

GABARAP Antibody (N-term)

Affinity Purified Rabbit Polyclonal Antibody (Pab) Catalog # AP1821a

Specification

GABARAP Antibody (N-term) - Product Information

Application Primary Accession Other Accession Reactivity Predicted Host Clonality Isotype Antigen Region WB, IHC-P, IF, IHC-P-Leica,E <u>095166</u> <u>P60517</u>, <u>08MK68</u>, <u>09DCD6</u>, <u>09GJW7</u> Human, Mouse, Rat Bovine, Rabbit Rabbit Polyclonal Rabbit IgG 1-30

GABARAP Antibody (N-term) - Additional Information

Gene ID 11337

Other Names Gamma-aminobutyric acid receptor-associated protein, GABA(A) receptor-associated protein, MM46, GABARAP, FLC3B

Target/Specificity

This GABARAP antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 1-30 amino acids of human GABARAP.

Dilution WB~~1:1000-1 :2000 IHC-P~~1:25 IF~~1:25 IHC-P-Leica~~N/A E~~Use at an assay dependent concentration.

Format

Purified polyclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is purified through a protein A column, followed by peptide affinity purification.

Storage

Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.

Precautions

GABARAP Antibody (N-term) is for research use only and not for use in diagnostic or therapeutic procedures.

GABARAP Antibody (N-term) - Protein Information



Name GABARAP (<u>HGNC:4067</u>)

Synonyms FLC3B

Function Ubiquitin-like modifier that plays a role in intracellular transport of GABA(A) receptors and its interaction with the cytoskeleton (PubMed:<u>9892355</u>). Involved in autophagy: while LC3s are involved in elongation of the phagophore membrane, the GABARAP/GATE-16 subfamily is essential for a later stage in autophagosome maturation (PubMed:<u>15169837</u>, PubMed:<u>20562859</u>, PubMed:<u>22948227</u>). Through its interaction with the reticulophagy receptor TEX264, participates in the remodeling of subdomains of the endoplasmic reticulum into autophagosomes upon nutrient stress, which then fuse with lysosomes for endoplasmic reticulum turnover (PubMed:<u>31006538</u>). Also required for the local activation of the CUL3(KBTBD6/7) E3 ubiquitin ligase complex, regulating ubiquitination and degradation of TIAM1, a guanyl-nucleotide exchange factor (GEF) that activates RAC1 and downstream signal transduction (PubMed:<u>25684205</u>). Thereby, regulates different biological processes including the organization of the cytoskeleton, cell migration and proliferation (PubMed:<u>25684205</u>). Involved in apoptosis (PubMed:<u>15977068</u>).

Cellular Location

Cytoplasmic vesicle, autophagosome membrane. Endomembrane system {ECO:0000250|UniProtKB:P60517}. Cytoplasm, cytoskeleton {ECO:0000250|UniProtKB:P60517}. Golgi apparatus membrane {ECO:0000250|UniProtKB:P60517}. Cytoplasmic vesicle {ECO:0000250|UniProtKB:P60517}. Note=Largely associated with intracellular membrane structures including the Golgi apparatus and postsynaptic cisternae. Colocalizes with microtubules (By similarity) Also localizes to discrete punctae along the ciliary axoneme (By similarity). {ECO:0000250|UniProtKB:P60517, ECO:0000250|UniProtKB:Q9DCD6}

Tissue Location

Heart, brain, placenta, liver, skeletal muscle, kidney and pancreas.

GABARAP Antibody (N-term) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- <u>Western Blot</u>
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- <u>Immunofluorescence</u>
- Immunoprecipitation
- Flow Cytomety
- <u>Cell Culture</u>

GABARAP Antibody (N-term) - Images





Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Hela (human cervical epithelial adenocarcinoma cell line)(Hela-C:Serum-starve overnight;Hela-chloroquine[]50 μ M, overnight; right) cells labeling GABARAP with AP1821a at 1/25 dilution, followed by Dylight® 488-conjugated goat anti-rabbit IgG (1583138) secondary antibody at 1/200 dilution (green). Immunofluorescence image showing cytoplasm and autophagic vacuoles staining on HeLa cell line. Cytoplasmic actin is detected with Dylight® 554 Phalloidin (PD18466410) at 1/100 dilution (red). The nuclear counter stain is DAPI (blue).



Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervical epithelial adenocarcinoma cell line) cells labeling GABARAP with AP1821a at 1/25 dilution, followed by Dylight® 488-conjugated goat anti-rabbit IgG (NK179883) secondary antibody at 1/200 dilution (green). Immunofluorescence image showing cytoplasm and weak nucleus staining on HeLa cell line. Cytoplasmic actin is detected with Dylight® 554 Phalloidin (PD18466410) at 1/100 dilution (red).The nuclear counter stain is DAPI (blue).





All lanes : Anti-Autophagy GABARAP Antibody (N-term) at 1:1000-1 :2000 dilution Lane 1: human fetal kidney lysate Lane 2: SH-SY5Y whole cell lysate Lane 3: mouse brain lysate Lane 4: rat brain whole cell lysate Lane 5: HepG2 whole cell lysate Lane 6: human fetal brain lysate Lane 7: C2C12 whole cell lysate Lysates/proteins at 20 μ g per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution. Predicted band size : 14 kDa Blocking/Dilution buffer: 5% NFDM/TBST.



AP1821a staining GABARAP in human brain tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 3% BSA for 0. 5 hour at room temperature; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody (1/25) for 1 hours at 37°C. A undiluted biotinylated goat polyvalent antibody was used as the secondary antibody.





AP1821a staining GABARAP in human heart tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 3% BSA for 0. 5 hour at room temperature; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody (1/25) for 1 hours at 37°C. A undiluted biotinylated goat polyvalent antibody was used as the secondary antibody.

GABARAP Antibody (N-term) - Background

Gamma-aminobutyric acid A receptors [GABA(A) receptors] are ligand-gated chloride channels that mediate inhibitory neurotransmission. GABARAP is GABA(A) receptor-associated protein, which is highly positively charged in its N-terminus and shares sequence similarity with light chain-3 of microtubule-associated proteins 1A and 1B. This protein clusters neurotransmitter receptors by mediating interaction with the cytoskeleton.

GABARAP Antibody (N-term) - References

References for protein:

1.Nemos, C., et al., Brain Res. Mol. Brain Res. 119(2):216-219 (2003).

2.Stangler, T., et al., J. Biol. Chem. 277(16):13363-13366 (2002).

3.Knight, D., et al., J. Biol. Chem. 277(7):5556-5561 (2002).

4.Tanida, I., et al., J. Biol. Chem. 277(16):13739-13744 (2002).

5.Harris, R., et al., J. Biomol. NMR 21(2):185-186 (2001).

References for U251 cell line:

1. Westermark B.; Pontén J.; Hugosson R. (1973)." Determinants for the establishment of permanent tissue culture lines from human gliomas". Acta Pathol Microbiol Scand A. 81:791-805. [PMID: 4359449].

2. Pontén, J., Westermark B. (1978)." Properties of Human Malignant Glioma Cells in Vitro". Medical Biology 56: 184-193.[PMID: 359950].

3. Geng Y.;Kohli L.; Klocke B.J.; Roth K.A.(2010). "Chloroquine-induced autophagic vacuole accumulation and cell death in glioma cells is p53 independent". Neuro Oncol. 12(5): 473–481.[PMID: 20406898].

GABARAP Antibody (N-term) - Citations

- Phosphorylation of the LIR Domain of SCOC Modulates ATG8 Binding Affinity and Specificity
- A conserved ATG2-GABARAP family interaction is critical for phagophore formation
- <u>Selective autophagy maintains centrosome integrity and accurate mitosis by turnover of centriolar satellites.</u>
- Molecular determinants regulating selective binding of autophagy adapters and receptors to ATG8 proteins.



- ATG9A shapes the forming autophagosome through Arfaptin 2 and phosphatidylinositol <u>4-kinase IIIβ</u>.
- <u>Recycling endosomal CD133 functions as an inhibitor of autophagy at the pericentrosomal region.</u>
- Autophagy Pathway Mapping to Elucidate the Function of Novel Autophagy Regulators Identified by High-Throughput Screening.
- Lentiviral-Mediated shRNA Approaches: Applications in Cellular Differentiation and Autophagy.
- <u>Centriolar Satellites Control GABARAP Ubiquitination and GABARAP-Mediated Autophagy.</u>
- K63-Linked Ubiquitination Targets Toxoplasma gondii for Endo-lysosomal Destruction in IFNy-Stimulated Human Cells.
- Induction of autophagy is a key component of all-trans-retinoic acid-induced differentiation in leukemia cells and a potential target for pharmacologic modulation.
- Small Molecule Inhibition of the Autophagy Kinase ULK1 and Identification of ULK1 Substrates.