Anti-Phospho- Ser31 Tyrosine Hydroxylase Antibody Immunohistofluorescence Protocol

Catalog #: p1580-31
Species: Rabbit
Tissue: Rat retina

Fixation: 4% Paraformaldehyde in 1X PBS for 60 minutes
Antibody incubation: Primary Antibody- 4C, 12-16 hours Secondary Antibody- RT, 2 hours

Materials Required

- ✓ **Fixative:** 4% Paraformaldehyde in freshly prepared 1X PBS
- ✓ **1X PBS:** 137 mM NaCl, 28 mM Na_2HPO_4, 5.4 mM KCl, 2.9 mM KH_2PO_4 , pH 7.6
- ✓ **30% sucrose buffer:** 30g of sucrose in 100mls of 1xPBS
- ✓ **Blocking/Incubation buffer:** 1X PBS with 1% Bovine Serum Albumin (BSA), 0.1% Triton X-100, 0.1% NaN_3 also use for secondary incubation buffer
- ✓ **Secondary Antibody:** example used is Donkey-Anti-Rabbit FITC from Jackson ImmunoResearch Laboratories Cat# 711-095-152
- ✓ **Mounting media:** ProLong Gold Antifade Mountant Medium Cat #: H-1200

Before you begin

This protocol was intended for retinas that have undergone light sensitivities and drug treatment before fixing. For more information reference Witkovsky et al, 2000. For optimal fixation slices must be placed in fixative volume 20x greater than the mass. For optimal antibody epitope binding, tissues should not be stored in fixative. It is best to store fixed tissue in cryoprotectant solution of 30% sucrose buffer.

Protocol

1. Following experimental treatment fix rat eyecups with freshly prepared 4% paraformaldehyde in 1X PBS for 1 hour at room temperature.
2. Wash the sections 6 times with 1X PBS in 10 minute intervals.
3. Place fixed tissue specimen into 30% sucrose in 1X PBS overnight at room temperature.
   Tech Tip:
   a. To prevent ice crystals from forming on tissue and destroying antibody epitope binding sites, do not remove the tissue until it has sunk to the bottom of the beaker to ensure complete sucrose infiltration.
4. Mount tissue onto cryostat and cut tissue into 12-16 micron thick sections at -20C. Mount tissue sections onto gelatin coated slides. Air dry the slides and store at -20C.
5. Thaw sections for 10 minutes at RT.
6. Wash the sections 6 times with 1X PBS in 10 minute intervals.

7. Block slides with blocking buffer for 1 hour at RT.
8. Dilute Anti-Phospho-Ser[31] Tyrosine Hydroxylase Antibody (Cat. # p1580-31) to 1:300 in blocking buffer. Incubate sections for 12-16 hours or overnight at 4C.
   Tech Tip:
   a. In this protocol a cocktail mixture was used of a mouse monoclonal tyrosine hydroxylase and the phosphospecific antibody. When mixing primary antibodies only mix antibodies of different species.
9. Wash the sections 6 times with 1X PBS in 10 minute intervals.
10. Dilute secondary antibody in blocking buffer per manufacturer's recommendation. Incubate sections for 2 hours at room temperature.
    Tech Tip:
    a. For this protocol a donkey anti-rabbit FITC secondary diluted to 1:100 was used.
11. Remove secondary antibody, wash the sections 6 times with 1X PBS in 10 minute intervals.
12. 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, an example is ProLong Anti-Fade medium. Cat #: H-1200.

Reference:

Witkovsky, P., Gabriel, R., Haycock, J.W. and Meller, E., 2000. Influence of light and neural circuitry on tyrosine hydroxylase phosphorylation in the rat retina. *Journal of chemical neuroanatomy*, 19(2), pp.105-116.