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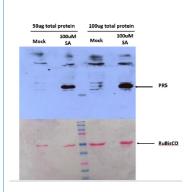
## Product no AS12 2373 Anti-PR-5 | Pathogenesis-related protein 5 (A.thaliana)

## **Product information**

Immunogen	KLH-conjugated synthetic peptide derived from Arabidopsis thaliana UniProt: P28493, TAIR: AT1G75040 The peptide is not found in other thautamin-like proteins.
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 μl
Reconstitution	For reconstitution add 50 $\mu$ l, 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.

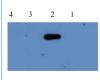
## **Application information**

Recommended dilution	
Expected   apparent MW	Propeptide 25.3 kD, processing aa 1-23, mature peptide 22.8 kD
Confirmed reactivity	Arabidopsis thaliana
Predicted reactivity	Arabidopsis thaliana
Selected references	Bernacki et al. (2024).METACASPASE8 (MC8) Is a Crucial Protein in the LSD1-Dependent Cell Death Pathway in Response to Ultraviolet Stress. Int. J. Mol. Sci. 2024, 25(6), 3195



50 and 100 µg of total protein from *Arabidopsis thaliana* wild-type plants treated with water and salicylic acid (SA) analog BTH (Benzothiadiazole) were extracted with buffer containing 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 10 mM MgCl2, 5 mM EDTA, 10% glycerol, 0.1% NP-40, 0.1% protease inhibitor cocktail . The proteins were denatured by boiling for 5 min were separated on 10% SDS-PAGE and blotted 30 min to PVDF using tank transfer. Blots were blocked with TBST containing 5% non-fat dry milk for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 10 000 for for 4 h/RT with agitation in TBS-T. The antibody solution was decanted and the blot was rinsed briefly twice, then washed 3 times for 15 min each in TBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1:20 000 in for 1h at RT with agitation. The blot was washed as above and developed with Clarity MAX (Bio-RAD). Exposure time was 3 minutes.

Courtesy Ms. Ruiying Liu and Dr. Pradeep Kachroo, University of Kentucky, USA





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*Arabidopsis thaliana* Col-0 treated with water (1) Col-0 treated with 0.5 mM SA (2), npr1-2 treated with water (3), npr1-2 treated with 0.5 mM SA (4). Samples were collected at 24 hours after treatment. 0.1g of total protein was collected from four-week-old plants. Protein extracted with 150 µL protein extraction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.1% Triton X-100, 0.2% Nonidet P-40, 1 mM PMSF, 1×PIC) and denatured with SDS at 70 for 10 min. 60 µg protein were separated on 4-12% SDS-PAGE and blotted 1h to nitrocellulose membrane using tank transfer. Blots were blocked with 5% non-fat dry milk in TBST for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1 000 overnight at 4 with agitation in TBST. 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) diluted to 1:2500 in 5% non-fat dry milk in TBST for 1h at RT with agitation. The blot was washed as above and developed for 5 min with SuperSignaITM WEST Pico PLUS Chemiluminescent Substrate from Thermo Scientific. Exposure time was 60 seconds.

Courtesy Msc Min Li, University of South Carolina, USA