Basic immunofluorescence protocol for adherent cells

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ABSTRACT

Adherent cells were cultured in flat bottom plates and fixed in situ. This is a protocol to detect specific proteins by immunofluorescence in these cells. Immunofluoroscence is a common laboratory technique where specific proteins within cells can be detected using antibodies coupled to fluorophores and then visualized using a microscope. The cells were counterstained with Hoechst dye to label nuclei.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

Secondary antibodies usually target species specific epitopes on the primary antibody. Thus, if the primary antibody was raised in goats, then the secondary should say "anti-goat". Further, antibodies can be of different isotypes such as IgM or IgG kappa (k) vs. lambda, or IgG1 vs. IgG2. It is important to make sure that the chosen secondary detects the specific isotype of your primary or all isotypes (for example, anti-goat IgG will detect both IgG1 and IgG2 or anti-goat Ig will all goat antibodies).

Anti-luciferase antibody

for 96 well plate, use 100 µL of buffers

MATERIALS

NAME	CATALOG #	VENDOR
Triton X-100	1610407	Bio-rad Laboratories
PBS		
Hoechst 33342, Trihydrochloride, Trihydrate - 10 mg/mL Solution in Water	H3570	Invitrogen - Thermo Fisher
4% Paraformaldehyde in PBS	J61899-AK	Alfa Aesar

STEPS MATERIALS

NAME	CATALOG #	VENDOR
4% Paraformaldehyde in PBS	J61899-AK	Alfa Aesar

BEFORE STARTING

1. Make sure the stock of paraformaldehyde (PFA) has not expired and is dissoved in an isotonic buffer such PBS.
2. Prepare or check that you have enough of the following buffers
   a. Permeabilization buffer: 0.1% Titron X, 1-2%FBS or BSA in PBS
   b. Staining buffer without detergent: 1-2%FBS or BSA in PBS

Fixation

1 Fix the cells using 4% paraformaldehyde (PFA) by removing media and submerging cells in PFA for 00:15:00 at

20 °C room temp . Cells can be left in fixative for a few days at 4 °C Fridge

4% Paraformaldehyde in PBS
by Alfa Aesar
Catalog #: J61899-AK

2 Aspirate fixative and replace with PBS. Cells can be stored at this step for upto 1 month.

Permeabilization

3 For the antibody to be able to penetrate the cells, the membranes need to be permeabilized. This may be necessary even if the protein to be detected is expressed on the surface if the antibody binds an intracellular region. Aspirate PBS from previous step and replace with 100 µl permeabilization buffer and incubate for 00:10:00 20 °C Room temp

Store staining buffer in 4 °C fridge

4 Aspirate permeabilization buffer and replace with 100 µl PBS. Aspirate PBS and proceed to next step or store in the fridge in fresh PBS.

Primary antibody staining

5 Incubate in primary antibody (the antibody that detects the protein of interest) at the recommended dilution (1:100) in staining buffer 00:00:00 Overnight 4 °C . See guidelines for dilutions of specific antibodies and duration of incubation.

Store staining buffer in 4 °C fridge

6 If you plan to reuse the diluted antibody for another experiment, use a pipette to transfer it into a collection tube for storage. Otherwise, aspirate primary antibody from the cells, and add PBS and leave plate on shaker for 00:05:00 at room temp. Repeat twice.

Secondary staining

7 For greater flexibility, primary antibodies are left unconjugated and detected using a secondary antibody which is specific to primary antibody (see guidelines) and are labeled with a fluorophore. Incubate in secondary antibody at the recommended dilution in staining buffer. See guidelines for dilutions of specific antibodies and duration of incubation.

Post-secondary washing and counterstaining for nuclei/DNA

8 Aspirate secondary antibody from the cells, and add PBS and leave plate on shaker for 00:05:00 at room temp.

9 Replace PBS with 1:2000 dilution of Hoechst dye and incubate at room temp for 00:20:00

10 Conduct two washes with PBS as in step 8. The cells are ready for visualization on the microscope.