Cell Surface Immunofluorescent Staining of Whole Blood

Kelsey Knight[1]

[1]BioLegend

Sep 13, 2018 dx.doi.org/10.17504/protocols.io.tjwekpe

BioLegend

Version 3

Working

Kelsey Knight
BioLegend

EXTERNAL LINK

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

PROTOCOL STATUS

Working

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

NAME	CATALOG #	
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

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

2 Incubate at room temperature for 15-20 minutes in the dark.
00:20:00

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. Add 2ml of 1X RBC lysis solution to whole blood/antibody mixture. Incubate at room temperature for 10 minutes. 00:10:00

4 Centrifuge at 350 X g for 5 minutes, discard the supernatant.
00:05:00

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

00:05:00

REAGENT
Cell Staining Buffer
by BioLegend
Catalog #: 420201

6 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.

00:20:00

7 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. 00:20:00

8 Repeat step 5.

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

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

REAGENT
Cell Staining Buffer
by BioLegend
Catalog #: 420201

10 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.