Immunofluorescent Staining of Whole Blood Version 2

BioLegend, Inc.

Abstract

Citation: BioLegend, Inc. Immunofluorescent Staining of Whole Blood. **protocols.io**
dx.doi.org/10.17504/protocols.io.hwdb7a6
Published: 09 May 2017

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.

Protocol

Step 1.

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

Step 2.

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

DURATION
00:20:00

Step 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.

REAGENTS
Red Cell Lysis Buffer 420301 by BioLegend

Step 4.

Add 2 ml of 1X RBC lysis solution to whole blood/antibody mixture.

Step 5.
Incubate at room temperature for 10 minutes.

DURATION
00:10:00

Step 6.

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

DURATION
00:05:00

Step 7.

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

REAGENTS
Cell Staining Buffer 420201 by BioLegend

DURATION
00:05:00

Step 8.

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.

DURATION
00:15:00

Step 9.

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.

Step 10.
Wash 1X with at least 2 ml of Cell Staining Buffer by centrifugation at 350 x g for 5 minutes.

DURATION
00:05:00

Step 11.
Resuspend cells in 0.5 ml Cell Staining Buffer or 0.5 ml 2% paraformaldehyde-PBS fixation buffer.

REAGENTS
Cell Staining Buffer 420201 by BioLegend

NOTES
Kelsey Knight 09 May 2017

Tip: For gentler fixation (particularly with tandem fluors), FluoroFix™ Buffer (Cat. No. 422101) may be used.

Step 12.

Analyze with a Flow Cytometer.

NOTES

Kelsey Knight 09 May 2017

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.