Anti-GAP43 Antibody

ER40201

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 25 kDa

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. As a consequence, in the cerebellum, the neuronal precursor pool fails to expand normally and the cerebellum is significantly smaller.

Immunogen: Synthetic peptide within mouse GAP43 aa 178-227 / 227.

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

Subcellular location: Cell membrane, Cell projection, Cytoplasm, Membrane, Synapse.

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

Recommended Dilutions:

WB	1:100,000
IF-Cell	1:200
IHC-P	1:1,000
FC	1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Immunogen affinity purified.

Images

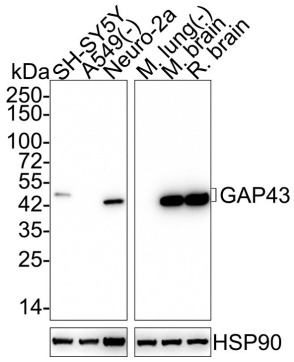

Fig1: Western blot analysis of GAP43 on different lysates with Rabbit anti-GAP43 antibody (ER40201) at 1/100,000 dilution.

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 (ER40201) at 1/100,000 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.

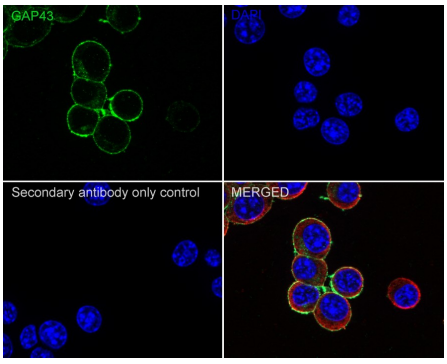

Fig2: Immunocytochemistry analysis of Neuro-2a cells labeling GAP43 with Rabbit anti-GAP43 antibody (ER40201) at 1/200 dilution.

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 (ER40201) at 1/200 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.

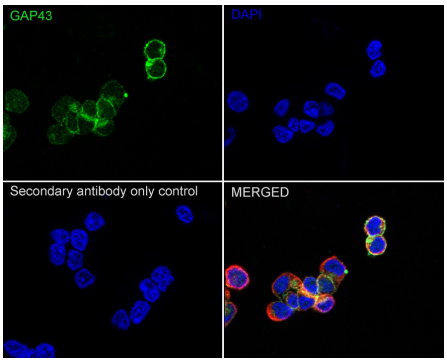

Fig3: Immunocytochemistry analysis of SH-SY5Y cells labeling GAP43 with Rabbit anti-GAP43 antibody (ER40201) at 1/100 dilution.

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 (ER40201) at 1/100 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.

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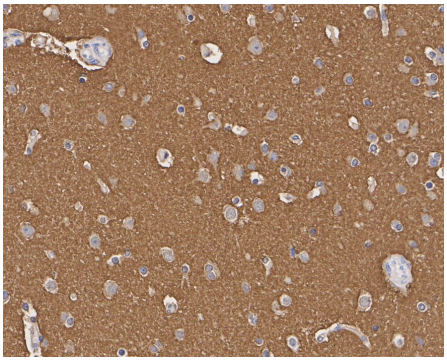

Fig4: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-GAP43 antibody (ER40201) at 1/1,000 dilution.

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 (ER40201) 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.

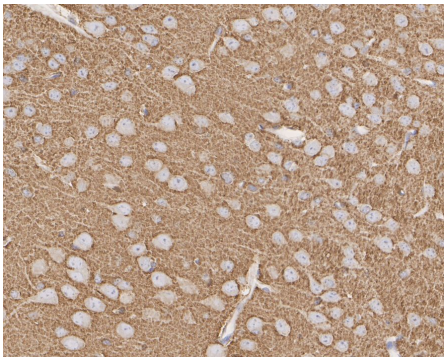

Fig5: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-GAP43 antibody (ER40201) at 1/1,000 dilution.

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 (ER40201) 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.

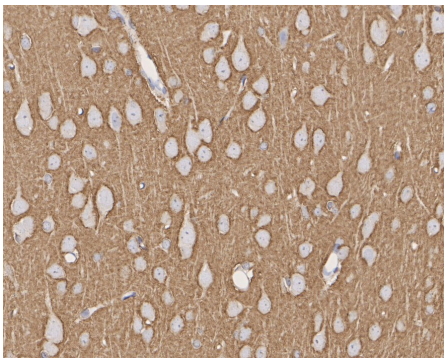

Fig6: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-GAP43 antibody (ER40201) at 1/1,000 dilution.

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 (ER40201) 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.

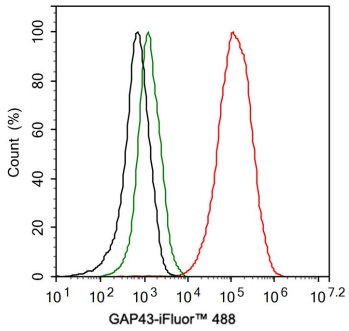

Fig7: Intracellular Flow Cytometry analysis of SH-SY5Y labeling GAP43 with purified ER40201 at 1/1,000 dilution (1 µg/ml) (red).

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.

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

Background References

1. "N-CAM modulates tumour-cell adhesion to matrix by inducing FGF-receptor signalling." Cavallaro U., Niedermeyer J., Fuxa M., Christofori G. Nat. Cell Biol. 3:650-657(2001)
2. "Acyl-protein thioesterase 2 catalyzes the deacylation of peripheral membrane-associated GAP-43." Tomatis V.M., Trenchi A., Gomez G.A., Daniotti J.L. PLoS ONE 5:E15045-E15045(2010)