

Anti-Atg4A Monoclonal Antibody

Catalog # ABO14469

Product Information

Application	WB, IHC, IP
Primary Accession	Q8WYN0
Host	Rabbit
Isotype	Rabbit IgG
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Liquid
Description	Anti-Atg4A Monoclonal Antibody . Tested in WB, IHC, IP applications. This antibody reacts with Human, Mouse, Rat.

Additional Information

Gene ID	115201
Other Names	Cysteine protease ATG4A, 3.4.22.-, AUT-like 2 cysteine endopeptidase, Autophagy-related cysteine endopeptidase 2, Autophagin-2, Autophagy-related protein 4 homolog A, HsAPG4A, hAPG4A, ATG4A {ECO:0000303 Ref.20, ECO:0000312 HGNC:HGNC:16489}
Calculated MW	45378
Application Details	WB 1:500-1:2000 IHC 1:50-1:200 IP 1:50
Source	Eukaryota
Contents	Rabbit IgG in phosphate buffered saline, pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol, 0.4-0.5mg/ml BSA.
Clone Names	Clone: ACHB-1
Immunogen	A synthesized peptide derived from human Atg4A The cysteine protease Atg4 is pivotal to autophagosome membrane generation and regulation. Atg4 primes the Atg8 homologue for lipidation by cleaving its carboxy terminus and exposing its glycine residue for E1-like enzyme Atg7. The Atg8 homologue is transferred to the E2-like enzyme Atg3 before forming the Atg8-PE conjugate. During later stages of autophagy, Atg4 can reverse this lipidation event by cleaving PE, thereby recycling the Atg8 homologue.
Purification	Affinity-chromatography
Storage	Store at -20°C for one year. For short term storage and frequent use, store at 4°C for up to one month. Avoid repeated freeze-thaw cycles.

Protein Information

Name	ATG4A {ECO:0000303 Ref.20, ECO:0000312 HGNC:HGNC:16489}
Function	Cysteine protease that plays a key role in autophagy by mediating both proteolytic activation and delipidation of ATG8 family proteins (PubMed:12473658, PubMed:15169837, PubMed:17347651, PubMed:21177865, PubMed:21245471, PubMed:22302004, PubMed:32732290). The protease activity is required for proteolytic activation of ATG8 family proteins: cleaves the C-terminal amino acid of ATG8 proteins to reveal a C-terminal glycine (PubMed:12473658, PubMed:15169837, PubMed:17347651, PubMed:21177865, PubMed:21245471, PubMed:22302004). Exposure of the glycine at the C-terminus is essential for ATG8 proteins conjugation to phosphatidylethanolamine (PE) and insertion to membranes, which is necessary for autophagy (PubMed:12473658, PubMed:15169837, PubMed:17347651, PubMed:21177865, PubMed:21245471, PubMed:22302004). Preferred substrate is GABARAPL2 followed by MAP1LC3A and GABARAP (PubMed:12473658, PubMed:15169837, PubMed:17347651, PubMed:21177865, PubMed:21245471, PubMed:22302004). Protease activity is also required to counteract formation of high-molecular weight conjugates of ATG8 proteins (ATG8ylation): acts as a deubiquitinating- like enzyme that removes ATG8 conjugated to other proteins, such as ATG3 (PubMed:31315929, PubMed:33773106). In addition to the protease activity, also mediates delipidation of ATG8 family proteins (PubMed:29458288, PubMed:33909989). Catalyzes delipidation of PE-conjugated forms of ATG8 proteins during macroautophagy (PubMed:29458288, PubMed:33909989). Compared to ATG4B, the major protein for proteolytic activation of ATG8 proteins, shows weaker ability to cleave the C-terminal amino acid of ATG8 proteins, while it displays stronger delipidation activity (PubMed:29458288). Involved in phagophore growth during mitophagy independently of its protease activity and of ATG8 proteins: acts by regulating ATG9A trafficking to mitochondria and promoting phagophore-endoplasmic reticulum contacts during the lipid transfer phase of mitophagy (PubMed:33773106).
Cellular Location	Cytoplasm {ECO:0000250 UniProtKB:Q8BGE6}.

Images

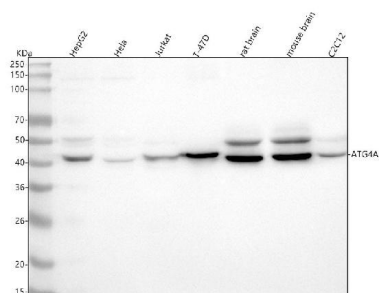


Figure 1. Western blot analysis of Atg4A using anti-Atg4A antibody (M06539).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human HepG2 whole cell lysates,
Lane 2: human Hela whole cell lysates,
Lane 3: human Jurkat whole cell lysates,
Lane 4: human T-47D whole cell lysates,
Lane 5: rat brain tissue lysates,
Lane 6: mouse brain tissue lysates,
Lane 7: mouse C2C12 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit

anti-Atg4A antigen affinity purified monoclonal antibody (Catalog # M06539) at 1:500 overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for Atg4A at approximately 45 kDa. The expected band size for Atg4A is at 45 kDa.

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