, and make sure not to leave more than 100ul of supernatant in the tube. Alternatively, you can place the tubes in a magnetic rack and incubate them for 5 minutes, to allow the magnetic beads (together with the exosomes attached to them) to migrate towards the magnet. The supernatant is then usually manually decanted, taking care not to disturb the bead pellet. Alternatively, the sample can be centrifuged at 2500 × g for 5 minutes to separate the microspheres. After centrifugation, the supernatant should be removed, preferably by aspiration, to avoid disturbing the pellet.

Stain exosomes for Flow Cytometry

8. After overnight incubation, add the suggested volume indicated of the primary detection antibody (5µL 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. Incubate in the dark 60 minutes at 2-8ºC, without stirring.
10. Wash the sample (bead-bound exosomes) by adding 1ml of Assay Buffer 10X.
11. 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 (Fig.4) or by aspiration. Take care not to disturb the microspheres, and make sure not to leave more than 100ul of supernatant in the tube.
12. Remove the tubes from the magnetic rack and resuspend the microspheres in the remaining 100µL of Assay Buffer 10X. Mix gently by pipetting.
13. Add 5µL of the secondary detection reagent to each tube. Mix the reactions gently by pipetting up and down several times with a micropipette.
14. Incubate in the dark 30 minutes at 2-8ºC, without stirring.
15. Wash the sample (bead-bound exosomes) by adding 1ml of Assay Buffer 10X.
16. 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 (Fig.4) or by aspiration. Take care not to disturb the microspheres, and make sure not to leave more than 100ul of supernatant in the tube.
17. Resuspend the sample in 350µL Assay Buffer 10X and acquire on a flow cytometer or store in the dark max up to 2 hours at 2-8ºC, until the analysis is carried out.

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.

1. Gate on the single population(s) on a forward scatter vs. side scatter plot in linear scale (Figure 3A).
2. Gate on the single population(s) on a FL3 vs. FL4 channel (bead auto fluorescence) in logarithmic scale (Figure 3B).
3. Using the FL2 channels, determine whether or not any or not any bead populations tested "positive" for the exosome. Note: A positive bead will produce a fluorescent peak in the FL2 channel.

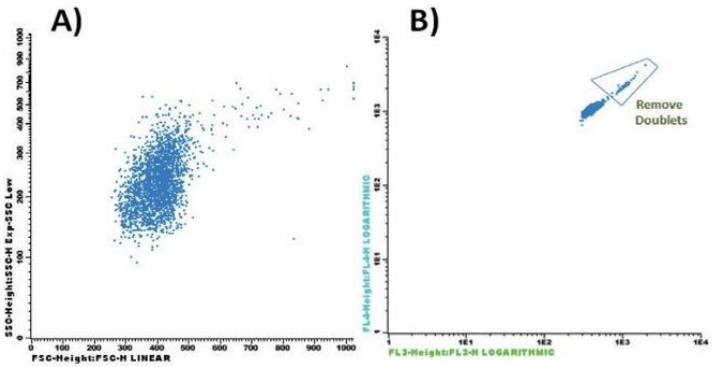

Figure 3: Dot-plot gating strategy for acquisition and analysis. FSC vs SSC (A) and FL3 vs FL4 (B)

Technical Support

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For all technical or commercial enquiries please go to:
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