Cell Surface Immunofluorescence Staining Protocol

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[1]BioLegend

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BioLegend

Version 3

Working

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BioLegend

EXTERNAL LINK

http://www.biolegend.com/media_assets/support_protocol/BioLegend_Surface_Staining_Flow_Protocol_060215.pdf

PROTOCOL STATUS

Working

GUIDELINES

- 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)

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.

REAGENT
Cell Staining Buffer
by BioLegend
Catalog #: 420201

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

00:05:00

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.

REAGENT
Red Cell Lysis Buffer
by BioLegend
Catalog #: 420301

00:05:00

4 Stop cell lysis by adding 10 ml Cell Staining Buffer to the tube. Centrifuge for 5 minutes at 350 x g and discard supernatant.
00:05:00

5 Repeat wash as in step 2.
00:05:00

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

Block Fc-Receptors

7 Reagents that block Fc receptors may be useful for reducing nonspecific immunofluorescent staining. In the mouse, TruStain fcX™ (anti-mouse CD16/32) Antibody specific for FcγR III/II (BioLegend Cat. No. 101319, clone 93) can be used to block nonspecific staining of antibodies. In this case, block Fc receptors by pre-incubating cells with 1.0µg of TruStain fcX™ (anti-mouse CD16/32) Antibody per 10^6 cells in a 100µl volume for 5-10 minutes on ice.
00:10:00

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.

Note: Mouse TruStain fcX 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.
00:10:00

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.
00:20:00

10 Wash 2X with at least 2ml of Cell Staining Buffer by centrifugation at 350xg for 5 minutes.
00:05:00

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.
00:20:00

12 Repeat step 10.
00:05:00

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.

00:05:00

15 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.