FC22 Direct Immunofluorescence Staining of Cells with StarBright Dyes

Protocol

Abstract

This method provides a general procedure for use with StarBright Dyes, for staining cells in tubes or a 96-well plate. In some cases, specific recommendations are provided on product datasheets, and these methods should always be used in conjunction with the product and batch specific information provided with each vial. A certain level of technical skill and immunological knowledge is required for the successful design and implementation of these techniques; these are guidelines only and may need to be adjusted for particular applications or cell types.

Reagents

- Fixation Buffer (optional) (Cat. #BUF071)
- Human Seroblock (#BUF070A) or Mouse Seroblock (#BUF041A) depending on the cells being stained
- Staining Buffer (#BUF073)

Method

1. Prepare cells appropriately; refer to protocol “FC2 Preparation of Human Peripheral Blood Mononuclear Cells” for further information. Adjust the cell suspension to a concentration of 1 x 10^7 cells/ml with cold (4°C) staining buffer. Staining buffer for StarBright Dyes can be PBS/1% BSA buffer, or commercial staining buffers such as #BUF073.
2. Aliquot 100 µl of the cell suspension into as many tubes (or wells of a 96-well plate) as required.
3. Incubate in Fc block (for example Human Seroblock #BUF070A, or Mouse Seroblock #BUF041A) or 10% serum of the cell species you are using, for 10-30 min.
4. Centrifuge at 300-400 g for 5 min at 4°C and discard the supernatant.
5. Add antibody or multiple antibodies at the recommended dilution in 100-200 µl of staining buffer and incubate for 1 hr at 4°C or RT avoiding direct light.

   StarBright Dyes are available as a 5 µl test but you may wish to titrate all the antibodies in your experiment for your particular cells, as it may improve the stain index.

   StarBright Dyes do not require a special buffer when combining with any other StarBright Dye, organic fluorophore, or protein-based fluorophore, including tandem dyes. However you may need to use recommended staining buffers for certain polymer dyes, when combining with one or more polymer dyes.
   Staining cells on ice will result in a lower staining index so we recommend staining at 4°C or RT to improve resolution.
6. Wash the cells with 2 ml cold (4°C) staining buffer (200 µl for 96-well plates) and centrifuge at 300-400 g for 5 min. Discard the supernatant and repeat for a total of three washes.
7. Resuspend cells in 200 µl of cold (4°C) PBS or in 200 µl of a fixative such as Fixation Buffer #BUF071 or 2-4% paraformaldehyde. Do not leave for long periods of time in the fixative. For long-term storage, transfer to PBS and store in a fridge avoiding direct light.
8. Acquire data by flow cytometry.

BIO-RAD

Notes

The following should be considered when designing your flow cytometry experiments.

Appropriate controls should always be carried out. This may include unstained cells to help instrument setup and autofluorescence detection, and a positive and negative control. For multicolor staining, we recommend using a fluorescence minus one control, which shows the influence of other fluorophores in the missing channel and helps set gating boundaries.

Visit **bio-rad-antibodies.com/starbright** for more information on StarBright Dyes.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Website *bio-rad-antibodies.com*

Bulletin 3215 Ver A US/EG *0421 Sig 0121*