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product AS14 2769

ATG8 | Autophagy-related protein

product information

Background	**ATG8 (Autophagy-related protein 8)** is involved in degradation and recycling of intracellular components in a process of autophagy. ATG8 is a molecular autophagy marker in *Chlamydomonas reinhardtii* (Pérez-Pérez et al. 2010, Plant Physiol. 152: 1874-88).
Immunogen	Fragment of recombinant ATG8 from *Chlamydomonas reinhardtii*, UniProt: A8JB85, conserved from 70-80 % in following ATG protein from Arabidopsis: 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: Q8S925,
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 tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.
Tested applications	Immunolocalization (IL), Western blot (WB)
Related products	AS19 4275 | Anti-ATG3 | Autophagy-related protein 3, rabbit antibodies AS15 2831 | Anti-ATG4 | Autophagy protein 4, rabbit antibodies AS19 4276 | Anti-ATG5 | Autophagy protein 5, rabbit antibodies AS19 4277 | Anti-ATG7 | Autophagy-related protein 7, rabbit anitbodies AS14 2811 | Anti-ATG8A | Autophagy-related protein 8a, rabbit antibodies AS14 2769PRE | ATG8 | Autophagy-related protein, pre-immune serum AS16 4071 | Anti-ATG9 | Autophagy-related protein 9 (N-terminal), rabbit antibodies AS16 4072 | Anti-ATG9 | Autophagy-related protein 9 (C-terminal), rabbit antibodies AS19 4278 | Anti-ATG12b | Autophagy-related protein 12b, rabbit antibodies AS19 4279 | Anti-ATG13a | Autophagy-related protein 13a, rabbit antibodies AS19 4280 | Anti-ATG16 | Autophagy-related protein 16, rabbit antibodies AS14 2805 | Anti-NBR1 | Autophagy substrate NBR1, rabbit antibodies AS19 4281 | Anti-NBR1 | Autophagy substrate NBR1, rabbit antibodies
Additional information	This product can be sold containing ProClin if requested. This antibody is recognizing 1 ng of recombinant CrATG8

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, Populus trichocarpa, Solanum lycopersicum*

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Predicted reactivity

Brassica napus, Micromonas sp., Physcomitrella patens, Pinus sitchensis, Solanum tuberosum, Volvox carteri

Not reactive in

No confirmed exceptions from predicted reactivity are currently known.

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.

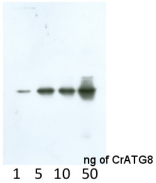
Important note:
It is recommendable to use bigger gels in order to get a better resolution of ATG8 bands. Midi-protean gels are better than mini-gels. There are 9 ATG8 isoforms and this antibody will likely recognizes all of them.

For immunolocalization protocol, please inquire.

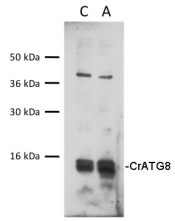
Selected references

Shull et al. (2019). Anatase TiO2 nanoparticles induce autophagy and chloroplast degradation in thale cress (Arabidopsis thaliana). Environ Sci Technol. 2019 Jul 29. doi: 10.1021/acs.est.9b01648. Wojciechowska et al. (2018). Autophagy counteracts instantaneous cell death during seasonal senescence of the fine roots and leaves in Populus trichocarpa. BMC Plant Biol. 2018 Oct 29;18(1):260. doi: 10.1186/s12870-018-1439-6. (immunolocalization)
Chen et al. (2016). The role of nitric oxide signalling in response to salt stress in Chlamydomonas reinhardtii. Planta. 2016 Sep;244(3):651-69. doi: 10.1007/s00425-016-2528-0. Epub 2016 Apr 26.
Gorovits et al. (2016). Tomato yellow leaf curl virus confronts host degradation by sheltering in small/midsized protein aggregates. Virus Res. 2016 Feb 2;213:304-13. doi: 10.1016/j.virusres.2015.11.020. Epub 2015 Dec 1

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

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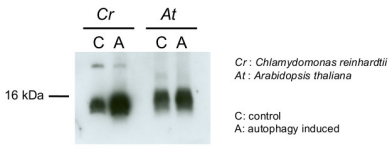

15 µg of total protein from *Chlamydomonas* reinhardtii and *Arabidopsis thaliana* were separated on 15 % SDS-PAGE and blotted 1h to nitrocellulose membrane using semi-dry transfer. Blots were blocked with 5 % dry milk in PBS for 1h at room temperature (RT) with agitation. Blot was incubated in the primary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