

Mature Neuron Marker Antibody Kit

HAK21007



Contains Product	Specification	Applications	Species reactivity	MW(kDa)
NeuN[ET1602-12]	20μl	WB,IF-Ce,IF-Tissue,IHC-P,FC,IHC-Fr,mIHC	H,M,R	34 kDa
GAP43[ET1610-94]	20μl	WB,IF-Ce,IF-Tissue,IHC-P,FC,IP	H,M,R	25 kDa
MAP2[HA500177]	20μl	WB,IF-Ce,IHC-P,mIHC	H,M,R	200 kDa
NF-L[HA721538]	20μl	WB,IHC-P,IF-Ce,IF-Tissue	H,M,R	62 kDa
Beta III Tubulin[ET1604-17]	20μl	WB,IHC-P,IF,FC,IHC-Fr,IF-Cell	H,M,R	50 kDa
Synaptophysin[ET1606-56]	20μl	WB,IF-Ce,IF-Tissue,IHC-P	H,M,R	34 kDa
PSD95[ET1602-20]	20μl	WB,IF-Ce,IF-Tissue,IHC-P,FC,IP	H,M,R	80 kDa
PGP9.5[ET1703-22]	20μl	WB,IF-Ce,IF-Tissue,IHC-P,IP	H,M,R	25 kDa
HRP-Apaca anti-Rabbit IgG Fc, Recombinant VHH[HA1031]	100μl	IP,ELISA,IHC-P,WB	Rab	

Description:

Neuronal nuclei (NeuN, Fox-3, RBFOX3) is a nuclear protein expressed in most post-mitotic neurons of the central and peripheral nervous systems. NeuN is not detected in Purkinje cells, sympathetic ganglion cells, Cajal-Retzius cells, INL retinal cells, inferior olivary, and dentate nucleus neurons. This neuronal protein was originally identified by immunoreactivity with a monoclonal antibody also called NeuN. Using MS-analysis, NeuN was later identified as the Fox-3 gene product. Fox-3 contains an RNA recognition motif and functions as a splicing regulator. Fox-3 regulates alternative splicing of NumB, promoting neuronal differentiation during development.

Storage Buffer:

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

Storage Instruction:

Store at +4°C after thawing. Aliquot store at -20°C. Avoid repeated freeze / thaw cycles.

Background

The antibodies in this kit serve to characterize and identify mature neurons. Neuronal nuclei (NeuN, Fox-3, RBFOX3) is a nuclear protein expressed in most post-mitotic neurons of the central and peripheral nervous systems.

GAP43 is a nervous system specific, growth-associated protein enriched in growth cones and areas of high plasticity. β 3-tubulin is one of six β -tubulin isoforms that make up the building blocks of microtubules. Microtubule-associated protein 2 (MAP2) is a neuronal phosphoprotein that regulates the structure and stability of microtubules, neuronal morphogenesis, cytoskeleton dynamics, and organelle trafficking in axons and dendrites. Neurofilaments are the major intermediate filaments found in neurons and consist of light (NFL), medium (NFM), and heavy (NFH) subunits. Synaptophysin is a neuronal synaptic vesicle glycoprotein. Synaptophysin is responsible for targeting synaptobrevin 2/VAMP2 to synaptic vesicles, and is a critical component and marker for the presynaptic fusion complex. PSD95 is a scaffolding protein involved in the assembly and function of mature postsynaptic density complexes.

Database links:

UniProt ID: A6NFN3, Q8BIF2, 143966, P17677, P06837, P07936, P11137, P20357, P15146, P07196, P08551, P19527, Q13509, Q9ERD7, Q4QRB4, P08247, Q62277, P07825, P78352, Q62108, P31016, P09936, Q9R0P9, Q00981

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

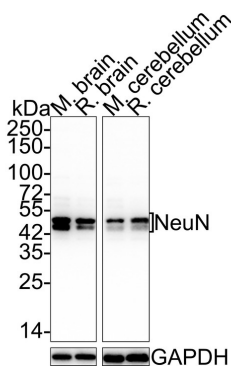


Fig1: Western blot analysis of NeuN on different lysates with Rabbit anti-NeuN antibody (ET1602-12) at 1/5,000 dilution.

- Lane 1: Mouse brain tissue lysate
- Lane 2: Rat brain tissue lysate
- Lane 3: Mouse cerebellum tissue lysate
- Lane 4: Rat cerebellum tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 34 kDa
Observed band size: 45/50 kDa

Exposure time: 43 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 (ET1602-12) at 1/5,000 dilution was used in 5% NFDm/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

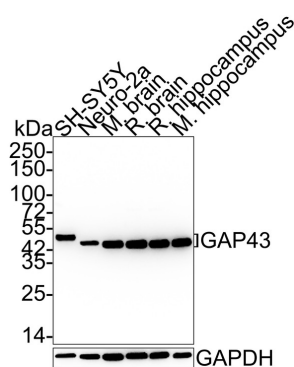


Fig2: Western blot analysis of GAP43 on different lysates with Rabbit anti-GAP43 antibody (ET1610-94) at 1/5,000 dilution.

Lane 1: SH-SY5Y cell lysate

Lane 2: Neuro-2a cell lysate

Lane 3: Mouse brain tissue lysate

Lane 4: Rat brain tissue lysate

Lane 5: Rat hippocampus tissue lysate

Lane 6: Mouse hippocampus tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 25 kDa

Observed band size: 43/45 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 (ET1610-94) at 1/5,000 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.

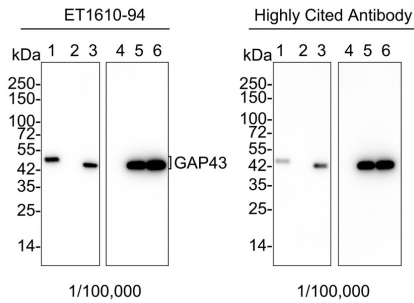


Fig3: Western blot analysis of GAP43 on different lysates with Rabbit anti-GAP43 antibody (ET1610-94) at 1/100,000 dilution and competitor's antibody at 1/100,000 dilution.

- Lane 1: SH-SY5Y cell lysate
- Lane 2: A549 cell lysate (negative)
- Lane 3: Neuro-2a cell lysate
- Lane 4: Mouse lung tissue lysate (negative)
- Lane 5: Mouse brain tissue lysate
- Lane 6: Rat brain tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 25 kDa
Observed band size: 43/45 kDa

Exposure time: 3 minutes 20 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 (ET1610-94) at 1/100,000 dilution and competitor's antibody at 1/100,000 dilution were 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.

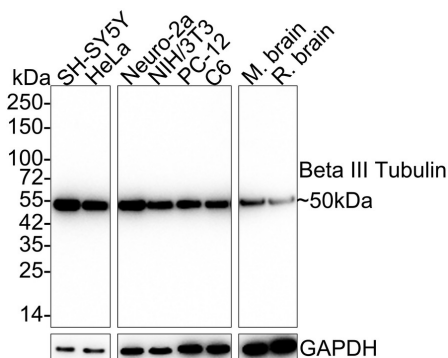


Fig4: Western blot analysis of Beta III Tubulin on different lysates with Rabbit anti-Beta III Tubulin antibody (ET1604-17) at 1/20,000 dilution.

Lane 1: SH-SY5Y cell lysate (15 µg/Lane)
 Lane 2: HeLa cell lysate (15 µg/Lane)
 Lane 3: Neuro-2a cell lysate (15 µg/Lane)
 Lane 4: NIH/3T3 cell lysate (15 µg/Lane)
 Lane 5: PC-12 cell lysate (15 µg/Lane)
 Lane 6: C6 cell lysate (15 µg/Lane)
 Lane 7: Mouse brain tissue lysate (20 µg/Lane)
 Lane 8: Rat brain tissue lysate (20 µg/Lane)

Predicted band size: 50 kDa

Observed band size: 50 kDa

Exposure time: 3 minutes 54 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 (ET1604-17) at 1/20,000 dilution was used in 5% NFDm/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

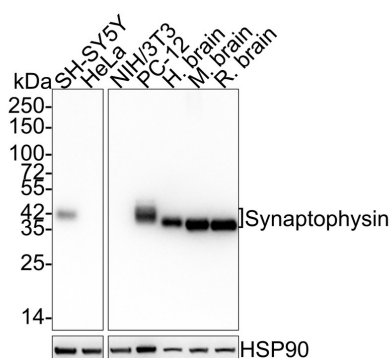


Fig5: Western blot analysis of Synaptophysin on different lysates with Rabbit anti-Synaptophysin antibody (ET1606-56) at 1/5,000 dilution.

Lane 1: SH-SY5Y cell lysate
 Lane 2: HeLa cell lysate (negative)
 Lane 3: NIH/3T3 cell lysate (negative)
 Lane 4: PC-12 cell lysate
 Lane 5: Human brain tissue lysate
 Lane 6: Mouse brain tissue lysate
 Lane 7: Rat brain tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 34 kDa

Observed band size: 34/40 kDa

Exposure time: 43 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

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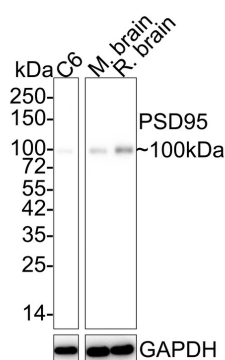


Fig6: Western blot analysis of PSD95 on different lysates with Rabbit anti-PSD95 antibody (ET1602-20) at 1/2,000 dilution.

Lane 1: C6 cell lysate (15 µg/Lane)

Lane 2: Mouse brain tissue lysate (20 µg/Lane)

Lane 3: Rat brain tissue lysate (20 µg/Lane)

Predicted band size: 80 kDa

Observed band size: 100 kDa

Exposure time: 5 minutes 10 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 (ET1602-20) 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.

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

Background References

1. Mullen, R.J. et al. (1992) Development 116, 201-11.
2. Kim, K.K. et al. (2009) J Biol Chem 284, 31052-61.
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