Anti-GAP43 Antibody [SC60-06]

ET1610-94

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 25 kDa
Clone number:	SC60-06

Description: GAP43, is a nervous tissue-specific cytoplasmic protein that can be attached to the membrane via a dual palmitoylation sequence on cysteines 3 and 4. This sequence targets GAP43 to lipid rafts. It is a major protein kinase C (PKC) substrate and is considered to play a key role in neurite formation, regeneration, and plasticity. The role of GAP-43 in CNS development is not limited to effects on axons: It is also a component of the centrosome, and differentiating neurons that do not express GAP-43 show mislocalization of the centrosome and mitotic spindles, particularly in neurogenic cell divisions. GAP43, the consensus choice for its designation, is a nervous system-specific protein that is attached to the membrane via a dual palmitoylation sequence on cysteines 3 and 4, though it can exist in the non-bound form in the cytoplasm. This dual sequence enables the association of phosphatidylinositol-4,5-bisphosphate [PI(4,5)P2] or PIP2, with actin, facilitating the latter's polymerization thereby regulating neuronal structure. GAP-43 is also a protein kinase C (PKC) substrate. Phosphorylation of serine-41 on GAP-43 by PKC regulates neurite formation, regeneration, and synaptic plasticity.

Immunogen: Synthetic peptide within Human GAP43 aa 189-238 / 238.

Positive control: SH-SY5Y cell lysate, Neuro-2a cell lysate, mouse brain tissue lysate, rat brain tissue lysate, human glioma tissue, Neuro-2a, rat hippocampus tissue lysate, mouse hippocampus tissue lysate, mouse brain tissue, rat brain tissue, rat hippocampus tissue, SH-SY5Y.

Subcellular location: Cell membrane, growth cone membrane, filopodium membrane, Cytoplasm, synapse, perikaryon, dendrite, axon.

Database links: SwissProt: P17677 Human | P06837 Mouse | P07936 Rat

Recommended Dilutions:

WB	1:5,000-1:100,000
IF-Cell	1:500
IF-Tissue	1:100-1:500
IHC-P	1:10,000-1:15,000
FC	1:1,000
IP	Use at an assay dependent concentration.

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

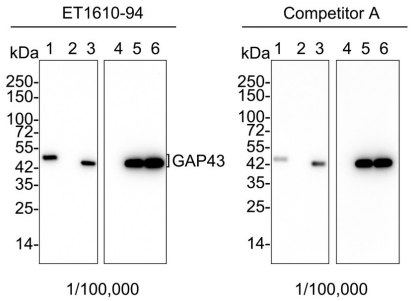

Fig1: Western blot analysis of GAP43 on different lysates with Rabbit anti-GAP43 antibody (ET1610-94) at 1/100,000 dilution and competitor's antibody at 1/100,000 dilution.

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℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

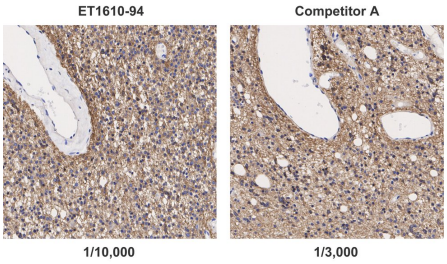

Fig2: Immunohistochemical analysis of paraffin-embedded human glioma tissue with Rabbit anti-GAP43 antibody (ET1610-94) at 1/10,000 dilution and competitor's antibody at 1/3,000 dilution.

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 (ET1610-94) at 1/10,000 dilution and competitor's antibody at 1/3,000 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.

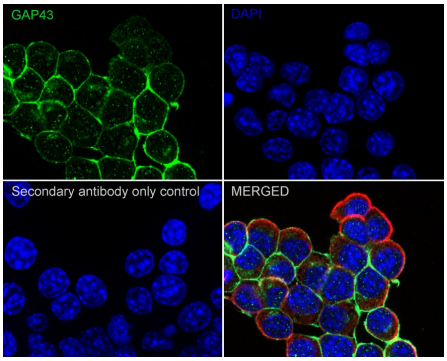

Fig3: Immunocytochemistry analysis of Neuro-2a cells labeling GAP43 with Rabbit anti-GAP43 antibody (ET1610-94) at 1/500 dilution.

Cells were fixed in 100% precooled methanol for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-GAP43 antibody (ET1610-94) at 1/500 dilution in 1% BSA in PBST 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.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

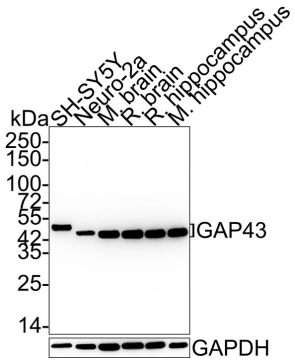

Fig4: Western blot analysis of GAP43 on different lysates with Rabbit anti-GAP43 antibody (ET1610-94) at 1/5,000 dilution.

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℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

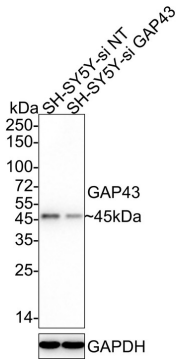

Fig5: Western blot analysis of GAP43 on different lysates with Rabbit anti-GAP43 antibody (ET1610-94) at 1/20,000 dilution.

Lane 1: SH-SY5Y-si NT cell lysate
Lane 2: SH-SY5Y-si GAP43 cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 30 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 1/20,000 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.

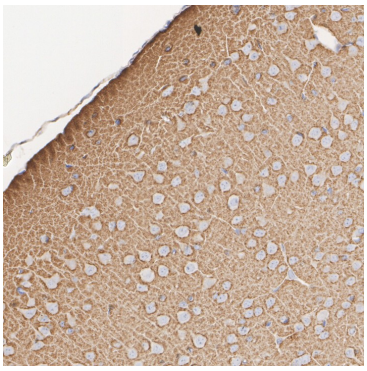

Fig6: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-GAP43 antibody (ET1610-94) at 1/15,000 dilution.

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 (ET1610-94) at 1/15,000 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.

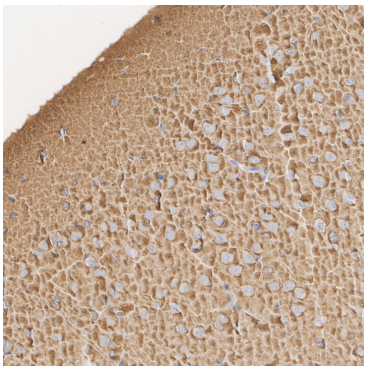

Fig7: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-GAP43 antibody (ET1610-94) at 1/15,000 dilution.

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 (ET1610-94) at 1/15,000 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.

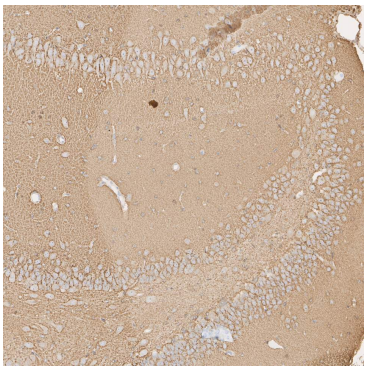

Fig8: Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue with Rabbit anti-GAP43 antibody (ET1610-94) at 1/15,000 dilution.

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 (ET1610-94) at 1/15,000 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.

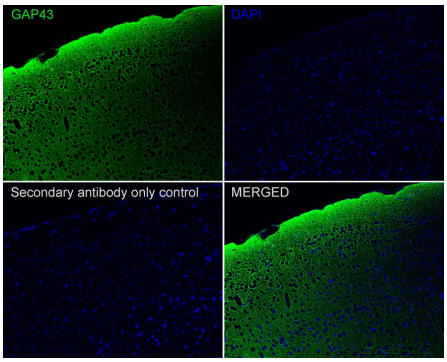

Fig9: Immunofluorescence analysis of paraffin-embedded mouse brain tissue labeling GAP43 with Rabbit anti-GAP43 antibody (ET1610-94) at 1/200 dilution.

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 (ET1610-94, 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).

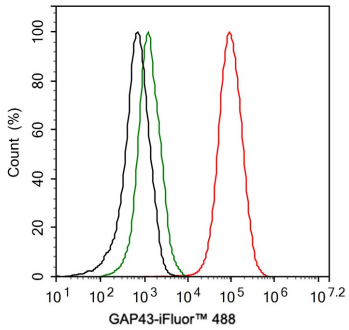

Fig10: Intracellular Flow Cytometry analysis of SH-SY5Y labeling GAP43 with purified ET1610-94 at 1/1,000 dilution (1 µg/ml) (red).

Cells were fixed with 4% PFA and permeabilised with 90% methanol. Rabbit monoclonal IgG (green) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (black) were used as the unlabeled control. A Goat anti-rabbit IgG iFluor™ 488 (HA1121)(1/1,000 dilution) was used as the secondary antibody.

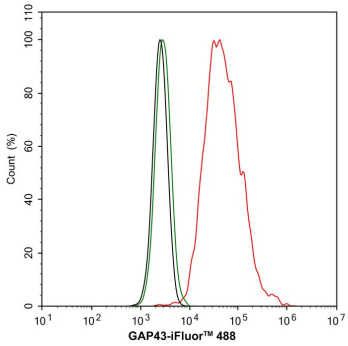

Fig11: Flow cytometric analysis of Neuro-2a cells labeling GAP43.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1610-94, 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. D'Agostino G et al. Prolyl endopeptidase-deficient mice have reduced synaptic spine density in the CA1 region of the hippocampus, impaired LTP, and spatial learning and memory. Cereb Cortex 23:2007-14 (2013).
2. Figueroa JD et al. Metabolomics uncovers dietary omega-3 fatty acid-derived metabolites implicated in anti-nociceptive responses after experimental spinal cord injury. Neuroscience 255C:1-18 (2013).