Anti- Phospho-Ser40 Tyrosine Hydroxylase Immunocytofluorescence Protocol

Catalog #: p1580-40
Species: rabbit
Tissue/ Cells: Rat PC-12 cells

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

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):** 0.1% Triton-X 100 in 1xPBS
- ✓ **Blocking buffer:** 5% FBS (fetal bovine serum), 0.3% saponin in 1xPBS
- ✓ **Incubation buffer:** 1% FBS in 1xPBS
- ✓ **Secondary Antibody:** example used is Goat-Anti-Rabbit Alexa Fluor 488 from Invitrogen
- ✓ **Mounting media:** ProLong Gold with DAPI (Molecular Probes)
- ✓ **Glass cover slip**

Before you begin

This protocol is intended for PC-12 cells that were transfected with various recombinant tyrosine hydroxylase mutants. For preparing, growing, and propagating these cultured cells reference Jorge-Finnigan et al (2017).

Protocol

1. Draw off culture medium with aspirator and add 1 ml of fixative to the coverslip. Incubate at room temperature for 30 minutes.
2. Remove the fixative and wash with 1xPBS 3 times.
3. Permeabilize cover slips with permeabilization solution for 5 minutes.
4. Remove permeabilization solution and add blocking buffer. Incubate for 30 minutes at room temperature.
5. Rinse coverslips with 1xPBS 3 times, in 10 minute intervals.
6. Dilute the Anti- Phospho-Ser^{40} Tyrosine Hydroxylase (Cat. # p1580-40) to 1:50 in incubation buffer. Incubate cells overnight at 4C.
7. Remove primary antibody and wash with 1xPBS 3 times, in 10 minute intervals.
8. Dilute secondary antibody in incubation buffer per manufacturer's recommendation. Incubate cells for 1 hour at room temperature.
   Tech Tip:
   a. Alexa Fluor 488 dye diluted 1:200 was used in this protocol.
9. Remove secondary antibody and wash with 1xPBS 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 with DAPI (Molecular Probes).

Reference:

Jorge-Finnigan, A., Kleppe, R., Jung-KC, K., Ying, M., Marie, M., Rios-Mondragon, I., Salvatore, M.F., Saraste, J. and Martinez, A., 2017. Phosphorylation at serine 31 targets tyrosine hydroxylase to vesicles for transport along microtubules. *Journal of Biological Chemistry*, 292(34), pp.14092-14107.