ExoGenie Kit for Saliva Exosomes (BN00094)

(Catalog # BN00094; 20 reactions; Store at 4°C)

I. Introduction:

Exosomes are small endosome derived lipid nanoparticles (50-120 nm) actively secreted by exocytosis by most living cells. Exosome release occurs either constitutively or upon induction, under both normal and pathological conditions, in a dynamic, regulated and functionally relevant manner. Both the amount and molecular composition of released exosomes depend on the state of a parent cell. Exosomes have been isolated from diverse cell lines (hematopoietic cells, tumor lines, primary cultures, and virus infected cells) as well as from biological fluids in particular blood (e.g. serum and plasma from cancer patients) and other body fluids (broncho alveolar lavage fluid, pleural effusions, synovial fluid, urine, amniotic fluid, semen, saliva etc). Exosomes have pleiotropic physiological and pathological functions and an emerging role in diverse pathological conditions such as cancer, infectious and neurodegenerative diseases.

ExoGenie allows exosome isolation from biofluids or cell culture media and FACS analysis of exosome markers. The kit consists of components for exosome isolation, 4 µm beads for the overall capture of pre-isolated exosomes and lyophilized exosomes from cell culture supernatants or human biological fluids as the positive control. The characterization of exosomal proteins (membrane expressed or internal) is subsequently performed using appropriate detection antibodies against exosome associated antigens. Assay Genie offers different ExoGenie kits for staining of exosomal markers from human biofluids (plasma, urine, serum, saliva) and from cell culture supernatants. ExoGenie contains reagents for 20 reactions (lyophilized exosomes, beads, antibodies and buffers). Primary antibody included in the kit is against a common exosomal marker (CD9 or CD63) and can be used as a positive control for protein profiling via FACS analysis.

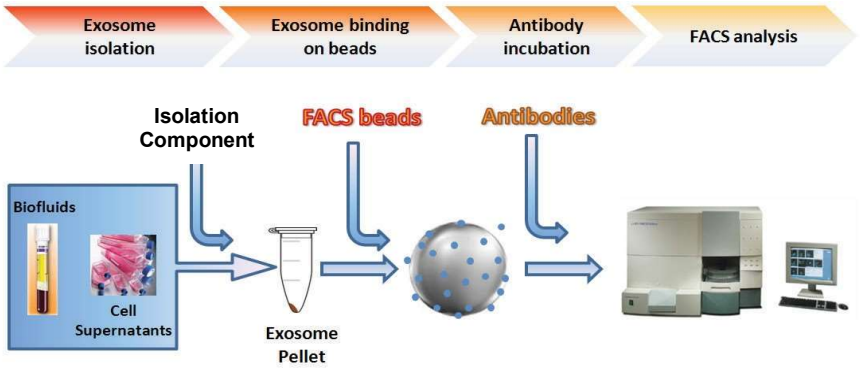

Figure 1. Exosome isolation and exosome marker characterization via FACS.

II. Application:

- ExoGenie Kits ensure exosome isolation and exosome marker characterization via FACS.
- Exosome comprehensive profiling.
- No initial exosome purification required.
- Lyophilized Exosome Standards for positive control included.
- User friendly and suitable for multiple marker analyses.

III. Sample Type:

- Human biological fluids: Saliva.

IV. Kit Contents (Ready to use kit for FACS analysis from human saliva):

Components	Description	BN00094	Part Number
Isolation Component	Exosome isolator	1 bottle (3 ml)	BN00094-1
FACS-beads	4 µm Aldehyde-Sulfate latex beads	1 vial (100 µl)	BN00094-2
Primary Antibody	Anti-human CD9 mouse antibody	1 vial (20 µl)	BN00094-3
Secondary Antibody	Secondary antibody Alexa 488	1 vial (10 µl)	BN00094-4
Sample Buffer (1X)	Buffer for antibody incubation	2 bottles (2 X 10 ml))	BN00094-5
Exosome Standards (100 µg)	Lyophilized exosomes from healthy donors (Saliva)	1 vial (100 µg)	BN00094-6

V. User Supplied Reagents and Equipment:

- 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

VI. Shipment and Storage:

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!

VII. Reagent Preparation and Storage Conditions:

- ExoGenie 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), from human saliva (number of particles/ml $1x10^{10}$).
- 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.

VIII. ExoGenie Assay Protocol:

1. Human Saliva sample preparation: Add 1X PBS in saliva samples in ratio 1/1 (5 ml of saliva + 5 ml of 1X PBS). Mix together and proceed to the following centrifugation steps:

a) 15 min at 2600g.

b) 20 min at 15,550g. filter through 0.22 um filter.

2. Exosome isolation from Saliva:

Fluid	Minimum volume required	Volume suggested
Saliva	200 µl	250 µl -500 µl

a) Add Isolation Component solution to your sample in ratio 1/4.

b) Mix well by pipetting and inverting tube.

c) Incubate on ice for 1 hr.

d) Centrifuge 20 min at 10,000g (centrifuge can be performed at 4°C or at RT).

e) Discard the supernatant.

f) Centrifuge for 2 min at 1500g to eliminate entirely the supernatant.

g) Resuspend the pellet in 100 µl* of 1X PBS. * Volume of resuspension can be defined by the user.

3. Lyophilized Exosome Standard reconstitution:

a) Reconstitute lyophilized exosome standard by adding 100 µl of deionized water to get a final concentration of 1 µg/µl.

b) Resuspend exosomes pipetting the solution up and down 10-15 times, avoiding bubbles.

c) 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.

d) Use 5 µl of reconstituted Exosome Standard for each reaction.

4. Exosome binding onto latex FACS-Beads:

a) It is recommended to prepare the complex Exosome-Beads (Exo-Beads) in one single tube, then to divide in single reactions before the antibody incubation.

b) Latex FACS-Beads are ready to use. Resuspend well FACS-Beads prior to use by vortexing or pipetting several times.

c) 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 25 µl of FACS-Beads.

d) For exosome isolated using Isolation Component, mix 25 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).

e) Incubate for 15 min at room temperature (RT).

f) Add 0.7 ml of 1X PBS and incubate in rotator or shaker for 2 hr at RT or overnight (ON) at 4°C.

g) Centrifuge the complex Exosomes-Beads (Exo-Beads) for 5 min at 4500g at 4°C and discard the supernatant.

h) Add 1 ml of Sample Buffer, resuspend Exo-Beads for 5-6 times and incubate for 15 min at RT.

i) Centrifuge for 5 min at 4500g at 4°C, discard the supernatant.

5. Antibody Incubation:

a) 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.

b) 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.

c) Prepare the FACS tubes (not provided in the kit), one tube for each reaction.

d) Divide the Exo-Beads resuspended in sample buffer in each FACS tube, pipetting 100 µl of suspension in each tube.

e) Add primary antibody in ratio 1:200 (0.5 µl 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 µl of Sample buffer.
j) Add secondary antibody in ratio 1:2000. (mix 57 µl of 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).
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 µl 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: ExoGenie is a useful tool for exosome protein profiling by using FACS technique.** ExoGenie 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.

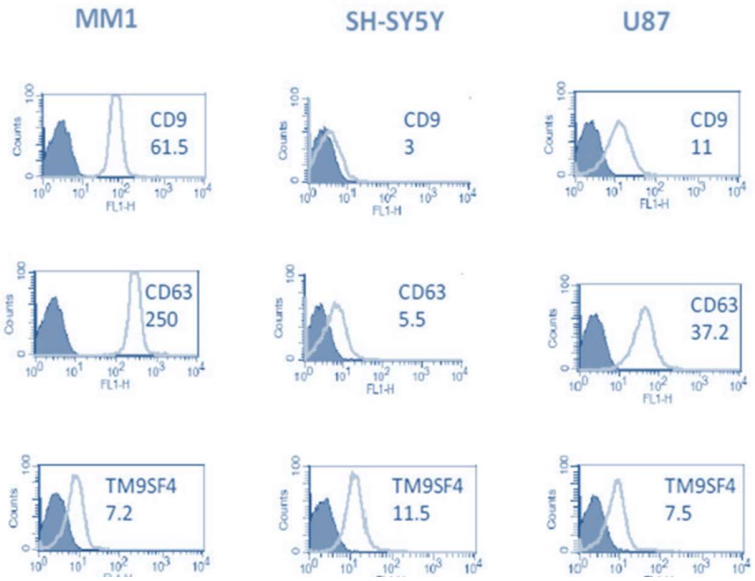

Figure 2. FACS profiling of exosomal markers CD9, CD63 and TM9SF4 in purified exosomes from MM1, SH-SY5Y and U87 cell lines.

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