

## Fluorescence microscopy (Direct Immunofluorescence) for cell suspensions

Protocol

IF

For use with Bio-Rad's directly-conjugated Alexa Fluor® 405/488/647, DyLight® 405/488/550/650 and Fluorescein Isothiocyanate (FITC) antibodies.

This method provides a general procedure for use with the majority of Bio-Rad reagents. 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. Please note that 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.

### Reagent

1. **PBS/BSA** Phosphate Buffered Saline, pH 7.4, 1% Bovine Serum Albumin (BSA)

### Method

1. Prepare cells appropriately. Adjust cell suspension to a concentration of  $1 \times 10^6$  cells/ml in PBS/BSA.
2. Aliquot 100  $\mu$ l of cell suspension into the required number of test tubes.
3. Add the appropriate volume of antibody at the recommended dilution (see specific datasheet for details). Mix well, and incubate at room temperature for 30 minutes.
4. Wash twice with 2 ml of PBS/BSA, centrifuge at 400 g for 5 minutes. Discard supernatant.
5. Resuspend cells in 0.2 ml of PBS/BSA containing 50% v/v glycerol.  
*Note: It may be necessary to use a commercially available "anti-fading" reagent in place of PBS/Glycerol as a mounting medium.*
6. Place a drop of the resuspended cells on the microscope slide. Gently add a coverslip (taking care to avoid bubbles), and view under a fluorescence microscope using a suitable filter set.

Are you interested in learning more about which reagents to use in your immunofluorescence experiments? Bio-Rad's immunofluorescence application page enables you to quickly and easily find antibodies, kits and reagents tested in immunofluorescence. Find out more at [bio-rad-antibodies.com/if-resources](https://www.bio-rad-antibodies.com/if-resources)

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