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Phospho-ULK1(S556) Antibody

Affinity Purified Rabbit Polyclonal Antibody (Pab)
Catalog # AP3804a

Product Information

Application	IF, DB, E
Primary Accession	O75385
Other Accession	NP_003556.1
Reactivity	Human
Host	Rabbit
Clonality	Polyclonal
Isotype	Rabbit IgG
Clone Names	RB30585
Calculated MW	112631

Additional Information

Gene ID	8408
Other Names	Serine/threonine-protein kinase ULK1, Autophagy-related protein 1 homolog, ATG1, hATG1, Unc-51-like kinase 1, ULK1, KIAA0722
Target/Specificity	This ULK1 Antibody is generated from rabbits immunized with a KLH conjugated synthetic phosphopeptide corresponding to amino acid residues surrounding S556 of human ULK1.
Dilution	IF~~1:200 DB~~1:500 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	Phospho-ULK1(S556) Antibody is for research use only and not for use in diagnostic or therapeutic procedures.

Protein Information

Name	ULK1 {ECO:0000303|PubMed:9693035, ECO:0000312|HGNC:HGNC:12558}
Function	Serine/threonine-protein kinase involved in autophagy in response to starvation (PubMed:18936157, PubMed:21460634, PubMed:21795849, PubMed:23524951, PubMed:25040165, PubMed:29487085, PubMed:31123703). Acts upstream of phosphatidylinositol 3-kinase PIK3C3 to

	regulate the formation of autophagophores, the precursors of autophagosomes (PubMed:18936157, PubMed:21460634, PubMed:21795849, PubMed:25040165). Part of regulatory feedback loops in autophagy: acts both as a downstream effector and negative regulator of mammalian target of rapamycin complex 1 (mTORC1) via interaction with RPTOR (PubMed:21795849). Activated via phosphorylation by AMPK and also acts as a regulator of AMPK by mediating phosphorylation of AMPK subunits PRKAA1, PRKAB2 and PRKAG1, leading to negatively regulate AMPK activity (PubMed:21460634). May phosphorylate ATG13/KIAA0652 and RPTOR; however such data need additional evidences (PubMed:18936157). Plays a role early in neuronal differentiation and is required for granule cell axon formation (PubMed:11146101). Also phosphorylates SESN2 and SQSTM1 to regulate autophagy (PubMed:25040165, PubMed:37306101). Phosphorylates FLCN, promoting autophagy (PubMed:25126726). Phosphorylates AMBRA1 in response to autophagy induction, releasing AMBRA1 from the cytoskeletal docking site to induce autophagosome nucleation (PubMed:20921139). Phosphorylates ATG4B, leading to inhibit autophagy by decreasing both proteolytic activation and delipidation activities of ATG4B (PubMed:28821708).
Cellular Location	Cytoplasm, cytosol. Preautophagosomal structure. Note=Under starvation conditions, is localized to puncate structures primarily representing the isolation membrane that sequesters a portion of the cytoplasm resulting in the formation of an autophagosome.
Tissue Location	Ubiquitously expressed. Detected in the following adult tissues: skeletal muscle, heart, pancreas, brain, placenta, liver, kidney, and lung

Background

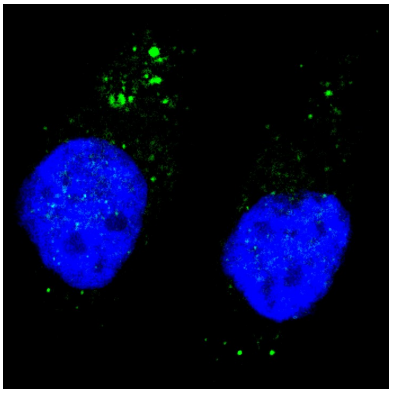
Involved in autophagy. Required for autophagosome formation (By similarity). Target of the TOR kinase signaling pathway that regulates autophagy through the control of phosphorylation status of ATG13/KIAA0652 and ULK1, and the regulation of the ATG13-ULK1-RB1CC1 complex (By similarity). Phosphorylates ATG13/KIAA0652. Involved in axon growth (By similarity). Plays an essential role in neurite extension of cerebellar granule cells (By similarity).

References

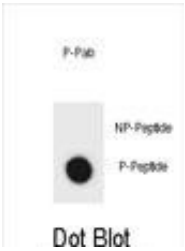
References for protein:
1.Mercer, C.A., et al. Autophagy 5(5):649-662(2009)
2.Ganley, I.G., et al. J. Biol. Chem. 284(18):12297-12305(2009)
3.Jung, C.H., et al. Mol. Biol. Cell 20(7):1992-2003(2009)
4.Hosokawa, N., et al. Mol. Biol. Cell 20(7):1981-1991(2009)
5.Chan, E.Y. Sci Signal 2 (84), PE51 (2009)
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].

Images

Fluorescent image of U251 cells stained with ULK1



(phospho S556) antibody. U251 cells were treated with Chloroquine (50 μM,16h), then fixed with 4% PFA (20 min), permeabilized with Triton X-100 (0.2%, 30 min). Cells were then incubated with AP3804a ULK1 (phospho S556) primary antibody (1:200, 2 h at room temperature). For secondary antibody, Alexa Fluor® 488 conjugated donkey anti-rabbit antibody (green) was used (1:1000, 1h). Nuclei were counterstained with Hoechst 33342 (blue) (10 μg/ml, 5 min). ULK1 (phospho S556) immunoreactivity is localized to autophagic vacuoles in the cytoplasm of U251 cells.



Dot blot analysis of ULK1 Antibody (Phospho S556) Phospho-specific Pab (Cat. #AP3804a) on nitrocellulose membrane. 50ng of Phospho-peptide or Non Phospho-peptide per dot were adsorbed. Antibody working concentrations are 0.6ug per ml.