Astrocyte Markers Antibody Sampler Kit

HAK21083

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
GFAP [ET1601-23]	20μl	WB,IHC-P,IF-Tissue,IHC-Fr,mIHC,IP	H,M,R	50 kDa
EAAT1 [ET1704-54]	20μl	WB,IHC-P,IF-Tissue,IHC-Fr	H,M,R	60 kDa
Survivin [ET1604-34]	20μl	WB,IF-Cell,IF-Tissue,IHC-P,FC	H,M,R	16 kDa
EAAT2 [HA721323]	20μl	WB,IHC-P,IHC-Fr	M,R	62 kDa
ALDH1L1 [ET7106-64]	20μl	WB,IHC-P,IF-Tissue,IHC-Fr	H,M,R	99 kDa
Aquaporin 4 [HA722672]	20μl	WB,IHC-P,IF-Tissue,IHC-Fr,IP	H,M,R	35 kDa
GABA Transporter 1 / GAT 1 [HA721496]	20μl	WB,IHC-P,IHC-Fr,IF-Tissue	H,M,R	67 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100μl	WB,ELISA,IHC-P	Rab	

Description: The Astrocyte Markers Antibody Sampler Kit provides an economical means of detecting astrocyte markers by Immunofluorescence or Western Blot. The kit includes enough antibodies to perform at least two western blot or twenty IF tests 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 Astrocytes have a regulatory role in brain functions that are implicated in neurogenesis and synaptogenesis.AQP4 is present in the brain and it is enriched in astrocytes to regulate water homeostasis, preventing cerebral edema caused by solute imbalance. GABA transmitters are Na+/Cl--dependent transporters that regulate neurotransmitter transport, including GAT1 (SLC6), GAT2, GAT3, and BGT1. GAT1 expresses in the brain, preferentially in glial cells, but is also found in neurons, regulating the uptake and release of neurotransmitters in terminal clefts. EAAT2 is primarily expressed in astrocytes accounting for up to 90% of the total glutamate transport in the brain. EAAT1 upregulates increased concentrations of glutamate in astrocytes and has a neuroprotective potential following ischemia. ALDH1L1 has also been shown to be a useful astrocyte marker throughout the grey and white matter of the brain, labeling both the cell body and processes of astrocytes. Survivin is a 16 kDa anti-apoptotic protein highly expressed during fetal development and cancer malignancy.

Database links: UniProt ID: P14136, P03995, P47819, P43003, P56564, P24942, O15392, O70201, Q9JHY7, P43004, P43006, P31596, O75891, Q8R0Y6, P28037, P55087, P55088, P47863, P30531, P31648, P23978

Images

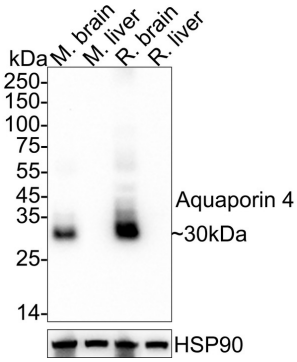

Fig1: Western blot analysis of Aquaporin 4 on different lysates with Rabbit anti-Aquaporin 4 antibody (HA722672) at 1/2,000 dilution.

Lane 1: Mouse brain tissue lysate (no heat) (40 µg/Lane)
Lane 2: Mouse liver tissue lysate (negative) (40 µg/Lane)
Lane 3: Rat brain tissue lysate (no heat) (40 µg/Lane)
Lane 4: Rat liver tissue lysate (negative) (40 µg/Lane)

Notice: no heat means the lysate is not boiled.

Predicted band size: 35 kDa
Observed band size: 30 kDa

Exposure time: 16 seconds; 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 (HA722672) at 1/2,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

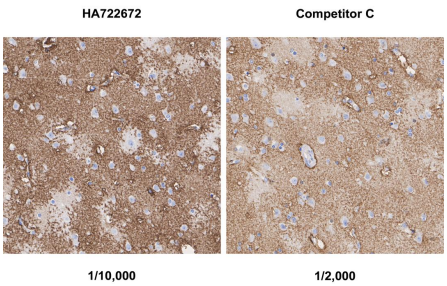

Fig2: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-Aquaporin 4 antibody (HA722672) at 1/10,000 dilution and competitor's antibody at 1/2,000 dilution.

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 (HA722672) at 1/10,000 dilution and competitor's antibody 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.

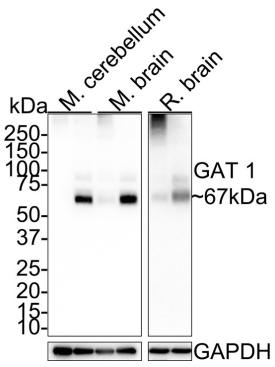

Fig3: Western blot analysis of GABA Transporter 1 / GAT 1 on different lysates with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/1,000 dilution.

Lane 1: Mouse cerebellum tissue lysate
Lane 2: Mouse cerebellum tissue lysate (70℃ heat)
Lane 3: Mouse brain tissue lysate
Lane 4: Mouse brain tissue lysate (70℃ heat)
Lane 5: Rat brain tissue lysate
Lane 6: Rat brain tissue lysate (70℃ heat)

Lysates/proteins at 30 µg/Lane.

Predicted band size: 67 kDa
Observed band size: 67 kDa

Exposure time: Lane 1-4: 1 minute 40 seconds; Lane 5-6: 5 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 (HA721496) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

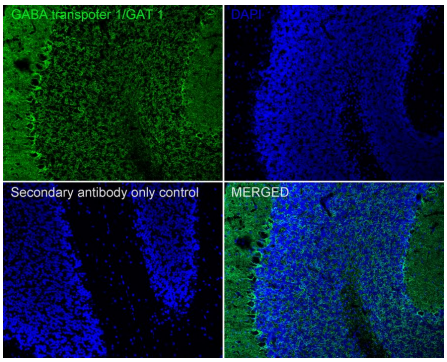

Fig4: Immunofluorescence analysis of frozen mouse cerebellum tissue with Rabbit anti-GABA Transporter 1 / GAT 1 antibody (HA721496) at 1/200 dilution.

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 (HA721496, 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).

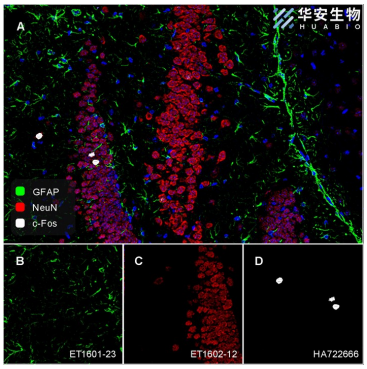

Fig5: Fluorescence multiplex immunohistochemical analysis of mouse hippocampus (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-GFAP (ET1601-23, Green), anti-NeuN (ET1602-12, Red) and anti-c-Fos (HA722666, White) on hippocampus. HRP Conjugated UltraPolymer Goat Polyclonal 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 ET1601-23 (1/1,000 dilution), ET1602-12 (1/1,000 dilution) and HA722666 (1/200 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.

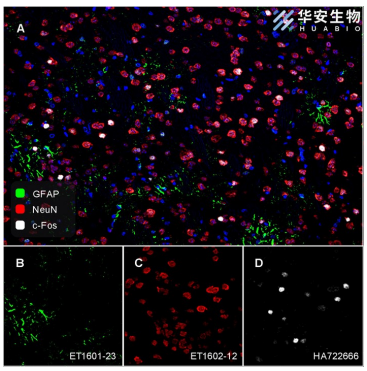

Fig6: Fluorescence multiplex immunohistochemical analysis of mouse brain (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-GFAP (ET1601-23, Green), anti-NeuN (ET1602-12, Red) and anti-c-Fos (HA722666, White) on brain. HRP Conjugated UltraPolymer Goat Polyclonal Antibody 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 ET1601-23 (1/1,000 dilution), ET1602-12 (1/1,000 dilution) and HA722666 (1/200 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.

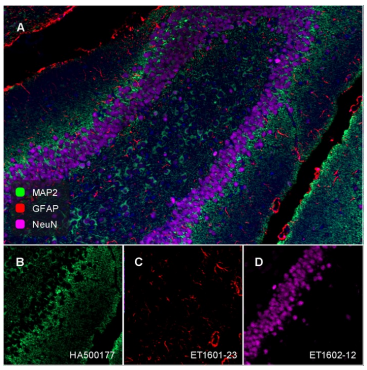

Fig7: Fluorescence multiplex immunohistochemical analysis of mouse hippocampus (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-MAP2 (HA500177, Green), anti-GFAP (ET1601-23, Red) and anti-NeuN (ET1602-12, Magenta) on Mouse hippocampus. HRP Conjugated UltraPolymer Goat 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 HA500177 (1/1,000 dilution), ET1601-23 (1/1,000 dilution) and ET1602-12 (1/10,000 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°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

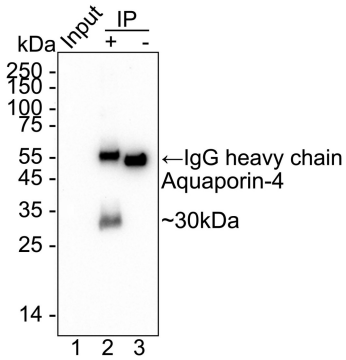

Fig8: Aquaporin 4 was immunoprecipitated from 0.2 mg mouse brain tissue lysate with HA722672 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA722672 at 1/1,000 dilution. Mouse anti Rabbit IgG heavy chain (Fc) secondary antibody (M1003-7) at 1/100,000 dilution was used for 1 hour at room temperature.

Lane 1: Mouse brain tissue lysate (input)
Lane 2: HA722672 IP in mouse brain tissue lysate
Lane 3: Rabbit IgG instead of HA722672 in mouse brain tissue lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 1 minute; ECL: K1801

Background References

1. Michalovicz LT, Kelly KA, Vashishtha S, Ben-Hamo R, Efroni S, Miller JV, Locker AR, Sullivan K, Broderick G, Miller DB, O'Callaghan JP. Astrocyte-specific transcriptome analysis using the ALDH1L1 bacTRAP mouse reveals novel biomarkers of astrogliosis in response to neurotoxicity. J Neurochem. 2019 Aug;150(4):420-440.

2. Siracusa R, Fusco R, Cuzzocrea S. Astrocytes: Role and Functions in Brain Pathologies. Front Pharmacol. 2019 Sep 27;10:1114.