

ab287876 – ExoFACS Kit

For exosome isolation and overall capture of pre-isolated exosomes and lyophilized exosomes from cell culture supernatants or human biological fluids.
For research use only - not intended for diagnostic use.

Storage and Stability

All the reagents are shipped and stored at 4°C for up to 12 months, if unopened. Briefly centrifuge small vials prior to opening. DO NOT FREEZE!

Materials Supplied

Item	Quantity	Storage Condition
5X Sample Buffer	10 ml	4 °C
ExoPure Reagent	20 ml	4 °C
Exosome Standards (100 ug)	100 µg	4 °C
FACS-beads	1 vial	4 °C
Primary Antibody (Mouse Anti-human CD63 antibody)	20 µl	4 °C
Secondary Antibody (Alexa 488)	3 µl	4 °C

Materials Required, Not Supplied

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

- Single-use and/or pipettes with disposable tips 2-100 µl
- Polypropylene tubes
- Pipettes 1 ml and 5 ml for reagent preparation
- Deionized water
- PBS
- BSA or FBS or FCS
- Disposable pipetting reservoirs
- Prepare the Washing buffer (not provided in the kit)
- FACS tubes

Reagent Preparation

- Cap all the bottles well immediately after each use, to prevent evaporation.
- ExoFACS contains reagents and antibodies for 20 reactions.
- Exosome standards must be reconstituted in 100 µl of deionized water.
- Isolation Component is included in the kit for exosome isolation. Beads are ready to use for exosome capture.
- Primary and secondary antibody must be appropriately diluted in sample buffer.
- 1 vial (100 µg) of exosome standards (lyophilized), (possibility to choose from the list of lyophilized exosome standards from cell media available (number of particles/ml 1×10^{10})).
- Exosome standards: The remaining reconstituted standard stock solution should be aliquoted into polypropylene vials (preferably low binding) and stored at -20°C for up to one month or at -80°C for up to six months. Strictly avoid repeated freeze-and-thaw cycles.
- Store opened and diluted reagents at 4°C up to 12 months if unopened.

Assay Procedure

Human Cell culture medium sample preparation:

1. Prepare cell supernatants by 3 centrifugation steps. After each step, transfer the supernatant to a new tube and discard the pellet.
 - a) 10 min at 300g at 4°C (save supernatant; discard pellet).
 - b) 20 min at 1600g at 4°C (save supernatant; discard pellet).
 - c) 30 min at 10,000g at 4°C (save supernatant; discard pellet).
2. Exosome isolation from Cell Culture Medium:

Fluid	Minimum volume required	Volume requested
Cell medium	1 ml	1 ml – 5 ml

3. Add Isolation Component to your sample in ratio 1:1 (i.e. 1 ml of cell medium + 1 ml of Isolation Component).
4. Mix well by pipetting and inverting tube.
5. Incubate on ice for 1 hr.
6. Centrifuge 20 min at 10,000g (centrifuge can be performed at 4°C or at RT).
7. Discard the supernatant.
8. Centrifuge for 2 min at 1500g to eliminate entirely the supernatant.
9. Resuspend the pellet in 100 µl* of 1X PBS.
* **Δ Note:** Volume of resuspension can be defined by the user on the base of downstream analysis.

Lyophilized Exosome Standard reconstitution:

1. Reconstitute lyophilized exosome standard by adding 100 µl of deionized water to get a final concentration of 1 µg/µl.
2. Resuspend exosomes pipetting the solution up and down 10-15 times, avoiding bubbles.
3. Vortex the reconstituted standard for 60 secs. Briefly centrifuge the tubes containing the standard to ensure that the solution is collected at the bottom of the tube. Pipette the solution up and down 10 times, avoiding the introduction of bubbles. After this step, the standard is ready to use.
4. Use 5 µl of reconstituted Exosome Standard for each reaction.

Exosome binding onto latex FACS-Beads:

1. Dilute 5X Sample buffer with 1X PBS to prepare 1X Sample buffer.
2. Latex FACS-Beads are ready to use. Resuspend well FACS-Beads prior to use by vortexing or pipetting several times.
3. For each reaction, mix together 5 µl of Exosome Standards and 5 µl of FACS-beads in an eppendorf tube (preferably low binding). Mix well by pipetting 5-6 times. Example: if you want to run 10 reactions, mix into the same eppendorf low binding tube 50 µl of Exosome Standards and 50 µl of FACS-Beads.
4. For exosome isolated using Isolation Component, mix 5 µl of FACS-Beads with the volume of resuspended exosomes suggested (volumes are indicative only. The user should define the appropriate volumes on the base of exosome yield). Cell medium: 50 µl - 100 µl /reaction.
5. Incubate for 15 min at room temperature (RT).
6. Add 0.7 ml of 1X PBS and incubate in rotator or shaker for 2 hr at RT or overnight (ON) at 4°C.
7. Centrifuge the complex Exosomes-Beads (Exo-Beads) for 5 min at 4500g at 4°C and discard the supernatant.

8. Add 1 ml of 1X Sample Buffer, resuspend Exo-Beads for 5-6 times and incubate for 15 min at RT.
9. Centrifuge for 5 min at 4500g at 4°C and discard the supernatant.

Antibody Incubation:

1. Prepare the Washing buffer (not provided in the kit) diluting 2% of FBS (or FCS) in 1X PBS (consider that you need 8 ml of washing buffer for each reaction). Alternatively, if you don't have FBS or FCS, prepare the Washing buffer diluting 0.5% of BSA in 1X PBS. Keep on ice.
2. Resuspend the Exo-Beads in 100 µl of Sample buffer for each reaction. Example: if you are running 10 reactions resuspend Exo-Beads in 1 ml of Sample buffer.
3. Prepare the FACS tubes (not provided in the kit), one tube for each reaction.
4. Divide the Exo-Beads resuspended in sample buffer in each FACS tube, pipetting 100 µl of suspension in each tube.
5. Add primary antibody in ratio 1:200 (0.5 µl per each FACS tube).
 Δ **Note:** If other primary antibodies are used the correct dilution must be defined by the user. f) Incubate for 2 hr at 4°C in the dark). For negative control, PE or FITC-anti- Mouse IgG1 isotype or FITC or PE anti-Rabbit IgG1 can be used (isotype controls not provided in the kit). Otherwise, incubate control samples with secondary Abs only (either leave in ice or directly add sec Abs).
6. Add 4 ml of prepared Washing buffer to each FACS tube.
7. Centrifuge 5 min at 4000g and discard the supernatant.
8. Resuspend beads pellet into the FACS tubes in 100 µl of 1x Sample buffer.
9. Add secondary antibody in ratio 1:2000 (mix 57 µl of 1x Sample buffer with 3 µl of secondary antibody; add 1 µl of the received solution per each FACS tube to obtain the right dilution of antibody).
10. Incubate for 1 hr in the dark at 4°C.
10. Add 4 ml of Washing buffer in each FACS tube, centrifuge for 5 min at 4000g at 4°C. Discard supernatant by pouring it out. Vortex what has remained inside the FACS tubes. If not use immediately, put in the dark at 4°C.
11. Add 500 µl of Washing buffer per FACS tube.
12. Analyze the samples.
13. Read 10,000 events from the gated first population.
14. Alexa 488 is read in FL1 channel (green)

Reproducibility

ExoFACS is a useful tool for exosome protein profiling by using FACS technique. ExoFACS was used for a protein marker profile in exosomes derived from different sources. Exosome binding on FACSbeads was performed by incubation at 4°C overnight. Exosome-bead complex is ready to be labeled with fluorophore-conjugated antibodies for specific exosome markers. Figure 2 shows a profile of expression of three different exosome markers in exosomes purified from Melanoma (MM1), Neuroblastoma (SH) and Glioblastoma (U87) cell supernatants.

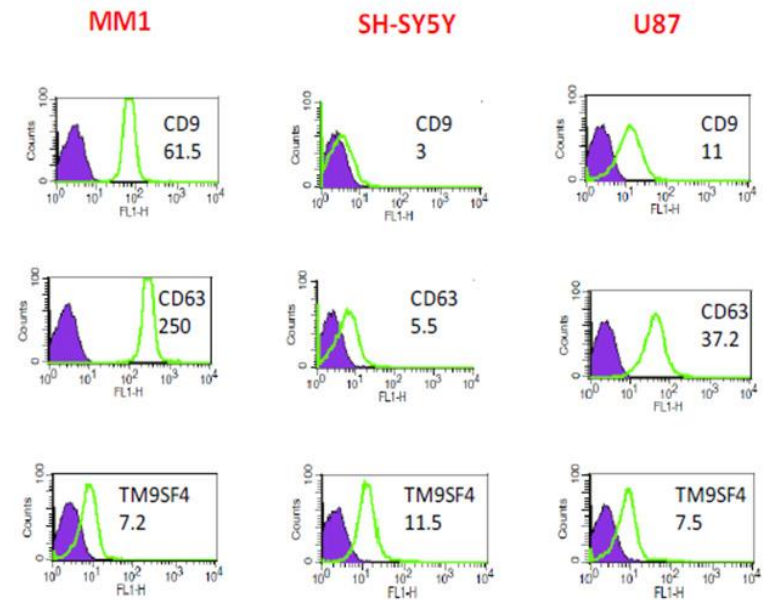


Figure 2. FACS profiling of exosomal markers CD9, CD63 and TM9SF4 in purified exosomes from MM1, SH-SY5Y and U87 cell lines.

Technical Support

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