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Cell Surface Flow Cytometry Staining of Whole Blood V.4

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[1]BioLegend

1 Works for me dx.doi.org/10.17504/protocols.io.babbiain

BioLegend

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EXTERNAL LINK

https://www.biolegend.com/protocols/cell-surface-flow-cytometry-staining-of-whole-blood/4240/

MATERIALS

NAME	CATALOG #	VENDOR
TruStain fcX™ (anti-mouse CD16/32) Antibody	101319	BioLegend
RBC Lysis Buffer	420301	BioLegend
Human TruStain FcX™ (Fc Receptor Blocking Solution)	422301, 422302	BioLegend
TruStain FcX™ PLUS (anti-mouse CD16/32)	156603, 156604	BioLegend
Cell Staining Buffer	420201	BioLegend
7-AAD Viability Staining Solution	420403, 420404	BioLegend

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

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

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.

4 Centrifuge at 350xg for 5 minutes, discard the supernatant.

5 Wash 1X with at least 2ml of Cell Staining Buffer by centrifugation at 350xg for 5 minutes.

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.

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.

8 Repeat step 5.

9 Resuspend cells in 0.5ml Cell Staining Buffer or 0.5ml 2% paraformaldehyde-PBS fixation buffer.Tip: For gentler fixation (particularly with tandem fluors), FluoroFix™ Buffer (Cat. No. 422101) may be used.

10 Perform fluorescence activated cell sorting (FACS), or flow cytometric analysis.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.