Anti-Tyrosine Hydroxylase Antibody [SN59-03] ET1611-12

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, IF-Tissue, mIHC, IHC-Fr
Molecular Wt:	Predicted band size: 59 kDa
Clone number:	SN59-03

Description: Tyrosine hydroxylase or tyrosine 3-monooxygenase is the enzyme responsible for catalyzing the conversion of the amino acid L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA). It does so using molecular oxygen (O_2), as well as iron (Fe^{2+}) and tetrahydrobiopterin as cofactors. L-DOPA is a precursor for dopamine, which, in turn, is a precursor for the important neurotransmitters norepinephrine (noradrenaline) and epinephrine (adrenaline). Tyrosine hydroxylase catalyzes the rate limiting step in this synthesis of catecholamines. In humans, tyrosine hydroxylase is encoded by the TH gene, and the enzyme is present in the central nervous system (CNS), peripheral sympathetic neurons and the adrenal medulla. Tyrosine hydroxylase, phenylalanine hydroxylase and tryptophan hydroxylase together make up the family of aromatic amino acid hydroxylases (AAAHs).

Immunogen: Synthetic peptide within C-terminal human Tyrosine Hydroxylase.

Positive control: Rat brain tissue lysates, PC-12 cell lysates, N2A, NIH/3T3, SH-SY5Y, mouse brain tissue, rat brain tissue, HEK-293.

Subcellular location: Cytoplasm.

Database links: SwissProt: P07101 Human | P24529 Mouse | P04177 Rat

Recommended Dilutions:

WB	1:1,000-1:10,000
IF-Cell	1:50-1:200
IHC-P	1:2,000
IHC-Fr	1:1,000
FC	1:1,000
IF-Tissue	1:200
mIHC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

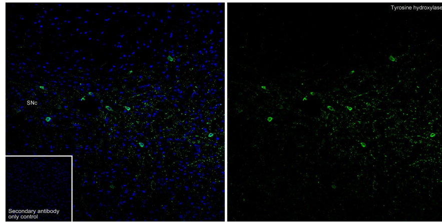

Fig1: Immunofluorescence analysis of frozen mouse brain tissue with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/1,000 dilution. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1611-12, green) at 1/1,000 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

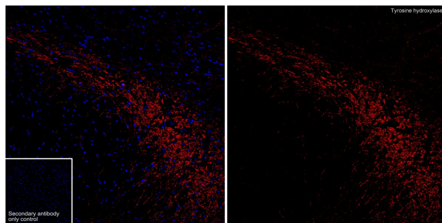

Fig2: Immunofluorescence analysis of frozen mouse brain tissue with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/1,000 dilution. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1611-12, red) at 1/1,000 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

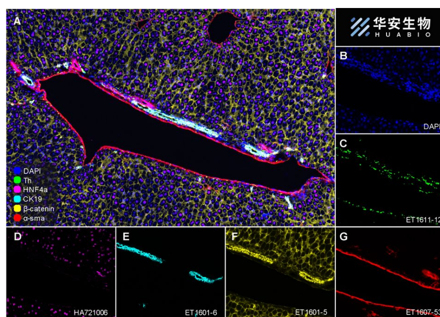

Fig3: Fluorescence multiplex immunohistochemical analysis of mouse liver (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Th (ET1611-12, Green), anti-HNF4a (HA721006, Magenta), anti-CK19 (ET1601-6, Cyan), anti-α-sma (ET1607-53, Red) and anti-β-catenin (ET1601-5, Yellow) on liver. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of ET1611-12 (1/1,000 dilution), HA721006 (1/2,000 dilution), ET1601-6 (1/3,000 dilution), ET1607-53 (1/10,000 dilution) and ET1601-5 (1/2,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

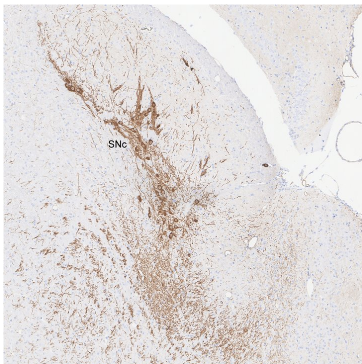

Fig4: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-12) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

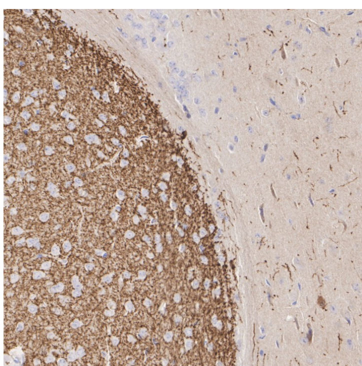

Fig5: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-12) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

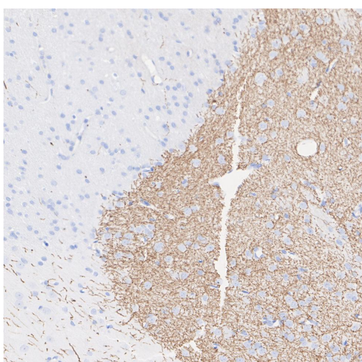

Fig6: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-12) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

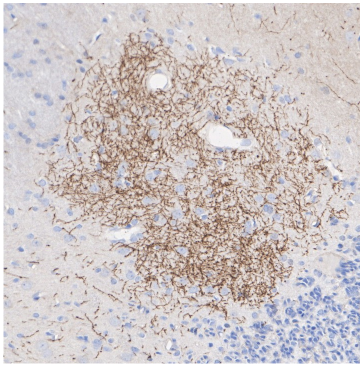

Fig7: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-12) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

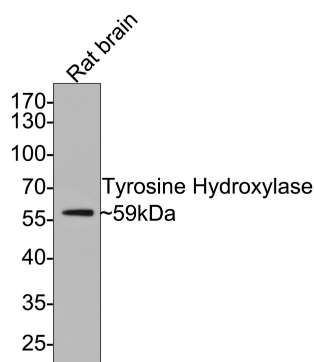

Fig8: Western blot analysis of Tyrosine Hydroxylase on rat brain tissue lysates with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/1,000 dilution.

Lysates/proteins at 20 µg/Lane.

Predicted band size: 59 kDa
Observed band size: 59 kDa

Exposure time: 2 minutes;

10% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1611-12) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

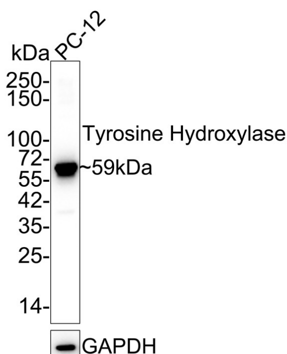

Fig9: Western blot analysis of Tyrosine Hydroxylase on PC-12 cell lysates with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/2,000 dilution.

Lysates/proteins at 15 µg/Lane.

Predicted band size: 59 kDa
Observed band size: 59 kDa

Exposure time: 24 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1611-12) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1

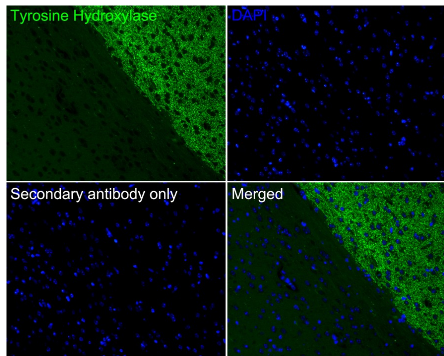

Fig10: Immunofluorescence analysis of paraffin-embedded mouse brain tissue labeling Tyrosine Hydroxylase with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1611-12, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

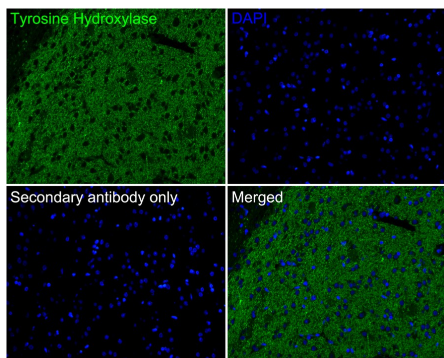

Fig11: Immunofluorescence analysis of paraffin-embedded rat brain tissue labeling Tyrosine Hydroxylase with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1611-12, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

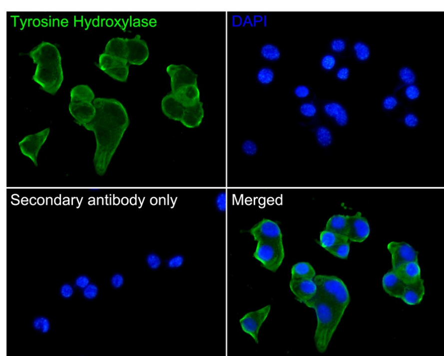

Fig12: Immunocytochemistry analysis of N2A cells labeling Tyrosine Hydroxylase with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

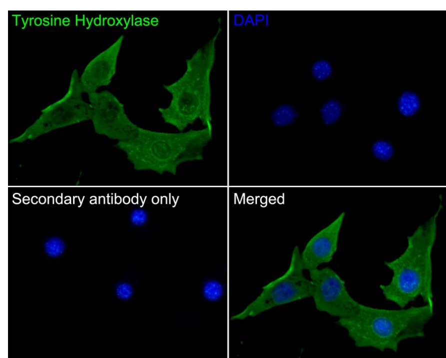

Fig13: Immunocytochemistry analysis of NIH/3T3 cells labeling Tyrosine Hydroxylase with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

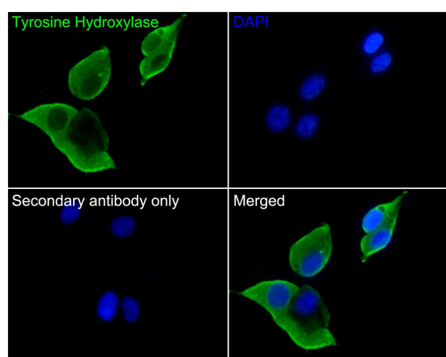

Fig14: Immunocytochemistry analysis of SH-SY5Y cells labeling Tyrosine Hydroxylase with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Tyrosine Hydroxylase antibody (ET1611-12) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

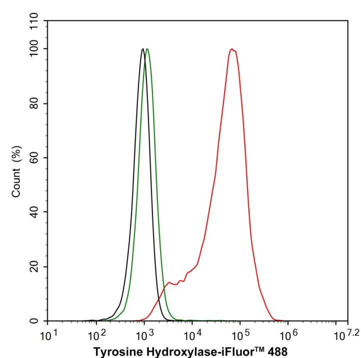

Fig15: Flow cytometric analysis of HEK-293 cells labeling Tyrosine Hydroxylase.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1611-12, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. De Miranda BR et al. Novel para-phenyl substituted diindolylmethanes protect against MPTP neurotoxicity and suppress glial activation in a mouse model of Parkinson's disease. Toxicol Sci 143:360-73 (2015).
2. Park J et al. Pain perception in acute model mice of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Mol Pain 11:28 (2015).