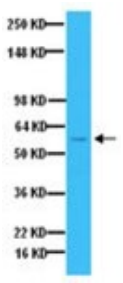
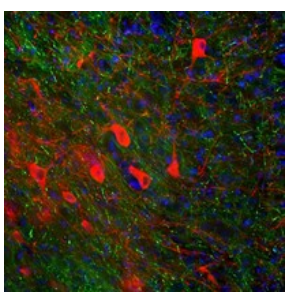
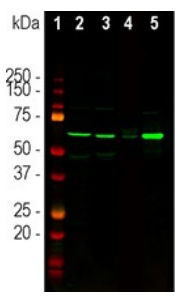
Product Data Sheet: Tyrosine Hydroxylase

Cat. No:	AB-10312
Conjugate:	Unconjugated
Size:	100 ul
Clone:	POLY
Concentration:	1mg/ml
Host:	Rb
Isotype:	IgG
Immunogen:	Two rabbits were immunized with sodium dodecyl sulfated-denatured tyrosine hydroxylase that was purified from rat pheochromocytoma. Immune sera were subjected to ammonium sulphate fractionation to purify immunoglobulins. Antibodies to tyrosine hydroxylase were then purified by affinity chromatography on a CNBr-Sepharose column to which purified tyrosine hydroxylase was bound. This antibody preparation has been compared to numerous other rabbit anti-tyrosine hydroxylase preparations with several light microscopic immunocytochemical procedures and was found to provide, as a rule, superior immunocytochemical reactivity. Preliminary data also indicate that this antibody preparation reacts well with glutaraldehyde-fixed tissue suggesting that it will be preferred antibody for ultrastructural localization studies. The antibody has also been characterized with Western blots.
Reactivity:	Hu, Ms, Rt
Applications:	Western Blot : 1:2500 Immunohistochemistry (Frozen Tissues) : 1:1000 Immunohistocmistry (Paraffin Embedded Tissues): 1:1000 Immunofluorescence: 1:1000 Immunocytochemistry: 1:1000 Optimal dilution must be determined by end-user
Purification:	Aff. Pur.
Background:	Specific for the □ 60 k tyrosine Hydroxylase in Western Blots of rat brain lysates of PG-12 cells stimulated by Okadaic Acid. The antibody cross-reacts with all mammalian and at least some non mammalian forms of the enzyme in immunolabeling and immunocytochemical studies.
Form:	Liquid
Buffer:	Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN3
Storage:	Store the antibody at -20°C for long term storage and refrigerate a 2-8°C for shorter term. Avoid freeze and thaw cycles. Stock solutions are stable for a minimum of 1 year at -20°C.





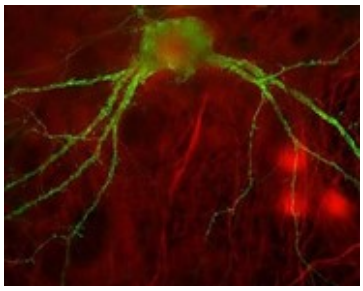


Product Data Sheet: Tyrosine Hydroxylase

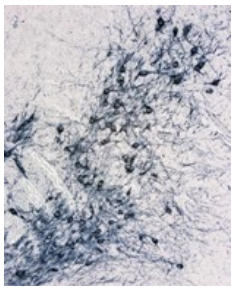
Western Blot Analysis ((~62 kDa):
PC12 lysate was resolved by electrophoresis, transferred to PVDF membrane and probed with anti-tyrosine hydroxylase
Proteins were visualized using a donkey anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

Immunofluorescent analysis of rat brain section stained with rabbit pAb to tyrosine hydroxylase, dilution 1:10,000, in red and costained with mouse mAb to pNF-H, dilution 1:1,000 in green.
The blue is Hoechst staining of nuclear DNA. Following transcardial perfusion of rat with 4% paraformaldehyde, brain was post fixed for 24 hours, cut to 45µM, and free-floating sections were stained with the above antibodies. The TH1 antibody stains the striatal TH expressing interneurons, while the pNF-H antibody labels axons from other neuronal cells.

Western blot analysis of tissue and cell lysates using rabbit polyclonal antibody to tyrosine hydroxylase, AB-10312 dilution 1:5,000 in green:
[1] protein standard (red), [2] rat brain caudate/putmen, [3] HeLa cell extract and [4] PC12 cells. The strong band at about 58kDa corresponds to TH protein.



Immunofluorescence: Representative image from a previous lot. Anti-Tyrosine Hydroxylase (TH) staining in mouse primary neural cultures using AB-10312 shown with an FITC fluorescent secondary (green). Fixation was 4% PFA and the primary antibody incubation was 1:1000, overnight at 4°C.



AB-10312 IHC(P) on Mouse Brain Tissues Substantia Nigra. Immunohistochemical performed by: Dr. Francesca Biagioni, lab. Neurobiology of Movement Disorders, I.R.C.C.S. INM Neuromed and Dr. Maria Teresa Calierno
I.R.C.C.S. INM Neuromed

AB-10312 IHC(P) on Mouse Brain Tissues Striatum. Immunohistochemical performed by: Dr. Francesca Biagioni, lab. Neurobiology of Movement Disorders, I.R.C.C.S. INM Neuromed and Dr. Maria Teresa Calierno
I.R.C.C.S. INM Neuromed

IMMUNOHISTOCHEMISTRY PROTOCOL (Paraffin Embedded Tissues)

Deparafinized section:
– Xilene 30’;
– ETOH 100□ 10’;
– ETOH 96□ 10’;
– ETOH 70□ 10’;
– H2Od 5’

-Antigen retrieval:
□ Acetic acid 5% in ETOH 100% at RT for 15’
□ Unmasking with pH 9 buffer (Microwave 10’): pH 9 buffer.
□ Unmasking with pH 8 buffer (Microwave 10’): pH 8 buffer

-Wash in PBS 3×10’
-Triton X100 0,1% (in PBS) 15’
– Wash in PBS 3×10’
-Hydrogen peroxide 3% 10’
– Wash in PBS 3×10’
-Normal Goat Serum (NGS) 10% in PBS 1h
-Incubation with anti-TH □1:200□ in PBS+NGS 2% overnight a +4□C in humid chamber
– Wash in PBS 3×10’
– Incubation with Ab II biotinilated anti-goat □1:200□ in PBS 1h

– Wash in PBS 3×10’
– Horseradish peroxidase streptavidin (1:100) in PBS 1h
– Wash in PBS 3×10’
-Develop with chromogen (DAB + metal enhancer) 3’

PROCEDURE OF IMMUNOFLUORESCENT STAINING OF FREE-FLOATING BRAIN TISSUE SECTIONSTISSUE PREPARATION:

1. Perfuse transcardially the animal (rat or mouse) with ice-cold PBS (pH7.4), followed by freshly made 4% paraformaldehyde fixative solution in PBS.
2. Postfix the removed brain in the same 4% paraformaldehyde fixative solution in PBS (4°C for 16 – 24 hours).
3. Cryoprotect the tissue by immersing it in sucrose solutions in PBS (15%, for 24 hours followed by 30% until tissue will sink, may take from 48 hours up to 1 week).
4. Cut 40 – 50µm sections on a cryostat.
5. Keep sections in PBS + 0.05M NaN3 at 4°C
until they were taken for staining. During the staining process, the sections should never be allowed to dry out.