Anti-TBR1 Antibody [JF10-00]

ET1702-97

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC, IF-Tissue, mIHC, IHC-Fr
Molecular Wt:	Predicted band size: 74 kDa
Clone number:	JF10-00

Description: A novel murine and human gene, TBR-1, encodes a putative transcription factor related to the Brachyrury (T) gene that is expressed only in postmitotic cells. T-brain-1 (TBR-1) mRNA is largely restricted to the cerebral cortex, where, during embryogenesis, it defines different regions that give rise to the palecortex, limbic cortex and neocortex. TBR-1, Pax-6 and Emx-1 are expressed in the mouse and chicken pallium. The pallio-subpallial boundary lies at the interface between the TBR-1 and Dlx-2 expression domains. Chicken genes homolgous to these mouse genes are expressed in topologically comparable patterns during development, suggesting that mouse and chicken may have similar histogenetic specification processes and field homologies. CASK/LIN-2, a membrane-associated guanylate kinase, is required for EGFR localization and signaling. In adult rat brain, CASK is concentrated at neuronal synapses and binds to the cell-surface proteins. CASK can interact with TBR-1, which is involved in forebrain development. CASK enters into the nucleus and binds to a specific DNA sequence (the T-element) in a complex with TBR-1. Thus, CASK acts as a coactivator of TBR-1 to induce transcription of T-element containing genes, including reelin.

Immunogen: Synthetic peptide within Human TBR1 aa 30-75 / 682.

Positive control: Human brain tissue lysates, mouse hippocampus tissue lysates, human brain tissue, mouse brain tissue, rat brain tissue, mouse hippocampus tissue, rat hippocampus tissue, SH-SY5Y, E14.5 mouse embryonic brain tissue.

Subcellular location: Nucleus.

Database links: SwissProt: Q16650 Human | Q64336 Mouse
Entrez Gene: 680427 Rat

Recommended Dilutions:

WB	1:1,000
FC	1:50-1:100
IHC-P	1:500-1:1,000
IF-Tissue	1:500
mIHC	1:1,000
IHC-Fr	1:500

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

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

Purity: Protein A affinity purified.

Images

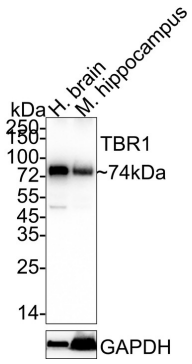

Fig1: Western blot analysis of TBR1 on different lysates with Rabbit anti-TBR1 antibody (ET1702-97) at 1/1,000 dilution.

Lane 1: Human brain tissue lysate (40 µg/Lane)
Lane 2: Mouse hippocampus tissue lysate (40 µg/Lane)

Predicted band size: 74 kDa
Observed band size: 74 kDa

Exposure time: 1 minute; ECL: K1801;

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 (ET1702-97) at 1/1,000 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 hour at room temperature.

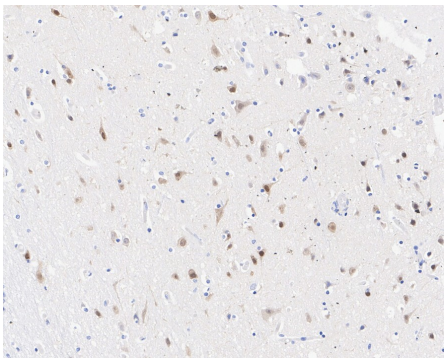

Fig2: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-TBR1 antibody (ET1702-97) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1702-97) at 1/1,000 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.

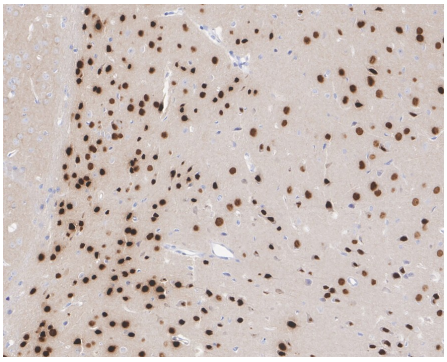

Fig3: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-TBR1 antibody (ET1702-97) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1702-97) at 1/1,000 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.

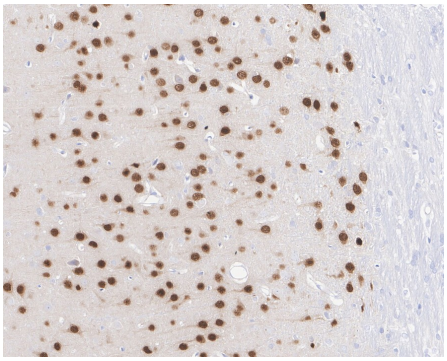

Fig4: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-TBR1 antibody (ET1702-97) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1702-97) at 1/1,000 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.

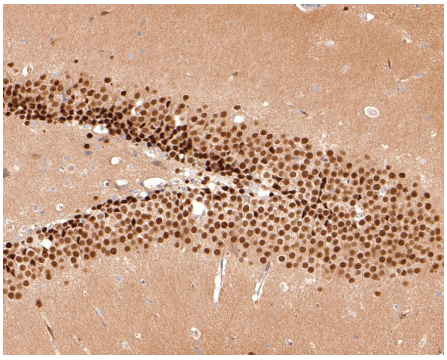

Fig5: Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue with Rabbit anti-TBR1 antibody (ET1702-97) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1702-97) at 1/500 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.

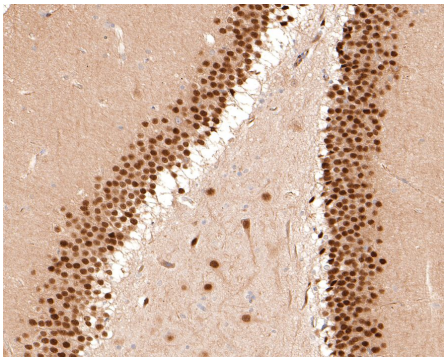

Fig6: Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue with Rabbit anti-TBR1 antibody (ET1702-97) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1702-97) at 1/500 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.

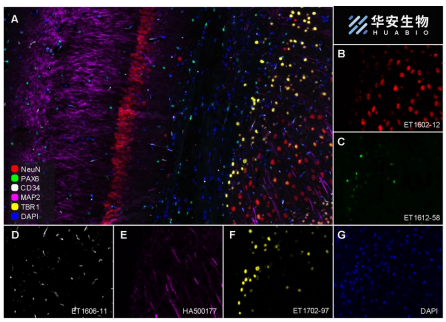

Fig7: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-NeuN (ET1602-12, red), anti-PAX6 (ET1612-58, green), anti-CD34 (ET1606-11, gray), anti-MAP2 (HA500177, magenta) and anti-TBR1 (ET1702-97, yellow) on mouse brain. 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 five rounds of staining: in the order of ET1602-12 (1/5,000 dilution), ET1612-58 (1/1,000 dilution), ET1606-11 (1/2,000 dilution), HA500177 (1/5,000 dilution) and ET1702-97 (1/1,000 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.

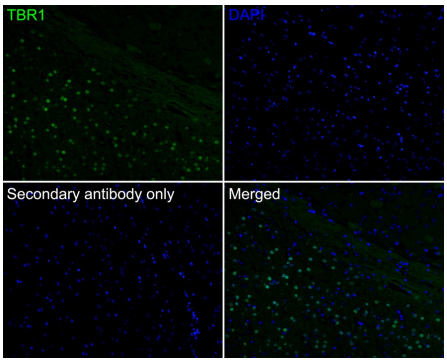

Fig8: Immunofluorescence analysis of paraffin-embedded mouse brain tissue labeling TBR1 with Rabbit anti-TBR1 antibody (ET1702-97) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1702-97, 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).

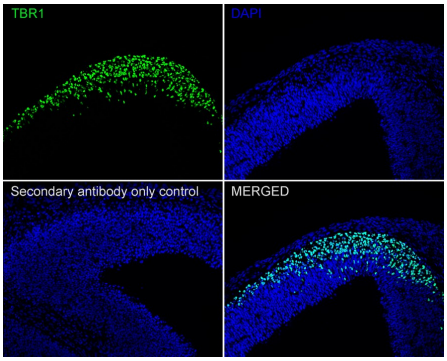

Fig9: Immunofluorescence analysis of frozen E14.5 mouse embryonic brain tissue with Rabbit anti-TBR1 antibody (ET1702-97) at 1/500 dilution.

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 (ET1702-97, green) at 1/500 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).

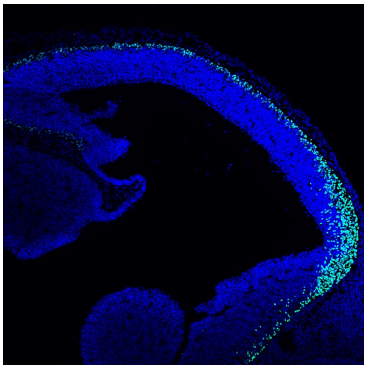

Fig10: Immunofluorescence analysis of frozen E14.5 mouse embryonic brain tissue with Rabbit anti-TBR1 antibody (ET1702-97) at 1/500 dilution.

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 (ET1702-97, green) at 1/500 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).

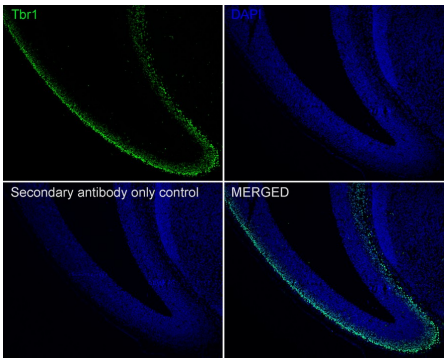

Fig11: Immunofluorescence analysis of paraffin-embedded E14.5 mouse embryonic brain tissue labeling TBR1 with Rabbit anti-TBR1 antibody (ET1702-97) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1702-97, green) at 1/500 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).

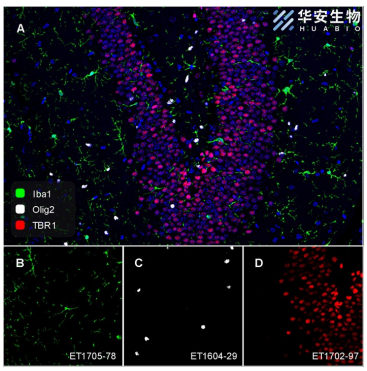

Fig12: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Iba1 (ET1705-78, Green), anti-Olig2 (ET1604-29, White) and anti-TBR1 (ET1702-97, Red) on brain. HRP Conjugated UltraPolymer Goat Polyclonal Antibody 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 ET1705-78 (1/2,000 dilution), ET1604-29 (1/1,000 dilution) and ET1702-97 (1/1,000 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 Zeiss Observer 7 Inverted Fluorescence Microscope.

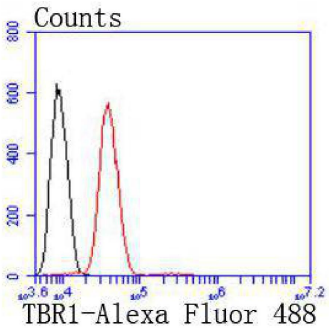

Fig13: Flow cytometric analysis of TBR1 was done on SH-SY5Y cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1702-97, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Nagao M et al. Zbtb20 promotes astrocytogenesis during neocortical development. Nat Commun 7:11102 (2016).
2. Notwell JH et al. TBR1 regulates autism risk genes in the developing neocortex. Genome Res 26:1013-22 (2016).