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Cell Surface Flow Cytometry Staining Protocol V.4

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[1]BioLegend

1 Works for me dx.doi.org/10.17504/protocols.io.baa9iah6

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EXTERNAL LINK

https://www.biolegend.com/protocols/cell-surface-flow-cytometry-staining-protocol/4283/

MATERIALS

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

Harvest Tissue or Cells:

1 Obtain desired tissue (e.g. spleen, lymph node, thymus, bone marrow) and prepare a single cell suspension in Cell Staining Buffer (BioLegend Cat. No. 420201). If using in vitro stimulated cells, simply resuspend previously activated cultures in Cell Staining Buffer and proceed to Step 2.

2 Add Cell Staining Buffer up to ~15ml and centrifuge at 350xg for 5 minutes, discard supernatant.

Lyse Red Cells:

3 If necessary (e.g. spleen), dilute 10X Red Blood Cell (RBC) Lysis Buffer (BioLegend Cat. No. 420301) to 1X working concentration with DI water and resuspend pellet in 3 ml 1X RBC Lysis Buffer. Incubate on ice for 5 minutes.

4 Stop cell lysis by adding 10ml Cell Staining Buffer to the tube. Centrifuge for 5 minutes at 350xg and discard supernatant.

5 Repeat wash as in step 2.

6 Count viable cells and resuspend in Cell Staining Buffer at 5-10 x 106 cells/ml and distribute 100µl/tube of cell suspension (5-10 x 105 cells/tube) into 12 x 75mm plastic tubes.

Block Fc-Receptors:

7 Reagents that block Fc Receptors may be useful for reducing non-specific immunofluorescent staining.

Note: Mouse TruStain FcX™ PLUS contains antibodies directed against CD16/32 (via the Fab portion of the antibody), while Human TruStain contains specialized human IgG that bind to Fc receptors via the Fc portion of the antibodies. Human TruStain is compatible with flow cytometric staining with anti-human CD16 (clone 3G8), CD32 (clone FUN-2), and CD64 (clone 10.1) antibodies.

For mouse samples, we recommend using TruStain FcX™ PLUS (anti-mouse CD16/32) Antibody specific for FcγR III/II (Cat. No. 156603, clone S17011E). Block Fc receptors by pre-incubating cells with 0.25µg of TruStain FcX™ PLUS (anti-mouse CD16/32) Antibody per 10^6cells in a 100µl volume for 5-10 minutes on ice.
Note: For step 7, 1µg of TruStain FcX™ (anti-mouse CD16/32) Antibody (Cat. No. 101319, clone 93) per 10^6cells in a 100µl volume can be used. However, we strongly recommend using TruStain FcX™ PLUS as indicated, based on in-house testing results that demonstrate its superior blocking capabilities.

8 In humans, cells can be pre-incubated with 5µl of Human TruStain FcX™ (Fc Receptor Blocking Solution, BioLegend Cat. No. 422301) per 100µl of cell suspension for 5-10 minutes at room temperature. In the absence of an effective/available blocking antibody, an alternative approach is to pre-block cells with excess irrelevant purified Ig from the same species and same isotype as the antibodies used for immunofluorescent staining.

Cell-Surface Staining with Antibody:

9 Add appropriately conjugated fluorescent, biotinylated, or purified primary antibodies at predetermined optimum concentrations (e.g. anti-CD3-FITC, anti-CD4-Biotin, and anti-CD8-APC) and incubate on ice for 15-20 minutes in the dark.

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

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

12 Repeat step 10.

13 Resuspend cell pellet in 0.5ml of Cell Staining Buffer and add 5µl (0.25µg)/million cells of 7-AAD Viability Staining Solution (BioLegend Cat. No. 420403) to exclude dead cells. Note: BioLegend recommends using the Spectra Analyzer to decide compatibility with other fluors.

14 Incubate on ice for 3-5 minutes in the dark.

15 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.