Anti-OLIG2
Immunohistofluorescence Protocol

Catalog #: 1538-OLIG2
Species: mouse
Tissue: Mouse spinal cord and brain

This antibody has successfully been used in immunohistochemistry on human oligodendroglioma, as well as rat brain and spinal cord. Additionally, this antibody has successfully been used in immunocytochemistry on human oligodendroglioma, rat primary neuroepithelial and mouse transfected cell lines.

Fixation: 4% paraformaldehyde in PBS overnight at 4C
Antibody incubation: Primary Antibody: 4C, overnight Secondary Antibody: RT, 1 hour
Antigen Retrieval: Warm 10mM citrate buffer (pH 6.0, 95C) and steam for 30 minutes. Cool slides in citrate bath to room temperature

Materials Required

- ✓ **Fixative:** 4% paraformaldehyde in PBS
- ✓ **1X PBS:** 137 mM NaCl, 28 mM Na_2HPO_4, 5.4 mM KCl, 2.9 mM KH_2PO_4, pH 7.6
- ✓ **PBST:** 0.4% Triton-X in 1X PBS
- ✓ **Blocking buffer:** 10% goat serum in PBST
- ✓ **Incubation buffer:** 2% goat serum in PBST
- ✓ **Secondary Antibody:** example used is Goat-Anti-Rabbit Cy 5, ThermoFisher (catalog # A10523)
- ✓ **Mounting media:** Permount, Fisher Scientific (catalog # SP15-100)
- ✓ **Counterstain:** DAPI, ThermoFisher (catalog # D1306)

Before you begin

This protocol was used for tissues fixed without perfusion following standard FFPE protocol. Cut tissue into 3-5mm sections and place in 4% paraformaldehyde in PBS overnight at 4C. For proper fixation, submerge sections into a 20x volume of fixative based on the mass of the tissue. After fixation of tissue, dehydrate and embed tissue into paraffin blocks according to standard protocol. Then section the blocks at 5 microns. Finally, transfer the sections onto positively charged slides (example: SFH1103, BioCare Medical) and dry overnight at room temperature.

Deparafinize

1. Warm slides for 10 minutes in a 60C oven.
2. Incubate slides in the following dehydrants in this order
   I. Xylene: 3 times, 10 minute intervals
   II. 100% ethanol: 2 times, 5 minute intervals
   III. 95% ethanol: 2 times, 3 minute intervals
   IV. 80% ethanol: 2 times, 1 minute interval
   V. H2O: 2 times, dip to rinse.

Antigen Retrieval

1. Place slides in 10mM citrate buffer (pH 6.0, 95C) and steam for 30 minutes. Let slides cool to room temperature in citrate bath.
2. Wash slides with 1X PBS.

Immunohistochemistry

1. Block slides with blocking buffer for 30 minutes at RT.
2. Wash slides with PBST 3 times, in 15 minute intervals.
3. Dilute Anti-OLIG2 (Cat. # 1538-OLIG2) to 1:1000 in incubation buffer. Incubate sections for 3 hours at room temperature or overnight at 4C.
4. Wash slides with PBST 3 times, in 15 minute intervals.
5. Dilute secondary antibody in incubation buffer per manufacturer's recommendation. Incubate sections for 1 hour at room temperature.

   Tech Tip:

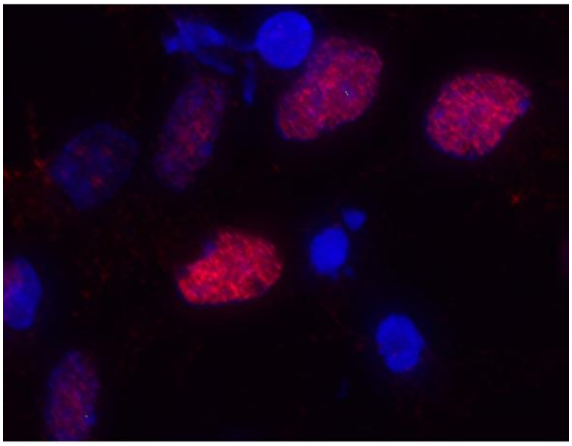
   a. An anti-rabbit Cy5 antibody was used to produce the image below, ThermoFisher (catalog # A10523, 1:500). Any anti-rabbit secondary can be used.
6. Remove secondary antibody and wash slides with PBST 3 times, in 15 minute intervals.
7. Prepare fresh DAPI solution per manufacturer's recommendation. Apply to slide and rinse 5 times with PBS.

   Tech Tip:

   a. Any nuclear counterstain can be used, for this protocol DAPI was used, catalog # D1306.
8. Apply mounting medium onto slide and gently place glass cover slip before viewing under the microscope.

   Tech Tip:

   a. Any mounting media can be used, for this protocol Permount was used, catalog # SP15-100.



Immunostaining of primary rat cortical neuroepithelial cells with anti-Olig2 (cat# 1538-OLIG2, red, 1:1000). The cells were treated with basic FGF for 30 hours prior to staining (this induces Olig2 expression in these primary cells). The blue is DAPI staining nuclear DNA.

References:

Ligon, K.L., Alberta, J.A., Kho, A.T., Weiss, J., Kwaan, M.R., Nutt, C.L., Louis, D.N., Stiles, C.D. and Rowitch, D.H., 2004. The oligodendroglial lineage marker OLIG2 is universally expressed in diffuse gliomas. *Journal of neuropathology and experimental neurology*, 63(5), pp.499-509.

Singh, S.K., Fiorelli, R., Kupp, R., Rajan, S., Szeto, E., Cascio, C.L., Maire, C.L., Sun, Y., Alberta, J.A., Eschbacher, J.M. and Ligon, K.L., 2016. Post-translational modifications of OLIG2 regulate glioma invasion through the TGF-β pathway. *Cell reports*, 16(4), pp.950-966.

Ligon KL, Huillard E, Mehta S, Kesari S, Liu H, Alberta JA, Bachoo RM, Kane M, Louis DN, Depinho RA, Anderson DJ, Stiles CD, Rowitch DH (2007) Olig2 regulated lineage-restricted pathway controls replication competence in neural stem cells and malignant glioma. *Neuron* 53(4):503-17.