

Anti-Tyrosine Hydroxylase

Polyclonal Antibody

Cat. # AB152

Lot # JC1658244

pack size: 100 µL

Store at -20 °C to -70°C

FOR RESEARCH USE ONLY



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, ELISA, IH, IH(P), IP, IF	Dr, Ft, H, M, R, Fe, MI, Sqd	N/A	N/A	Rb	62 kDa	N/A

Background

Tyrosine hydroxylase plays an important role in the physiology of adrenergic neurons. It is the first and rate-limiting enzyme involved in the biosynthesis of the catecholamines Dopamine and Norepinephrine from tyrosine. TH is, therefore, a useful marker for dopaminergic and noradrenergic neurons. The enzymatic activity of TH requires ferrous ions as cofactors and is believed to be regulated by phosphorylation. At least four isoforms of human TH have been identified which result from alternative splicing.

Presentation

Purified rabbit polyclonal in buffer containing liquid in 10 mM HEPES 150 mM NaCl (pH 7.5), 100 µg/mL BSA and 50% glycerol.

Specificity

Tyrosine hydroxylase. By western blot, AB152 selective labels a single band at approximately 62kDa (reduced) corresponding to Tyrosine Hydroxylase. It is expected that the antibody will react with most mammalian and many non-mammalian species. It has been reported that this antibody does not work on paraffin embedded human tissue.

Species Cross-reactivity

Rat, mouse, ferret, feline, Aplysia (marine mollusc). It is expected that the antibody will react with most mammalian and many non-mammalian species. It has been reported that this antibody does not work on paraffin embedded human tissue.

Immunogen

Denatured tyrosine hydroxylase from rat pheochromocytoma (denatured by sodium

dodecyl sulfate).

Molecular Weight

62 kDa

Method of Purification

Affinity purified

Storage and Handling

Maintain frozen at -20°C to -70°C in undiluted aliquots for up to 6 months after date of receipt. Avoid repeated freeze/thaw cycles. Do not store in a self defrosting freezer.

Control

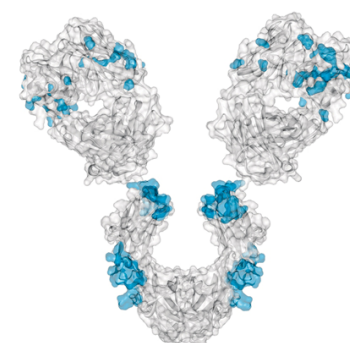
POSITIVE CONTROL: Brain (corpus striatum, sympathetic nerve terminals) and adrenal glands.

NEGATIVE CONTROL: Liver.

Quality Control Testing

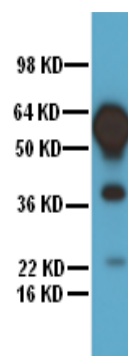
Routinely evaluated by Western Blot on PC12 lysates.

Western Blot Analysis: 1:1000 dilution of this lot detected tyrosine hydroxylase on 10 ug of PC12 lysates.



References

1. Tabuchi, Katsuhiko, *et al.* (2007). *Science*. 318: 71-6.
2. Nunez, Christine, *et al.* (2007). *Endocrinology*.
3. Pavon, Nancy, *et al.* (2006). *Biol Psychiatry*. 59: 64-74.
4. DeI Giacco, Luca, *et al.* (2006). *BMC Dev Biol*. 6: 50.
5. Hollis, J., *et al.* (2005). *J. Endocrinology*. 184: 393-406.



Western Blot Analysis:
Representative lot data. PC12 lysate was resolved by electrophoresis, transferred to PVDF membrane and probed with anti-tyrosine hydroxylase (1:1000 dilution of this lot). Proteins were visualized using a donkey anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

Arrow indicates protein tyrosine hydroxylase (~62 kDa).

APPLICATION LEGEND: WB Western Blotting ELISA Enzyme-linked Immunosorbent Assay IP Immunoprecipitation IH(P) Immunohistochemistry (Paraffin) IF Immunofluorescence IH Immunohistochemistry (Tissue)

SPECIES LEGEND: H Human M Mouse R Rat Rb Rabbit Dr Drosophila Ft Ferret Fe Feline (cat) MI Mollusk Sqd Squid

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Additional Research Applications

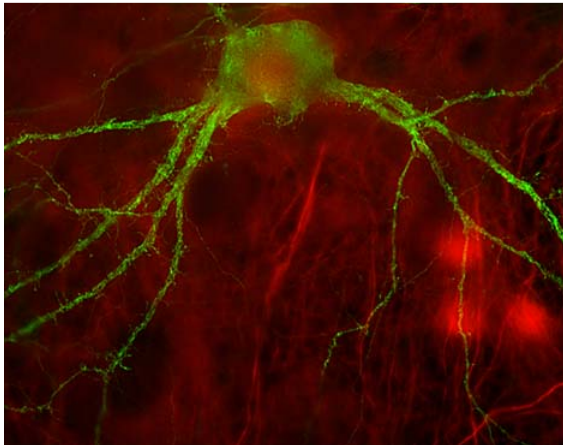
Immunohistochemistry: A previous lot of this antibody was used at 1:100-1:1,000 dilution, 4°C, for 1-3 days. Stains both fresh frozen and paraffin embedded tissue samples by indirect immunofluorescence and immunoperoxidase.

Immunofluorescence: A previous lot of this antibody was used at 1:100-1:1,000 dilution.

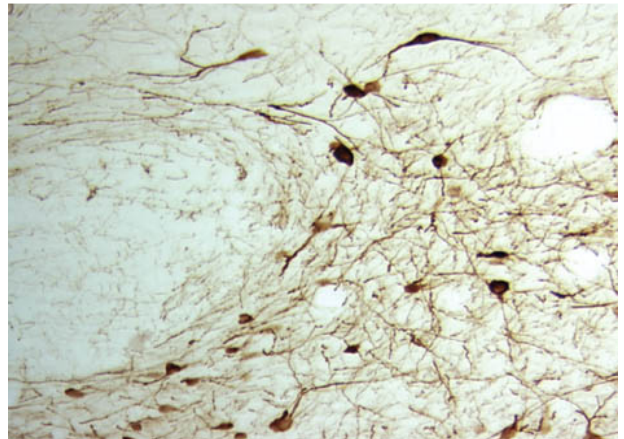
Immunoprecipitation: A previous lot of this antibody was used on Immunoprecipitation.

ELISA: A previous lot of this antibody was used in ELISA.

Optimal dilutions must be determined by the end user.



Immunofluorescence: Representative image from a previous lot. Anti-Tyrosine Hydroxylase (TH) staining in mouse primary neural cultures using AB152 shown with an FITC fluorescent secondary (green). Fixation was 4% PFA and the primary antibody incubation was 1:1000, overnight at 4°C.



Immunohistochemistry: Representative image from a previous lot. Rabbit anti-Tyrosine Hydroxylase (Catalog Number AB152) staining of rat brain stem.

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.

PROTOCOL

Western Blotting

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on cell lysate and transfer the proteins to a PVDF membrane. Wash the PVDF membrane twice with water.
2. Block the blotted PVDF membrane in freshly prepared 5% BSA with 0.05% Tween®-20 for 1 hour at room temp. with constant agitation.
3. Incubate the PVDF with the recommended dilution of anti-Tyrosine Hydroxylase at 1:1000 dilution in freshly prepared 5% BSA for 1 hour at room temperature or overnight with agitation at 2-8°C.
4. Wash the PVDF 3 times with TBST.
5. Incubate the PVDF in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348 1:1000 dilution was used) in 5% milk for 1 hour with agitation at room temperature.
6. Wash the PVDF 3-5 times with TBST.
7. Use Spray and Glow Catalog # 17-373 to visualize results. Use as directed.

■ antibodies ■ Multiplex products ■ biotools ■ cell culture ■ enzymes ■ kits ■ proteins/peptides ■ siRNA/cDNA products

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GENERAL METHODOLOGY FOR RAT BRAIN PERFUSION AND STAINING USING AB152

1. Perfuse the animal with PBS with 4% paraformaldehyde fixative. (Glutaraldehyde [0.1-0.5%] may be used.)
2. Remove brain and place it in cold fixative without glutaraldehyde (2-4 hours postfixation at 0-4°C).
3. Transfer the brain (or blocks therefrom) to PBS containing at least 5% sucrose. When ready to section, place the tissue on cryostat chucks and freeze them on crushed dry ice. Alternatively, freeze the sample on the stage of a sliding microtome or glue the sample onto a Vibratome chuck.
4. Make 15-30 micron sections and place them in PBS. Rinse the sections in PBS several times.
5. Place the sections in blocking solution (PBS containing 10% goat serum, 1% RIA-BSA, 0.3% Triton X-100 [high purity]) for 30-60 minutes at room temperature.
6. After a brief rinse in carrier solution (optional), place the sections in primary antibody that has been diluted in carrier solutions (PBS containing 1% goat serum, 1% RIA-BSA, 0.3% Triton X-100 [high purity]). Final working dilutions to be determined by end user. Suggested starting dilution is 1:100-1:500 of the antibody. Successful incubation conditions include 1-3 days at 0-4°C, 2-18 hours at room temperature, and 1-4 hours at 37°C.
7. Rinse the sections 3x with the carrier solution and place the sections in secondary antibody reagent. This is usually labeled or unlabeled goat anti-rabbit antibody, depending upon the visualization method to be used. For peroxidase reactions, the typical incubation is 1 hour at 37°C or 2 hours at room temperature.
8. Rinse the sections 3x with the carrier solution. For fluorescence labeling, rinse an additional time with PBS alone and mount from distilled water.
9. For peroxidase-anti-peroxidase reactions, place the sections in carrier solution containing the tertiary antibody (substitute Tween 20 or Nonidet P40 for Triton X-100). For avidin-biotin-complex reactions, place the sections in PBS containing either Tween 20 or Nonidet P40. Incubate the sections for 1-2 hours at room temperature and rinse them 3x in PBS alone.
10. For peroxidase development, place the sections in PBS containing diaminobenzide (50 mg/100 mL) and add 33 µL of 30% H₂O₂. After 2-30 minutes at room temperature, rinse the sections well with PBS, mount the sections from dilute PBS (e.g. 1:5, PBS:H₂O), and air dry. Rinse the slides in several changes of distilled water, dehydrate and clear.

RELATED PRODUCTS (specific)

cat #	description
MAB5278	■ Anti-Tryptophan Hydroxylase/Tyrosine Hydroxylase/Phenylalanine Hydroxylase, clone PH8-1
AB151	■ Anti-Tyrosine Hydroxylase
AB152	■ Anti-Tyrosine Hydroxylase
AB1542	■ Anti-Tyrosine Hydroxylase
AB5986	■ Anti-Tyrosine Hydroxylase
AB5986P	■ Anti-Tyrosine Hydroxylase
AB9702	■ Anti-Tyrosine Hydroxylase
MAB5280	■ Anti-Tyrosine Hydroxylase, clone 2/40/15
MAB5280X	■ Anti-Tyrosine Hydroxylase, Clone 2/40/15, AlexaFluor® 488 Conjugated
MAB318	■ Anti-Tyrosine Hydroxylase, clone LNC1
MAB358	■ Anti-Tyrosine Hydroxylase, clone TOHA1.1
AB5935	■ Anti-Tyrosine Hydroxylase, phosphoSer 40
AB5935-25UL	■ Anti-Tyrosine Hydroxylase, phosphoSer 40
AB5425	■ Anti-Tyrosine Hydroxylase, phosphoSer 19
AB5425-25UL	■ Anti-Tyrosine Hydroxylase, phosphoSer 19
AB5423	■ Anti-Tyrosine Hydroxylase, phosphoSer 31
AB5423-25UL	■ Anti-Tyrosine Hydroxylase, phosphoSer 31
IHCR1003-6	■ Anti-Tyrosine Hydroxylase, prediluted
IHCR1005-6	■ Anti-Tyrosine Hydroxylase, prediluted, clone LNC1
AG398	■ Tyrosine Hydroxylase pSer31, Control Peptide for AB5423
AP132P	■ Goat anti-Rabbit IgG, Peroxidase Conjugated

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0050	■ IMMOBILON WESTERN CHEMILUM HRP SUBSTRATE 50 mL
17-373SP	■ Spray & Glow™ ECL Western Blotting 40 mL
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G
2170	■ CHEMIBLOCKER-1LT
20-200	■ IMMUNOBLOT BLOCKING REAGENT 20G

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