Immature Neuron Marker Antibody Sampler Kit

HAK21046

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
Doublecortin [ET1701-98]	20μl	WB	H,M,R	41 kDa
NCAM1 [ET1702-43]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,FC,IP,mIHC	H,Z	95 kDa
NeuroD1 [ET1703-73]	20μl	WB,IP,IHC-P,IHC-Fr	H,M,R	40 kDa
Beta III Tubulin [ET1604-17]	20μl	WB,IHC-P,IP,FC,IHC-Fr,IF-Cell	H,M,R	50 kDa
TBR1 [ET1702-97]	20μl	WB,IHC-P,IF-Tissue,mIHC,IHC-Fr	H,M,R	74 kDa
Stathmin [HA721179]	20μl	IHC-P,WB,IF-Cell,IF-Tissue,FC	H,M,R	17 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100ul	WB,ELISA,IHC-P	Rab	

Description: The Immature Neuron Marker Antibody Sampler Kit provides an economical means for detecting immature neuron proteins by western blot. The kit includes enough antibodies to perform two western blot experiments with each primary antibody.

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℃. Avoid repeated freeze / thaw cycles.

Background

During development, radial glia (RG) cells located in the ventricular zone (VZ) of the brain divide asymmetrically, each producing a neuronal and RG daughter cell. The cytoskeleton of these cells plays an important role in generating neuronal processes. β3-tubulin is one of β-tubulin isoforms that make up the building blocks of microtubules. Stathmin is a tubulin binding protein that regulates microtubule dynamics in a phosphorylation dependent manner.

Doublecortin is a microtubule-associated protein that facilitates neurite outgrowth and cell migration. The dual expression of doublecortin and NCAM (neural cell adhesion molecule, CD56), combined with the lack of expression of mature neuronal markers, is evidence of an immature neuronal phenotype.

Transcription factors also play a key role in immature neuron growth and differentiation. NeuroD1 is a member of the basic helix-loop-helix (bHLH) family of transcription factors. Neuronal activity results in CaMKII-mediated phosphorylation of NeuroD1 at Ser336, which is necessary for the formation and growth of dendrites.As a member of the T-Box family of transcription factors, TBR1 is expressed in postmitotic glutamatergic projection neurons.

Database links: UniProt ID: O43602, O88809, Q9ESI7, P13591, Q13562, Q60867, Q64289, Q13509, Q9ERD7, Q4QRB4, Q16650, Q64336, 680427, P16949, P54227, P13668

Images

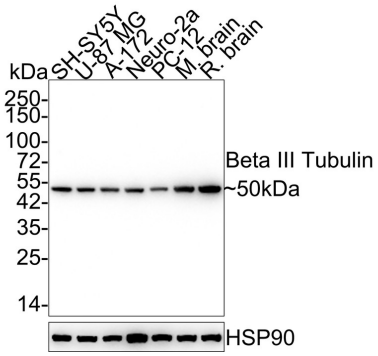

Fig1: Western blot analysis of Beta III Tubulin on different lysates with Mouse anti-Beta III Tubulin antibody (M0805-8) at 1/2,000 dilution.

Lane 1: SH-SY5Y cell lysate
Lane 2: U-87 MG cell lysate
Lane 3: A-172 cell lysate
Lane 4: Neuro-2a cell lysate
Lane 5: PC-12 cell lysate
Lane 6: Mouse brain tissue lysate
Lane 7: Rat brain tissue lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 50 kDa
Observed band size: 50 kDa

Exposure time: 11 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 (M0805-8) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

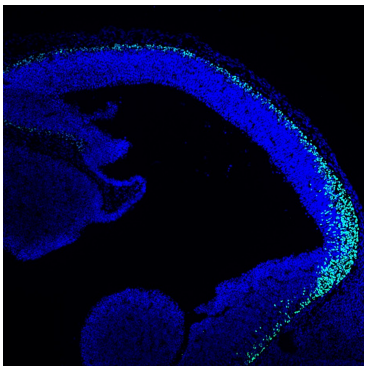

Fig2: Immunofluorescence analysis of frozen E14.5 mouse embryonic brain tissue with Rabbit anti-TBR1 antibody (ET1702-97) at 1/500 dilution.

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).

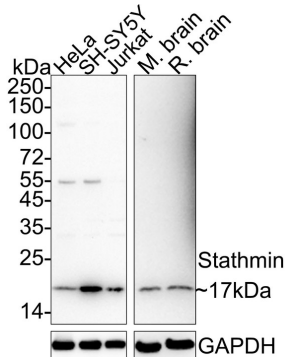

Fig3: Western blot analysis of Stathmin on different lysates with Rabbit anti-Stathmin antibody (HA721179) at 1/1,000 dilution.

Lane 1: HeLa cell tissue lysate (20 µg/Lane)
Lane 2: SH-SY5Y cell tissue lysate (20 µg/Lane)
Lane 3: Jurkat cell tissue lysate (20 µg/Lane)
Lane 4: Mouse brain tissue lysate (40 µg/Lane)
Lane 5: Rat brain tissue lysate (40 µg/Lane)

Predicted band size: 17 kDa
Observed band size: 17 kDa

Exposure time: 3 minutes; 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 (HA721179) 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.

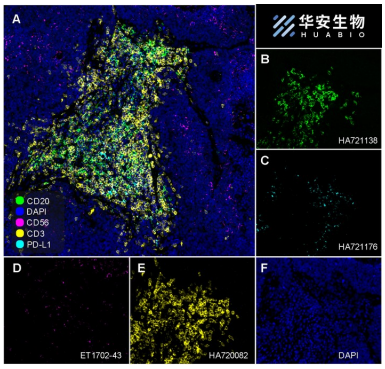

Fig4: Fluorescence multiplex immunohistochemical analysis of Tertiary Lymphoid Structures in Human Small Cell Lung Cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD20 (HA721138, green), anti-PD-L1 (HA721176, cyan), anti-CD56 (ET1702-43, magenta) and anti-CD3 (HA720082, yellow) on tertiary lymphoid structures. Panel B: anti- CD20 stained on B cells. Panel C: anti-PD-L1 stained on dendritic cells and macrophages cells. Panel D: anti-CD56 stained on NKT cells. Panel E: anti-CD3 stained on T cells. 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 four rounds of staining: in the order of HA721138 (1/1,500 dilution), HA721176 (1/1,000 dilution), ET1702-43 (1/1,000 dilution), and HA720082 (1/500 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.

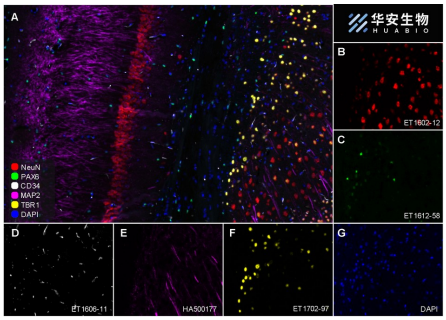

Fig5: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-NeuN (ET1602-12, red), anti-PAX6 (ET1612-58, green), anti-CD34 (ET1606-11, gray), anti-MAP2 (HA500177, magenta) and anti-TBR1 (ET1702-97, yellow) 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.

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

Background References

1. Coviello S, Gramuntell Y, Klimczak P, Varea E, Blasco-Ibañez JM, Crespo C, Gutierrez A, Nacher J. Phenotype and Distribution of Immature Neurons in the Human Cerebral Cortex Layer II. Front Neuroanat. 2022 Apr 8;16:851432.

2. Hagihara H, Murano T, Ohira K, Miwa M, Nakamura K, Miyakawa T. Expression of progenitor cell/immature neuron markers does not present definitive evidence for adult neurogenesis. Mol Brain. 2019 Dec 10;12(1):108.