

# Agrisera

This product is for research use only (not for diagnostic or therapeutic use)

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Product no **AS14 2769**

## ATG8 | Autophagy-related protein

### Product information

<b>Immunogen</b>	Fragment of recombinant ATG8 from <i>Chlamydomonas reinhardtii</i> , UniProt: <a href="#">A8JB85</a> , conserved from 70-80 % in following ATG protein from <i>Arabidopsis thaliana</i> : ATG8a UniProt: <a href="#">Q8LEM4</a> ATG8B UniProt: <a href="#">Q9XEB5</a> ATG8c UniProt: <a href="#">Q8S927</a> ATG8d UniProt: <a href="#">Q9SL04</a> , ATG8e UniProt: <a href="#">Q8S926</a> ATG8f UniProt: <a href="#">Q8VYK7</a> and conserved below 70 % in: ATG8g UniProt: <a href="#">Q9LZZ9</a> ATG8h UniProt: <a href="#">Q8S925</a>
<b>Host</b>	Rabbit
<b>Clonality</b>	Polyclonal
<b>Purity</b>	Serum
<b>Format</b>	Lyophilized
<b>Quantity</b>	50 µl
<b>Reconstitution</b>	For reconstitution add 50 µl of sterile water.
<b>Storage</b>	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.
<b>Additional information</b>	This product can be sold containing ProClin if requested. This antibody is recognizing 1 ng of recombinant CrATG8

### Application information

<b>Recommended dilution</b>	1 : 1000 (IL), 1 : 1000-1 : 2000 (WB)
<b>Expected   apparent MW</b>	15.2   15 kDa
<b>Confirmed reactivity</b>	<i>Arabidopsis thaliana</i> , <i>Aponogeton madagascariensis</i> , <i>Chlamydomonas reinhardtii</i> , <i>Populus trichocarpa</i> , <i>Solanum lycopersicum</i>
<b>Predicted reactivity</b>	<i>Brassica napus</i> , <i>Micromonas sp.</i> , <i>Physcomitrella patens</i> , <i>Pinus sitchensis</i> , <i>Solanum tuberosum</i> , <i>Volvox carteri</i> Species of your interest not listed? <a href="#">Contact us</a>
<b>Not reactive in</b>	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.

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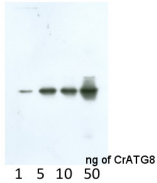
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For immunolocalization protocol, please [inquire](#).  
For high resolution images, please visit the specific product page at [www.agrisera.com](http://www.agrisera.com)

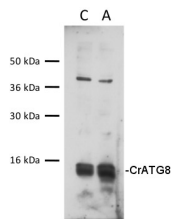
## Selected references

[Kazibwe](#) et al. (2020). TOR mediates the autophagy response to altered nucleotide homeostasis in a ribonuclease mutant. *J Exp Bot.* 2020 Sep 9;eraa410.doi: 10.1093/jxb/eraa410.  
[Upadhyaya](#) and Jagadeeshwar Rao (2019). Reciprocal regulation of photosynthesis and mitochondrial respiration by TOR kinase in *Chlamydomonas reinhardtii*. *Plant Direct* Volume 3, Issue 11.  
[Shull](#) et al. (2019). Anatase TiO<sub>2</sub> 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.

## Application example

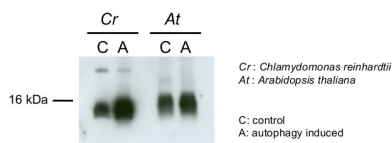


Anti-CrATG8 antibodies detect 1 ng of recombinant CrATG8 protein.



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 was incubated in the primary antibody at a dilution of 1:1 000 over night at 4 °C 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