Cell Surface Immunofluorescent Staining of Whole Blood V.3

Kelsey Knight¹

¹BioLegend

Sam Li
BioLegend

GUIDELINES

DOI:
[dx.doi.org/10.17504/protocols.io.tjwekpe](https://doi.org/10.17504/protocols.io.tjwekpe)

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

Protocol Citation: Kelsey Knight . Cell Surface Immunofluorescent Staining of Whole Blood. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.tjwekpe>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Created: Sep 13, 2018

Last Modified: Aug 29, 2019

PROTOCOL integer ID:
15702

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

MATERIALS

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

STEP MATERIALS

- ☒ Cell Staining Buffer **BioLegend Catalog #420201**
- ☒ Cell Staining Buffer **BioLegend Catalog #420201**

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

Cell Staining Buffer **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.

Cell Staining Buffer **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.