Cell Surface Immunofluorescent Staining of Whole Blood V.3

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Guidelines

Reagent List:

- Cell Staining Buffer (BioLegend Cat. No. 420201)
- Red Cell Lysis Buffer (BioLegend Cat. No. 420301)
- 7-AAD Viability Staining Solution (BioLegend Cat. No. 420403)
- TruStain FcX™ (anti-CD16/32, BioLegend Cat. No. 101319)
- Human TruStain FcX™ (Fc Receptor Blocking Solution, BioLegend Cat. No. 422301)

References:

Current Protocols in Cytometry (John Wiley & Sons, New York), Unit 6 Phenotypic Analysis.

Materials

MATERIALS

Cell Staining Buffer **BioLegend Catalog #**420201

Red Cell Lysis Buffer **BioLegend Catalog #**420301

7-AAD Viability Staining Solution **BioLegend Catalog #**420403

TruStain FcX™ **BioLegend Catalog #**101319

Human TruStain FcX™ **BioLegend Catalog #**422301

STEP MATERIALS

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- Cell Staining Buffer **BioLegend Catalog #**420201
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Troubleshooting

1 Add predetermined optimum concentrations of desired fluorochrome conjugated, biotinylated, or purified primary antibodies to 100 µl of anti-coagulated whole blood.

2 Incubate at room temperature for 15-20 minutes in the dark.
00:20:00

3 Dilute 10X Red Blood Cell (RBC) Lysis Buffer (BioLegend Cat. No.**420301**) to 1X working concentration with DI water. Warm to room temperature prior to use. Add 2ml of 1X RBC lysis solution to whole blood/antibody mixture. Incubate at room temperature for 10 minutes. 00:10:00

4 Centrifuge at 350 X g for 5 minutes, discard the supernatant.
00:05:00

5 Wash 1X with at least 2 ml of Cell Staining Buffer by centrifugation at 350 x g for 5 minutes.
00:05:00
Cell Staining Buffer **BioLegend Catalog #**420201

6 If using a purified primary antibody, resuspend pellet in residual buffer and add a previously determined optimum concentration of anti-species immunoglobulin fluorochrome conjugated secondary antibody(e.g. FITC anti-mouse Ig) and incubate in the dark for 15-20 minutes.
00:20:00

7 If using a biotinylated primary antibody, resuspend cell pellet in residual buffer and add a previously determined optimum concentration of fluorochrome conjugated Streptavidin (SAv) reagent (e.g. SAv-PE, BioLegend Cat. No.**405204**) and incubate for 15-20 minutes in the dark. 00:20:00

8 Repeat step 5.

9 Resuspend cells in 0.5 ml Cell Staining Buffer or 0.5 ml 2% paraformaldehyde-PBS fixation buffer.

Tip: For gentler fixation (particularly with tandem fluors), FluoroFix™ Buffer (Cat. No.**422101**) may be used.
Cell Staining Buffer **BioLegend Catalog #**420201

10 Analyze with a Flow Cytometer.

Note: If you are unable to immediately read your samples on a cytometer, keep them shielded from light and in a refrigerator set at 4-8°C. The samples should be resuspended in Cell Staining Buffer. Note that samples should not remain in a fixation buffer for extended periods of time as this can affect fluor conformation and fluorescence.