Anti-Nuclei
Immunocytofluorescence Protocol

Catalog #: 1590-NUC
Species: mouse
Tissue/ Cells: HeLa cells

Fixation: 4% paraformaldehyde, 15 minutes at room temperature
Antibody incubation: Primary Antibody- RT, 1 hour Secondary Antibody- RT, 30 minutes

Materials Required

- ✓ **Fixative:** 4% paraformaldehyde in 1xPBS
- ✓ **1x PBS:** 137 mM NaCl, 28 mM Na_2HPO_4, 5.4 mM KCl, 2.9 mM KH_2PO_4, pH 7.6
- ✓ **Permeabilization solution (PBST):** 0.4% Triton-X 100 in 1xPBS
- ✓ **Blocking and incubation buffer:** 3% horse serum in 1xPBS
- ✓ **Secondary Antibody:** example used is Goat-Anti-Mouse Alexa 488 from Invitrogen, cat #A-11001
- ✓ **Mountant:** Prolong Gold Antifade Mountant from ThermoFisher, Cat # P10144

Before you begin

This protocol is intended for adherent cells grown in 6 well plates or in 35mm dishes using a glass coverslip placed on top of the cells. These work well with most immunofluorescence microscopes using lenses up to 100X magnification. Antigen retrieval is not recommended as this may reveal cross reactive site not normally seen.

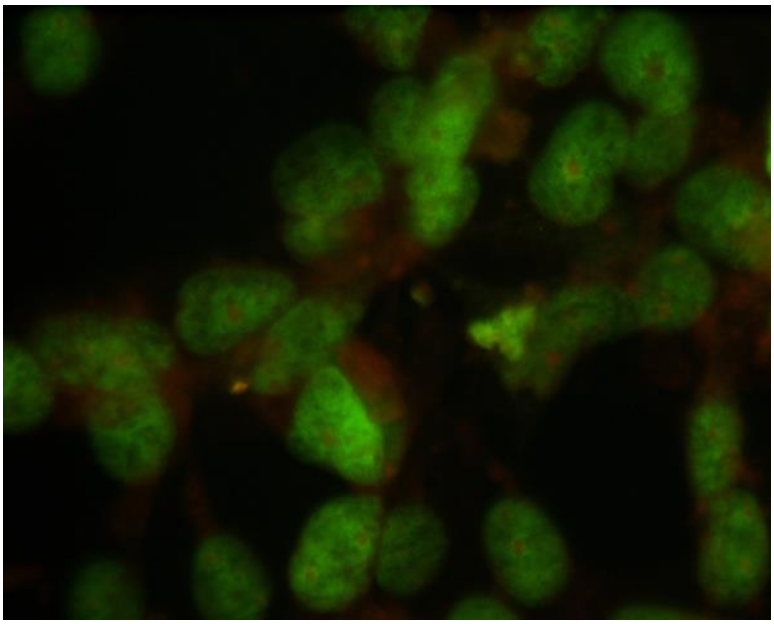
Protocol

1. Draw off culture medium with aspirator and wash cells with 1xPBS.
2. Remove 1xPBS and add 1 ml of fixative to the dish. Incubate at room temperature for 15 minutes.
3. Remove the fixative and wash with 1xPBS 3 times.
4. Permeabilize cells with permeabilization solution for 10 minutes.
5. Remove permeabilization solution and add blocking buffer. Incubate for 10 minutes at room temperature.
6. Rinse cells with 1xPBS 3 times, in 10 minute intervals.
7. Dilute the Anti-Nuclei (Cat. # 1590-NUC) to 1:100 in incubation buffer. Incubate cells for 1 hour at room temperature
8. Remove primary antibody and wash with 1xPBS 3 times, in 10 minute intervals.
9. Dilute secondary antibody in incubation buffer per manufacturer's recommendation. Incubate cells for 30 minutes at room temperature.
   Tech Tip:
   a. Alexa Fluor 488 dye diluted 1:2000 was used to produce image below.
10. Remove secondary antibody and wash with 1xPBS 3 times, in 5 minute intervals.

11. Apply mounting medium intended for fluorescence onto dish and gently place glass cover slip before viewing under the microscope.

Tech Tip:

a. There are various mounting medias for fluorescence that can be used, for this protocol the medium used was ProLong Gold (Molecular Probes).



Immunostaining of HeLa cells showing specific labeling of the nuclei using the anti-nuclei antibody (1:100, green).