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 Na₂HPO₄, 5.4 mM KCl, 2.9 mM KH₂PO₄, 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-Ser⁴⁰ 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](https://www.molecularprobes.com)).

**Reference:**