

# Phospho-ULK1(S556) Antibody

Affinity Purified Rabbit Polyclonal Antibody (Pab) Catalog # AP3804a

#### **Product Information**

**Application** IF, DB, E **Primary Accession** 075385 **Other Accession** NP 003556.1 Reactivity Human Host Rabbit Clonality Polyclonal Isotype Rabbit IgG **Clone Names** RB30585 **Calculated MW** 112631

#### **Additional Information**

8408 Gene ID

**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.

Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store Storage

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**

ULK1 {ECO:0000303 | PubMed:9693035, ECO:0000312 | HGNC:HGNC:12558} Name

**Function** Serine/threonine-protein kinase involved in autophagy in response to

starvation (PubMed: <u>18936157</u>, PubMed: <u>21460634</u>, PubMed: <u>21795849</u>,

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: <u>25040165</u>). 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: <u>25126726</u>). 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

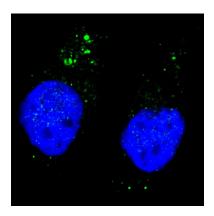
References for protein:

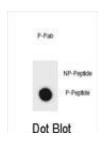
- 1.Mercer, C.A., et al. Autophagy 5(5):649-662(2009)
- 2.Ganley, I.G., et al. J. Biol. Chem. 284(18):12297-12305(2009)
- 3.Jung, C.H., et al. Mol. Biol. Cell 20(7):1992-2003(2009)
- 4. Hosokawa, N., et al. Mol. Biol. Cell 20(7):1981-1991(2009)
- 5.Chan, E.Y. Sci Signal 2 (84), PE51 (2009)

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**





(phospho S556) antibody. U251 cells were treated with Chloroquine (50  $\mu$ M,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  $\mu$ g/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.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.