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PKH67 Extracellular Vesicle Labeling

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We use this protocol and it's working

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Abstract

This protocol is designed to visualize extracellular vesicles with the lipid dye, PKH67.

PKH67 Extracellular Vesicle Labeling

1 In a microcentrifuge tube add 100 ug (100ul) of EVs in 1 mL of PBS

2 Centrifuge at 16,100 g for 10 minutes to form a pellet

3 Discard supernatant without disturbing pell and dissolve the pellet in 100 ul of diluent C and pipette up and down

4 In a new microcentrifuge tube add 4 ul of PKH67 to 100 ul of Diluent C and Vortex

5 Combine 100 ul of 2X EV with an equal volume of the pKH67 solution

6 Pipette the mixture 10 times

7 Incubate solution at 37°C for 5 minutes at 300 rpm away from light

8 To stop staining add 400 ul of 1% BSA to the mixture and incubate for a minute for excess dye to bind

9 Wash stained EVs with PBS 3 times and centrifuge the sample at 16,100 g for 5 minutes

10 Discard the supernatant and dissolve the pellet in 100 ul of PBS to get a final concentration of 1 ug/ul

11 Two days before transfer endothelial cells to a poly-L-lysine coated coverslip

12 Allow the cells to grow a monolayer on the coverslip

13 After the monolayer is formed removed the medium carefully and add 1 mL of DMEM to wash the cells twice

14 Add 50 ul of PKH67 stained EVs to the coverslip and incubate for 2 hours

15 After incubation aspirate the media and wash with PBS 3 times

16 Add 1 mL of 4% PFA (made in PBS) to coverslips and fix cells for 15 minutes covered in foil away from light shaking

17 Wash coverslips with 1X PBS 3X for 1 min each wash

18 Add 1 mL to each well of Perm buffer (Triton X, BSA, PBS) to permeabilize the membrane on the shaker for 10 minutes shaking

19 Aspirate the Perm Buffer and wash coverslips with 1X PBS 3X for 1 min each wash

20 Block with 1 mL of 5% Donkey Serum (DS) diluted in 1X PBS and incubate on the shaker for 30 min at room temperature shaking

21 Aspirate and add 800 ul Primary Antibody Staining in Blocking Buffer and incubate for 1 hour at room temperature shaking

22 Wash off unbound antibodies 3X with 1X PBS for 1 min each wash

23 Aspirate and add 800 ul of Secondary Antibody Staining in Blocking Buffer and incubate for 1 hour at room temperature shaking covered in foil

24 Wash off unbound antibodies with 1X PBS 3 × 10 min each wash shaking

25 Grab microscope slides and label them with the experiment, Ab used, initials, and date

26 Add (a small) drop of DAPI mounting media for every coverslip, and mount on glass slide

27 Cure slides overnight and image