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Product no AS12 1854-500

Anti-NPR1 | Nonexpresser of PR genes 1 (0,5 mg)

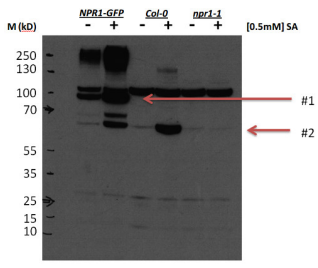
Product information

Immunogen	KLH-conjugated peptide, chosen from NPR1 sequence of *Arabidopsis thaliana*, TAIR: AT1G64280, UniProt: P93002
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	0.5 mg (10 x 50 µg)
Reconstitution	For reconstitution add 50 µl of sterile water to each tube.
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	For successful detection using NPR1 antibody please follow protocol suggested below. NPR1 protein readily oligomerizes, in addition to any naturally occurring oligomer, during extraction. Therefore 50 mM DTT has to be used as well as denaturation at 75°C for 15 minutes. Engogenous NPR1 level is very low, thereofre SA treatment is absolutely necessary for good detection. This antibody is recognizing NPR1-GFP in the 35S overexpression line.

Application information

Recommended dilution	1 : 1000 (WB)
Expected | apparent MW	66 | 66 kDa
Confirmed reactivity	*Arabidopsis thaliana*
Predicted reactivity	*Arabidopsis thaliana*
Not reactive in	*Nicotiana benthamiana, Nicotiana tabacum, Solanum lycopersicum, Solanum tuberosum*
Additional information	Please note that depending upon detection system you are using, longer exposure time may be required with this antibody

application example



Samples (0.2 g) were collected from leaf tissue (3 week old rosettes; 24 hours after +/- 0.5 mM sodium salicylate spray). Total protein was extracted in a buffer containing 50 mM Tris-Hcl, pH 7.5; 150 mM NaC; 0.5 mM EDTA; 0.1 % Triton X-100; 0.2 % nonidet P-40; 50 uM MG115 and samples were adjusted to equal total protein concentration. Samples were denatured with 4X SDS Sample buffer with 200 mM DTT (final sample concentration of 50 mM) at 75 C for 15 min. Protein samples (30 µg of total protein) were separated on 4-12% Bis-Tris gel and blotted to PVDF using an iBlot (semi-dry transfer system; Life technologies). The membrane was blocked with 1X PBS-T containing 5% low fat dry milk ans 0.1 % tween-20, for 1h at room temperature (RT) with agitation and incubated in the primary antibody (in blocking solution) at a dilution of 1: 1 000 over night at 4 C with agitation. The antibody solution was decanted and the membrane was washed with blocking solution, 3 times for 10 min each at RT with agitation. The membrane was then incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1:10 000 in blocking solution for 1h at RT with agitation. The membrane was washed as described above, rinsed with 1x PBS-T and developed for 2 min with Pierce West Dura extended duration substrate. Exposure time was 30 seconds.

** Note: 50 mM DTT concentration in the extraction buffer is necessary to reduce NPR1 oligomer formation.

** Note: The expression level of endogenous NPR1 is very low in extracts from healthy, untreated plants. A successful SA treatment or pathogen infection is absolutely necessary for good detection.

Courtesy Dr. John Withers, Duke University, USA