Immunofluorescent Staining of Whole Blood

BioLegend, Inc.

Abstract

Citation: BioLegend, Inc. Immunofluorescent Staining of Whole Blood. **protocols.io**
dx.doi.org/10.17504/protocols.io.exqbfmw
Published: 25 May 2016

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

Step 1.

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

Step 2.

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

DURATION
00:15:00

Step 3.

Dilute 10X Red Blood Cell (RBC) Lysis Buffer (BioLegend Cat. No. 420301) to 1X working concentrationwith 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 wholeblood/antibody mixture.

Step 5.
Incubate at room temperature for 10 minutes.

DURATION

00:10:00

Step 6.
Centrifuge at 350 X g 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 350 x g 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 determinedoptimum 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

NOTES

Kelsey Knight 06 May 2016
If using a biotinylated primary antibody, resuspend cell pellet in residual buffer and add a previouslydetermined 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 9.
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 10.
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

Step 11.
Analyze with a Flow Cytometer.