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Cell Staining for Flow Cytometry

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This protocol is a guide to surface and intracellular staining, including preparation of single-color controls. This is a protocol for Human cells for Mouse cells all centrifugation steps should be carried out at 300g for 10 minutes.

Materials:

Cells

Primary antibodies (e.g. directly conjugated)

Secondary antibodies (not required if using labeled primary antibody)

Flow cytometry tubes (12 x 75 mm polystyrene tubes)

DPBS

P1000 Pipette and respective Tips

P200 Pipette and respective Tips

P20 Pipette and respective Sterile P20 Pipette Tips

P2 Pipette and respective Tips

Plastic waste container

15 ml Falcon tubes

50 ml Falcon tubes

Paper Towels

(For intracellular staining: Invitrogen™ eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set Catalog number: 00-5523-00)

Surface Staining

1. Label FACS tubes.
2. Obtain each sample's desired number of cells (e.g. 500,000 cells) and transfer them to their respective FACS tubes.

    2a. Don't forget to prepare single-color controls for compensation **(refer to single-color control procedure)**, if you are using more than one color and an unstained control.

3. Centrifuge @500g x 5 minutes.
4. While your cells are centrifuging, make an antibody master mix by placing 100 ul PBS per sample + 1 extra sample in a 15ml Falcon tube.

    4a. Add desired surface antibodies in correct concentrations based on the 100 ul/ sample volume (e.g. 1:100 directly conjugated antibody, or 1 ul per 100 ul PBS).

    4b. If you are using secondary antibodies, make a separate master mix for those (e.g. 1:1000 secondary antibody, or 1 ul per 1 ml PBS).

5. Decant supernatant and blot excess on a paper towel.

    5a. Vortex each pellet gently.

6. Add 100 ul of Antibody Master Mix per tube.
7. Vortex each pellet gently.
8. Incubate at 4°C for 20 Minutes light protected.
9. After incubation, add 1000 ul of PBS on top of each sample to wash.
10. Centrifuge @500g x 5 Minutes.
11. Decant supernatant and blot excess on a paper towel.

    11a. Vortex each pellet gently.

    11b. If you are using secondary antibodies, repeat steps 6 through 10 with your secondary antibody master mix.

12. Add 200 ul of PBS per sample and vortex each pellet gently. *
13. Place samples on ice.
14. Read samples.

*Adjust your final resuspension volume appropriately for the total number of cells you are staining in each tube. In most cases, each sample should have the same number of cells.

Single Color Controls

1. Label FACS tubes. **Do not prepare a master mix.**
2. Obtain each sample's desired number of cells (e.g. 500,000 cells) and transfer them to their respective FACS tubes. Don't forget an unstained control.
3. Centrifuge @500g x 5 minutes.
    3a. Decant supernatant and blot excess on a paper towel.
        3b. Vortex each pellet gently.
4. Resuspend each sample in 100 ul of PBS and add the appropriate antibody to each single-color control. **Do not add any antibody to the unstained control.**
5. Incubate at 4°C for 20 Minutes light protected.
6. After incubation, add 1000 ul of PBS on top of each sample.
7. Centrifuge @500g x 5 Minutes.
8. Decant supernatant and blot excess on a paper towel.
    8a. Vortex each pellet gently.
9. Resuspend the pellets in 200 ul of PBS and place them on ice. *
10. Read single-color controls before the samples and set up compensation in flow cytometer.

*Adjust your final resuspension volume appropriately for the total number of cells you are staining in each tube. In most cases, each sample should have the same number of cells.

Intracellular Staining

(Invitrogen™ eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set Catalog number: 00-5523-00)

1. Before beginning, make a master mix by pipetting 100 ul PBS per sample + 1 extra sample in a 15ml Falcon tube.

    1a. Add desired surface antibodies (e.g. anti-CD4) in correct concentrations based on the 100 ul/ sample volume (e.g. 1:100 antibody, or 1 ul per 100 ul PBS). D**o not forget to add fixable viability dye (e.g. Ghost Viability Dye 1:500).**

2. Obtain each sample's desired number of cells (e.g. 500,000 cells) and transfer them to their respective FACS tubes.

    2a. Don't forget single-color controls (refer to the single-color control procedure if you are using more than one color and an unstained control.

3. Centrifuge @500g x 5 minutes

    3a. Decant supernatant and blot excess on a paper towel.

        3b. Vortex each pellet gently

4. Add 100 ul of Antibody Master Mix per tube.

    4a. Vortex gently.

5. Incubate at 4°C for 20 Minutes light protected.

6. After incubation, add 1000 ul of PBS on top of each sample to wash.

7. Centrifuge @500g x 5 Minutes

    7a. Decant supernatant and blot excess on a paper towel.

        7b. Vortex each pellet gently

8. Make Fix/Perm Buffer: 1 part concentrate (Fixation Permeabilization Concentrate, brown bottle): 3 parts diluent (Fixation Permeabilization Diluent, white bottle)

    8a. Add 100ul of Fix/Perm Buffer per sample and vortex gently.

9. Incubate at 4°C for 30 Minutes

    9a. While incubating, prepare Perm Buffer: 10% (Permeabilization Buffer 10x) and 90% (DPBS)

9b. Make intracellular antibody master mix with your intracellular antibodies (e.g. anti-FOXP3) ased on the 100 ul/ sample volume (e.g. 1:50 antibody, or 2 ul per 100 ul Perm Buffer).

10. After incubation, wash with 500 ul of **Perm Buffer.**
11. Centrifuge @500g x 5 minutes
    11a. Decant supernatant and blot excess on a paper towel.
    11b. Vortex each pellet gently
12. Add 100 ul of prepared Intracellular master mix to each tube and vortex gently.
13. **Incubate at ROOM TEMPERATURE for 30 Minutes in the dark.**
14. Wash with 500ul of **Perm Buffer made in step 9a.**
15. Centrifuge @500g x 5 Minutes
    15a. Decant supernatant and blot excess on a paper towel.
    15b. Vortex each pellet gently
16. Add 1000ul of DPBS to wash.
17. Centrifuge @500g x 5 Minutes
    17a. Decant supernatant and blot excess on a paper towel.
    17b. Vortex each pellet gently
18. Resuspend in 200 ul of PBS and read samples*

*Adjust your final resuspension volume appropriately for the total number of cells you are staining in each tube. In most cases, each sample should have the same number of cells.