Cell Surface Immunofluorescence Staining Protocol

BioLegend, Inc.

Abstract

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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

Cell Staining Buffer 420201 by BioLegend
Red Cell Lysis Buffer 420301 by BioLegend
7-AAD Viability Staining Solution 420403 by BioLegend
TruStain FcX™ 101319 by BioLegend
Human TruStain FcX™ 422301 by BioLegend

Protocol

Harvest Tissue or Cells

Step 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).

REAGENTS
Cell Staining Buffer 420201 by BioLegend

NOTES
Kelsey Knight 02 May 2016
If using in vitro stimulated cells, simply resuspend previously activated cultures in Cell Staining

Buffer and proceed to Step 2.

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If using in vitro stimulated cells, simply resuspend previously activated cultures in Cell Staining Buffer and proceed to Step 2.

Harvest Tissue or Cells

Step 2.

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

DURATION

00:05:00

Lyse Red Cells

Step 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.

REAGENTS

Red Cell Lysis Buffer 420301 by BioLegend

Lyse Red Cells

Step 4.

Incubate on ice for 5 minutes.

DURATION

00:05:00

Lyse Red Cells

Step 5.

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

DURATION

00:05:00

Lyse Red Cells

Step 6.

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

DURATION

00:05:00

Lyse Red Cells

Step 7.

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

NOTES

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Block Fc-Receptors:

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 1.0 µg of TruStain fcX™ (anti-mouse CD16/32) Antibody per 106 cells in a 100 µl volume for 5-10 minutes on ice. In humans, cells can be pre-incubated with 5 µl of Human TruStain FcX™ (Fc Receptor Blocking Solution, BioLegend Cat. No. 422301) per 100 µl of cellsuspension 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

Step 8.

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.

DURATION

00:15:00

Cell-Surface Staining with Antibody

Step 9.

Wash with at least 2 ml of Cell Staining Buffer by centrifugation at 350 x g for 5 minutes. (1/2)

DURATION

00:05:00

Cell-Surface Staining with Antibody

Step 10.

Wash with at least 2 ml of Cell Staining Buffer by centrifugation at 350 x g for 5 minutes. (2/2)

DURATION

00:05:00

Cell-Surface Staining with Antibody

Step 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.

DURATION

00:15:00

NOTES

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

Cell-Surface Staining with Antibody

Step 12.

Wash with at least 2 ml of Cell Staining Buffer by centrifugation at 350 x g for 5 minutes. (1/2)

DURATION

00:05:00

Cell-Surface Staining with Antibody

Step 13.

Wash with at least 2 ml of Cell Staining Buffer by centrifugation at 350 x g for 5 minutes. (2/2)

DURATION

00:05:00

Cell-Surface Staining with Antibody

Step 14.

Resuspend cell pellet in 0.5 ml of Cell Staining Buffer and add 5 µl (0.25 µg)/million cells of 7-AAD Viability Staining Solution (BioLegend Cat. No. 420403) to exclude dead cells.

REAGENTS

7-AAD Viability Staining Solution 420403 by BioLegend

NOTES

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Note, BioLegend does not recommend use of 7-AAD with either PE-Cy5 or PE-Cy7 antibody conjugates. For cells that must be fixed prior to staining, BioLegend recommends Zombie Live/Dead Fixable Dyes.

Cell-Surface Staining with Antibody

Step 15.

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

DURATION

00:03:00

Cell-Surface Staining with Antibody

Step 16.

Analyze with a Flow Cytometer.