Product Data Sheet:
Tyrosine Hydroxylase Mouse Monoclonal Antibody

Cat. No:	MAB-10263
Conjugate:	Unconjugated
Size:	100 ug
Clone:	TH-100
Concentration:	1mg/ml
Host:	Ms
Isotype:	IgG1 heavy, κ light
Immunogen:	Full length human TH as expressed in and purified from E. coli
Reactivity:	Human, Rat, Mouse
Applications:	Western Blot: 1:1000 Immunohistochemistry(Paraffin Embedded tissues): 1:250 Immunohistochemistry(F) formalin or acetone fixed tissues: 1:500 Immunofluorescence:1:500 Immunocytochemistry: 1:500
Molecular Weight:	~58kDa
Purification:	Immunogen Affinity Purified.
Background:	Tyrosine hydroxylase (TH) is a vital enzyme responsible for the generation of L-DOPA from the amino acid tyrosine. L-DOPA is the direct precursor of the neurotransmitter dopamine, and dopamine can itself be processed to produce the neurotransmitters adrenalin and noradrenalin (a.k.a. epinephrin and norepinephrin respectively). Neurons which use dopamine, adrenalin or noradrenaline, called collectively chatecholamines, must express TH. TH has a very restricted distribution in the brain but is highly expressed in the cells in which it is found. As a result antibodies to TH are useful for the identification of chatecholaminergic neurons. TH positive neurons in the rat are localized into clusters of cells most of which are in the brain stem, including notably the substantia nigra and locus ceruleus (1,2). The clusters of cells are usually referred to by a classification scheme based on that proposed by Dahlström and Fuxe, which labels cells in groups A1 – A17 and C1 to C3 (2). Subpopulations of neurons are localized in the olfactory bulb, habenula and retina. TH positive cells are also found in a subset of cells in the adrenal medulla, sympathetic ganglia, sensory ganglia and enteric ganglia (2). Numerous TH positive axons can be seen coursing through the striatum and to a much lesser degree the cortex originating from the mid brain A8, A9 and A10 nuclei. TH neurons have a huge impact on brain function and behavior but are relatively infrequent- the rat brain contains about 22,000 TH positive neurons in the A8, A9 and A10 nuclei out of a total of 200 million neurons (3). Parkinson's disease is caused by the loss of THpositive dopaminergic neurons in the substantia nigra, which are also relatively low in number (4), and perturbation of TH neurons has been implicated in Alzheimer's disease and schizophrenia (5-7). There is one mammalian gene which produces one mRNA transcript and one protein in rat but four alternate mRNA transcripts produce four slightly different forms of TH proteins in humans (8)
Form:	Liquid
Buffer:	Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN3
Storage:	Store at 4°C for short term, for longer term at -20°C

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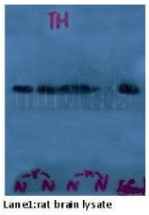
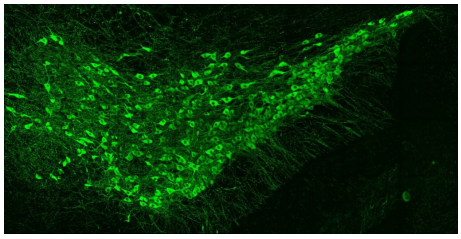
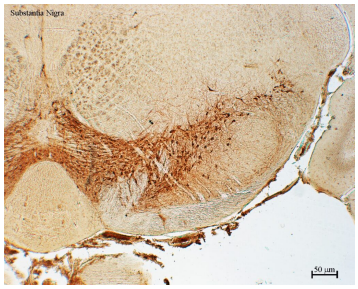


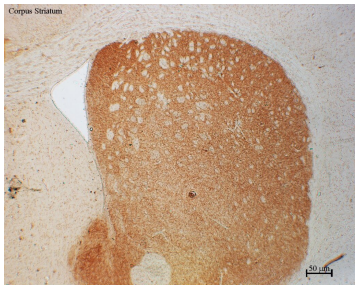
Lane1:rat brain lysate
Lane2:rat brain lysate
Lane3: mice brain lysate
Lane4: mice brain lysate
Lane5:mice IgG(molecule weight 55KD)



A section of mouse midbrain stained with mouse monoclonal antibody to tyrosine hydroxylase TH-100 in green. The cytoplasm and processes of these dopaminergic neurons are revealed.



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.



Anti-Tyrosine Hdroxylase Antibody in IHC (P) 1:100 on mouse brain tissue – (substantia nigra & striatum).

IMMUNOHISTOCHEMISTRY (Paraffin Embedded Tissues) SABC- METHODPreparation of tissue (SABC)

1) Dewax:
• Prepare three bottles of 90%, 95% and 100% dimethylbenzene and three bottles of 90%, 95% and 100% ethanol.
• Immerse paraffin sections into three bottles of dimethylbenzene orderly (from low to high), 7min each.
Then immerse into ethanol orderly at room temperature (from high to low), 7min each. Wash with water to remove ethanol.
Note: The process of dewaxing should be done in a fume hood at room temperature in summer. When the temperature is lower than 18°C, it is recommended to dewax at 50°C.
2) Inactivation
• Mix 30% H2O2 with distilled water(1:9). Immerse dewaxed paraffin section into the 3%H2O2 at room temperature for 10mim. Wash with distilled water for several times.
3) Repair
• Heat repair: Immerse the paraffin sections into Citrate buffer (pH6.0), heat until boiling in the microwave and then cut off the power, keep it in the microwave for nearly 5~10 mins. Repeat 1 or 2 times. Then cool at room temperature and wash 1 or 2 times with PBS(pH7.2-7.6)
Blocking
Add 5% BSA blocking solution or Normal goat serum and incubate at 37°C for 30min. Discard extra liquid, no washing.
4) Adding primary antibody
Dilute primary antibody: 1ug/ml is regarded as the concentration. Add some antibody diluents solution to primary antibody. Incubate at 37°C for 2 hours or overnight.
5) Wash with PBS for 2 times, 20min each. Add biotin-conjugated secondary antibody and incubate at 37°C for 30min.
6) Wash with PBS for 2 times, 20min each. Add SABC reagents and incubate at 37°C for 30 min. Wash with PBS for 3 times, 20min each.
7) Add DAB reagent and incubate at RT. Wash with distilled water for several times.
8) Counterstain with haematoxylin. Dehydration and then immerse paraffin sections into dimethylbenzene twice, 7min each. Observe with microscope after seal

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