Protocol: Characterization of Exosomal Protein - Flow Cytometry

Protocol Notes:

- Protocol has been modified from several references [1,2]
- BCA or Bradford total protein assay must be conducted on samples prior to flow cytometry preparation (see appropriate protocol).
- Pellet during spin steps will be difficult to detect. Place centrifuge tubes in centrifuge with hinge out to keep track of where the pellet is located.
- For further optimization of this assay, refer to Latex Bead protocol by Life Technologies, which is attached to this protocol.

Materials:

1. 4% w/v 4-micron aldehyde/sulfate latex beads, IDC Latex Particles Microspheres, Cat# A37304, location: refrigerator #1 rm. 231
2. Anti-CD63, purified 0.5 mg/ml, Cat# 556019, location: refrigerator #6 rm. 231
3. Anti-CD9, BD Pharmingen, 100 tests, Cat# 555372, location: refrigerator #6 rm. 231
4. Human IgG, Sigma Aldrich, Product No. I 4506, location: refrigerator #6 rm. 231
    - Stock was reconstituted to 5 mg/mL on 7/10/2012 with 150 mM NaCl (made on 7/10/2012)
    - Working solution for this assay is 1 mg/mL, stock diluted with 150 mM NaCl
5. 1x PBS, prepared by N/A on N/A, location: stored at room temperature in 1-L glass reagent bottle rm. 231
6. MES buffer, 0.025 M, pH 6, location: stored at room temperature rm. 231
    - MES hydrate, Sigma, Lot#118827, received 7/2012, opened 7/2012, location: reagent shelf rm. 231
7. Glycine, 200 mM, location: stored at room temperature rm. 231
    - Glycine, Sigma, Batch# 125K0124, CAS 56-40-6, received 4/2006, location: reagent shelf rm. 231
8. Wash buffer, 2% BSA in 1x PBS, location: refrigerator #1 rm. 231
    - Albumin, Bovine, Sigma, Lot# 29H06851, received 3/1999, location: refrigerator #7 rm. 226
9. Storage buffer, 0.1% (w/v) Glycine, 0.1% (w/v) Sodium Azide, 1x PBS, pH 7.2, location: stored at room temperature rm. 231

- Sodium Azide (currently not available in lab)

10. Ice

Methods:

1. Wash 25 mcL of latex beads (approximately $30x10^6$ beads) twice in MES buffer.
2. Spin at 3000 x g (6500 rpm on Eppendorf microcentrifuge) for 20 minutes and resuspend pellet in 100 mcL MES buffer.
3. Prepare antibody to be conjugated to latex bead (anti-CD63 in this case) by making a mixture with 12.5 mcg antibody in storage buffer, total volume of both antibody and storage buffer should be 100 mcL.
4. Add latex bead mixture to the antibody mixture (total volume of 200 mcL) and **incubate over night with gentle agitation** at room temperature.
5. Wash antibody coated latex beads three times with PBS by spinning at 3000 x g for 20 minutes.
6. Resuspend pellet in 100 mcL of storage buffer (final concentration is approximately 300,000 beads/mcL).
    - Antibody coated beads can be stored at 4 degrees C.
7. When adding exosomes to antibody coated latex beads, add approximately 100,000 beads per 30 mcg of exosomal protein (as determined by BCA or Bradford).
8. **Incubate over night** exosomes and beads in 300 mcL of PBS at 4 degrees C. If possible, incubate with gentle agitation.
9. Add 300 mcL of 200 mM glycine to block unbound anti-CD63 antibody and **incubate for 30 minutes** in at 4 degrees C.
10. Wash exosome bound beads twice in wash buffer by spinning at 600 x g (3000 rpm on Eppendorf microcentrifuge) for 10 minutes.
11. Resuspend pellet in 50 mcL IgG antibody at 1 mg/mL concentration and incubate at 4 degrees C for 30 minutes.
12. Wash exosome bound beads twice in wash buffer by spinning at 600 x g for 10 minutes
13. Resuspend pellet in 90 mcL wash buffer and 10 mcL fluorescent tagged antibody (anti-CD9 PE in this case) and **incubate for 40 minutes** at 4 degrees C. Incubate under gentle incubation if possible.
14. Wash exosome bound beads twice in wash buffer (repeat step 12).
15. Resuspend pellet in 300 mcL wash buffer and store covered at 4 degrees C.

References

1.Théry, C., Amigorena, S., Raposo, G. & Clayton, A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr Protoc Cell Biol* **Chapter 3**, Unit3.22 (2006).

2.Lässer, C., Eldh, M. & Lötvall, J. Isolation and characterization of RNA-containing exosomes. *J Vis Exp* **59**,)59(e3037 (2012).