

- d) Divide the Exo-Beads resuspended in sample buffer in each FACS tube, pipetting 100 μ l of suspension in each tube.
 - e) Add primary antibody in ratio 1:200 (0.5 μ l per each FACS tube) *. *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).
 - g) Add 4 ml of prepared Washing buffer to each FACS tube.
 - h) Centrifuge 5 min at 4000g and discard the supernatant.
 - i) Resuspend beads pellet into the FACS tubes in 100 μ l of Sample buffer.
 - j) Add secondary antibody in ratio 1:2000. (mix 57 μ l of Sample buffer with 3 μ l of secondary antibody; add 1 μ l of the received solution per each FACS tube to obtain the right dilution of antibody).
 - k) Incubate for 1 hr in the dark at 4°C.
 - l) 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.
 - m) Add 500 μ l of Washing buffer per FACS tube.
 - n) Analyze the samples.
 - o) Read 10,000 events from the gated first population.
 - p) Alexa 488 is read in FL1 channel (green)
6. **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 FACS-beads 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.

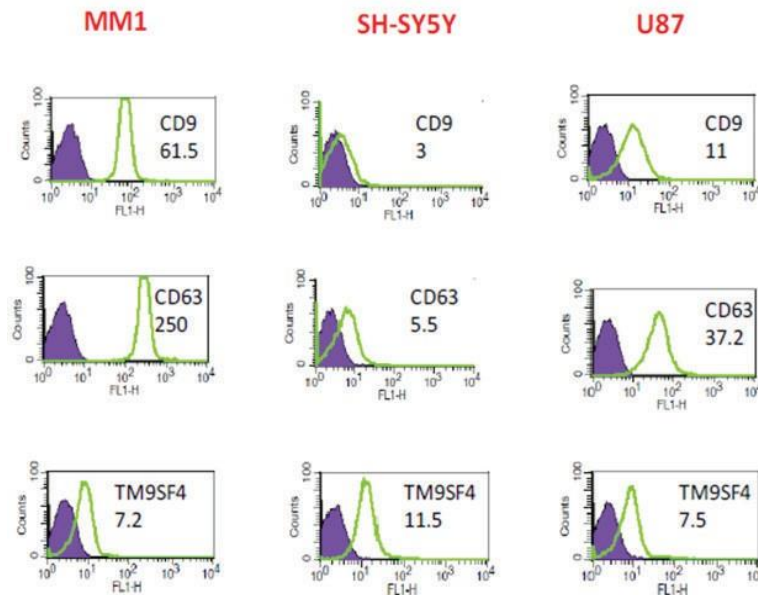


Figure 2. FACS profiling of exosomal markers CD9, CD63 and TM9SF4 in purified exosomes from MM1, SH-SY5Y and U87 cell lines.

IX. Related Products:

Products/Catalog Number
ExoFACS™ Kit for Plasma Exosomes # K1232-20
ExoFACS™ Kit for Serum Exosomes # K12333-20
ExoFACS™ Kit for Saliva Exosomes # K1234-20
ExoFACS™ Kit for Urine Exosomes # K1235-20
ExoFACS™ Kit for Cell Culture Media Exosomes # K1236-20

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