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Product no AS14 2769

ATG8 | Autophagy-related protein

Product information

Immunogen	Fragment of recombinant ATG8 from *Chlamydomonas reinhardtii*, UniProt: A8JB85
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 µl
Reconstitution	For reconstitution add 50 µl of sterile water
Storage	Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.
Additional information	This product can be sold containing ProClin if requested. This antibody is recognizing 1 ng of recombinant CrATG8. Antigen used to elicit this antibody is conserved from 70-80 % in following ATG protein from *Arabidopsis thaliana*: ATG8a UniProt: Q8LEM4 ATG8B UniProt: Q9XEB5 ATG8c UniProt: Q8S927 ATG8d UniProt: Q9SL04, ATG8e UniProt: Q8S926 ATG8f UniProt: Q8VYK7 and conserved below 70 % in: ATG8g UniProt: Q9LZZ9 ATG8h Uniprot: Q8S92 This antibody does not recognize all isoforms into the same degree.

Application information

Recommended dilution	1 : 1000 (IL), 1 : 1000-1 : 2000 (WB)
Expected | apparent MW	15,2 | 15 kDa
Confirmed reactivity	*Arabidopsis thaliana, Aponogeton madagascariensis, Chlamydononas reinhardtii, Chlorococcum dorsiventrale, Haematococcus lacustris, Nicotiana benthamiana, Populus trichocarpa, Solanum lycopersicum, Zea mays*
Predicted reactivity	*Ananas comosus, Brassica napus, Hordeum vulgare, Micromonas sp., Nelumbo nucifera, Oryza sativa, Panicum hallii, Phoenix dactylifera, Pyrus x bretschneideri, Physcomitrium patens, Pinus sitchensis, Solanum tuberosum, Triticum aestivum, Volvox carteri* Species of your interest not listed? Contact us
Not reactive in	*Cuscuta chinensis*, *Symbiodinium* sp.
Additional information	For *Arabidopsis thaliana* the signal obtained using ATG8 antibodies is cleaner in case of roots compare to leaf material. For best results please follow extraction protocol described in Álvarez et al. (2012). ATG8 signal corresponds to the two bands of 17 kDa. Preparation of a cell extract from *Arabidopsis thaliana*: A. Plants were first subjected to autophagy activating conditions: nutrient (nitrogen or carbon) limitation or oxidative stress in order to activate this degradative process. B. Total protein extracts can be obtained as described by Álvarez. Leaves are grinded in liquid nitrogen with a minimal volume of extraction buffer (100 mM Tris-HCl pH 8, 400 mM sucrose, 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mg/ml sodium deoxycholate, 10 µg/ml of leupeptin, 10 µg/ml of pepstatin A, 4% (v/v) protease inhibitor cocktail from Roche). C. Cell debris is removed by centrifuging at 500 g for 10 min at 4°C. **Important note:** It is recommendable to use bigger gels in order to get a better resolution of ATG8 bands. Midi-protean gels are better than mini-gels. There are 9 ATG8 isoforms and this antibody will likely recognizes all of them. For immunolocalization protocol, please inquire.
Selected references	Lan et al. (2024).Clathrin Light Chains negatively regulate plant immunity by hijacking the autophagy pathway. Plant Commun. 2024 Apr 30:100937.doi: 10.1016/j.xplc.2024.100937.

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Miklaszewska et al. (2023).CALEOSIN 1 interaction with AUTOPHAGY-RELATED PROTEIN 8 facilitates lipid droplet microautophagy in seedlings.Plant Physiol. 2023 Nov 22;193(4):2361-2380.doi: 10.1093/plphys/kiad471.
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Samperna et al (2022). Cyclopaldic Acid, the Main Phytotoxic Metabolite of Diplodia cupressi, Induces Programmed Cell Death and Autophagy in Arabidopsis thaliana. Toxins (Basel). 2022 Jul 11;14(7):474. doi: 10.3390/toxins14070474. PMID: 35878212; PMCID: PMC9325063.
Zharova et al. (2022) Role of Autophagy in Haematococcus lacustris Cell Growth under Salinity. Plants. 2022; 11(2):197. https://doi.org/10.3390/plants11020197 (immunofluorescence)
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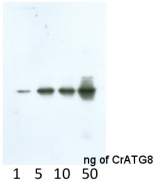
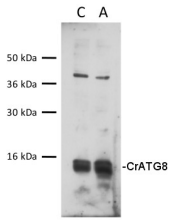
Application example

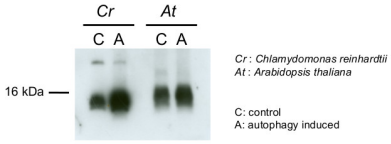


Anti-CrATG8 antibodies detect 1 ng of recombinant CrATG8 protein.



30 µg of total protein from *Chlamydomonas reinhardtii ,* control **(C)**, autophagy induced **(A)**, extracted with lysis buffer according to Perez-Perez et al. 2010 (Plant Physiology 152: 1874-1888) were separated on 15 % SDS-PAGE and blotted 1h to nitrocellulose membrane using semi-dry or tank transfer. Blots were blocked with 5% milk for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1:1000 for 1h at RT with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 5 min in TBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, from Agrisera AS09 602, diluted to 1:25 000) for 1h at RT with agitation. The blot was washed as above and developed for 5 min with chemiluminescent detection reagent, according to the manufacturer's instructions. Exposure time was 45 seconds.

Courtesy of Dr. María Esther Pérez-Perez, IBVF, Spain



15 µg of total protein from *Chlamydomonas* reinhardtii and *Arabidopsis thaliana* were separated on 15 % SDS-PAGE and blotted 1h to nitrocellulose membrane using semi-dry transfer. Blots were blocked with 5 % dry milk in PBS for 1h at room temperature (RT) with agitation. Blot

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was incubated in the primary antibody at a dilution of 1:1 000 over night at 4 ºC with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 5 min in TBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, AS09 602 from Agrisera) diluted to 1:10 000 in 5 % dry milk for 1h at RT with agitation. The blot was washed as above and developed for 5 min with chemiluminescent detection reagent, according to the manufacturer's instructions. Exposure time was 60 seconds.

Courtesy of Dr. María Esther Pérez-Pérez and Ana M. Laureano-Marín, IBVF, Spain