

Immunofluorescent Staining of Live Cells

DESCRIPTION

This protocol describes the procedure for direct immunofluorescent (IF) staining of live cells in culture using BioLite™ Antibodies. All steps should be performed in a sterile working environment using fresh culture medium. Please note:

- When using a primary antibody for the first time, it is recommended that the antibody be titrated for optimal performance specific to your cell type.
- Cells can be grown, treated, and stained directly in multi-well plates.
- Only open the antibody in a biological safety cabinet to prevent possible contamination.
- Protect fluorescent conjugates from light.

REQUIRED MATERIALS

- BioLite™ Antibody (Cat. No. ST11006 or Cat. No. ST11008)
- Cell Culture Medium
- Pluripotent Stem Cells

REQUIRED EQUIPMENT

- 37°C and 5% CO₂ Incubator
- Fluorescence Microscope

EXPERIMENTAL PROTOCOL

1. Refer to the Technical Data Sheet for the recommended starting concentration, and titrate the primary antibody for optimal performance on your cell type.
2. Dilute the primary antibody in fresh cell culture medium to the determined optimum concentration.
3. Aspirate the culture medium from the well of cells to be stained, and add the diluted antibody directly to the well of live cells.
4. Incubate the cells in a 5% CO₂ incubator at 37°C for 30 minutes.
5. Aspirate the primary antibody and gently wash the cells twice with fresh culture medium.
6. Add fresh cell culture medium to the well.
7. Examine the cells using a fluorescence microscope and appropriate filters.
8. Return cells to the incubator and continue to culture as normal.