

### 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  $\mu L$  of buffers

## MATERIALS

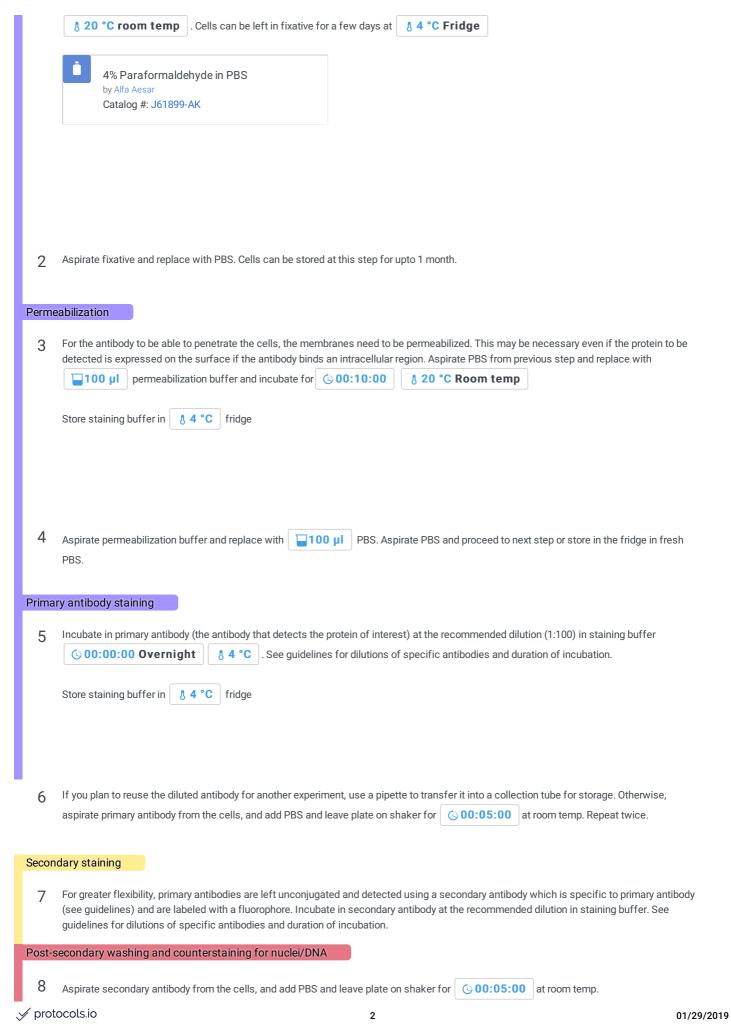
CATALOG #	VENDOR ~
1610407	Bio-rad Laboratories
H3570	Invitrogen - Thermo Fisher
J61899-AK	Alfa Aesar
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J61899-AK	Alfa Aesar
	1610407  H3570  J61899-AK  CATALOG #

## **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

Fix the cells using 4% paraformaldehyde (PFA) by removing media and submerging cells in PFA for 600:15:00



- 9 Replace PBS with 1:2000 dilution of Hoechst dye and incubate at room temp for © 00:20:00
- $10 \hspace{0.5cm} \hbox{Conduct two washes with PBS as in step 8. The cells are ready for visualization on the microscope.} \\$

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