

Anti-OLIG1

Immunocytofluorescence Protocol

Catalog #: 1537-OLIG1

Species: rabbit

Tissue/ Cells: Rat oligodendrocytes

Fixation: 4% paraformaldehyde, 15 minutes at room temperature

Antibody incubation: Primary Antibody- 4C, overnight Secondary Antibody- RT, 2 hours

Materials Required

- ✓ **Fixative:** 4% paraformaldehyde in 1xPBS
 - ✓ **1x PBS:** 137 mM NaCl, 28 mM Na₂HPO₄, 5.4 mM KCl, 2.9 mM KH₂PO₄, pH 7.6
 - ✓ **Wash Solution:** 0.4% Triton-X 100 in 1xPBS
 - ✓ **Blocking buffer:** 3% BSA in 1xPBS
 - ✓ **Incubation buffer:** 1% BSA in 1xPBS
 - ✓ **Secondary Antibody:** example used is Goat-Anti-Rabbit Alexa 647 from Invitrogen, cat [#A-21245](#)
 - ✓ **Mountant:** Prolong Gold Antifade Mountant from ThermoFisher, [Cat # P10144](#)
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Before you begin

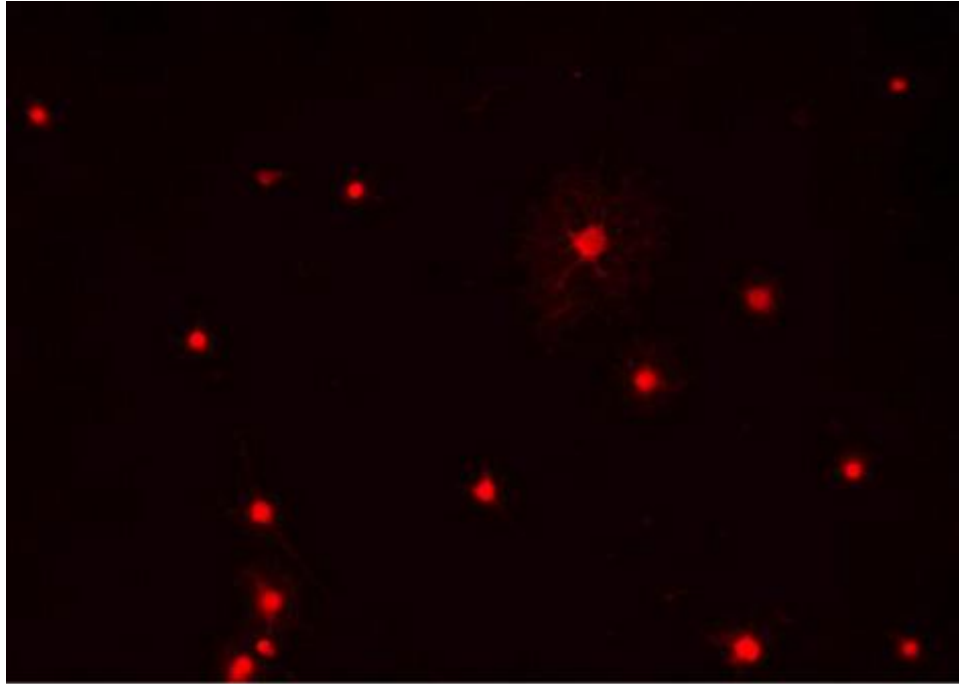
This protocol is intended for adherent cells grown in 6 well plates or in 35mm dishes using a glass coverslip placed on top of the cells. These work well with most immunofluorescence microscopes using lenses up to 100X magnification.

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 wash buffer 3 times.
4. Incubate blocking buffer for 30 minutes at room temperature.
5. Rinse cells with wash buffer 3 times, in 10 minute intervals.
6. Dilute the Anti-OLIG1 (Cat. # 1537-OLIG1) to 1:100 in incubation buffer. Incubate cells overnight at 4C.
7. Remove primary antibody and wash with wash buffer 3 times, in 10 minute intervals.
8. Dilute secondary antibody in incubation buffer per manufacturer's recommendation. Incubate cells for 2 hours at room temperature.
 - Tech Tip:**
 - a. Alexa Fluor 648 dye diluted 1:2000 was used to produce image below.
9. Remove secondary antibody and wash with wash buffer 3 times, in 5 minute intervals.
10. 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 primary rat oligodendrocytes using anti-Olig1 (cat. #1537-OLIG1, red, 1:100).