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### Product no AS14 2805 Anti-NBR1 | Autophagy substrate NBR1

### **Product information**

 Immunogen
 UBA2 domain of NBR1 of Arabidopsis thaliana, fused with GST, UniProt:Q9SB64, TAIR:AT4G24690

 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.

## **Application information**

Recommended dilution	1: 1000 (IL), 1 : 500-1 : 5000 (WB)
Expected   apparent MW	75   100 kDa
Confirmed reactivity	Arabidopsis thaliana, Physcomitrium patens
Predicted reactivity	Brassicaceae family, Zea mays
	Species of your interest not listed? Contact us
Not reactive in	Citrus sinensis, Nicotiana tabacum
Additional information	Specific extraction method and tissue type needs to be used as described in Minina et al, (2013), Dilution in western blot depends upon amount of NBR1 in the sample
Selected references	Julian et al. (2025). ATG8ylation of vacuolar membrane protects plants against cell wall damage. Nat Plants. 2025 Feb;11(2):321-339.doi: 10.1038/s41477-025-01907-z. Yan et al. (2024). Autophagy modulates Arabidopsis male gametophyte fertility and controls actin organization. Nat Commun. 2024 Nov 21;15(1):10071. doi: 10.1038/s41467-024-54468-8. Mosesso et al. (2024). Arabidopsis CaLB1 undergoes phase separation with the ESCRT protein ALIX and modulates autophagosome maturation. Nat Commun. 2024 Jun 19;15(1):5188. doi: 10.1038/s41467-024-49485-6. 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. <u>Rodriguez</u> et al. (2020). Autophagy mediates temporary reprogramming and dedifferentiation in plant somatic cells. bioRxiv doi.org/10.1101/747410 <u>Calero-MuÅ?Å+co</u> et al. (2019). Cadmium induces reactive oxygen species-dependent pexophagy in Arabidopsis leaves. Plant Cell Environ. 2019 Sep;42(9):2696-2714. doi: 10.1111/pce.13597. Jia et al. (2019). Noncanonical ATG8-ABS3 interaction controls senescence in plants. Nat Plants. 2019 Feb;5(2):212-224. doi: 10.1038/s41477-018-0348-x. <u>Hackenberg</u> et al. (2013). Catalase and NO CATALASE ACTIVITY1 promote autophagy-dependent cell death in Arabidopsis. Plant Cell. 2013 Nov;25(11):4616-26. doi: 10.1105/tpc.113.117192. Epub 2013 Nov 27. <u>Minina</u> et al. (2013). Autophagy mediates caloric restriction-induced lifespan extension in Arabidopsis. Aging Cell. 2013 Apr;12(2):327-9. doi: 10.1111/acel.12048. Epub 2013 Feb 28. (method description in supplemental materials) <u>Katisiarimopa</u> et al. (2011). Plant NBR1 is a selective autophagy substrate and a functional hybrid of the mammalian autophagic adapters NBR1 and p62/SQSTM1. Autophagy. 2011 Sep;7(9):993-1010. Epub 2011 Sep 1. (original reference)

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# Application example

8.5, 141 mM Tris Base, 2% lithium dodecyl sulfate, 0.51 mM EDTA pH 8.0, 10% glycerol, 0.22 mM Coomassie Blue G250, 0.166 mM Phenol Red) with 50 mM dithiothreitol and denatured at 100°C for 5 min. Proteins were separated by electrophoresis on a Bolt 10% Bis-Tris Plus gel (Invitrogen) and transferred for 40 min at 24 V to an Amersham Protran 0.45 µm nitrocellulose membrane (GE Healthcare Life Sciences) using semidry transfer. The membrane was blocked with 8% milk in TBS-T for 2 h at 4°C with agitation. The membrane was incubated in the primary antibody at a dilution of 1:4000 overnight at 4°C with agitation in 8% milk in TBS-T. The antibody solution was decanted and the blot was rinsed briefly twice, then washed 3 times for 5 min in 8% milk in TBS-T at 4°C with agitation. The membrane was incubated in secondary antibody (horse radish peroxidase conjugated goat anti-rabbit IgG) diluted to 1:5000 in 8% milk in TBS-T for 4 h at 4°C with agitation. The membrane was washed 3 times for 5 min in TBS-T at 4°C with agitation and incubated for 2 min with WesternBright chemiluminescent detection reagent (Advansta). Exposure time was 30 seconds.

Courtesy of Pierce Young, Rice University, USA