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Product no AS12 2602

Anti-RCD1 | Radical cell death 1

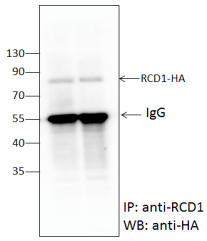
Product information

Immunogen	KLH-conjugated synthetic peptide derived from *Arabidopsis thaliana* RCD1 protein sequence UniProt: Q8RY59, TAIR: AT1G32230
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	100 µg
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 (WB) on samples following IP
Expected | apparent MW	65 kDa
Confirmed reactivity	*Arabidopsis thaliana*
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Additional information	Protein has three splice variants: 63.8 + 65.6 + 65.7 kD Peptide used to elicit this antibody was designed for detection of all (3) RCD1 splice variants but NOT the close relative SRO1.

application information



5 mg of total protein from *Arabidopsis thaliana* seedlings expressing RCD1-HA under native promoter extracted with RIPA buffer for protein extraction (50 mM Tris-HCl pH 7,5, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, protease inhibitor cocktail 1:100, 50 uM MG132) were used for immunoprecipitation and afterwards separated on 10% SDS-PAGE and blotted 1h to PVDF using tank transfer. Blots were blocked with 5% milk for 1h at room temperature (RT) with agitation. Blot was incubated in the anti-HA antibody (Roche) at a dilution of 1: 1 000 in 1% milk, TBS-T overnight at +4C 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-rat IgG horse radish peroxidase conjugated) diluted to 1:10 000 in 1% milk TBS-T for 1h at RT with agitation. The blot was washed as above and developed for 5 min with ECL Prime, GE Healthcare. Exposure time was 1 min.

Courtesy Dr. Julia Vainonen, Helsinki University, Finland

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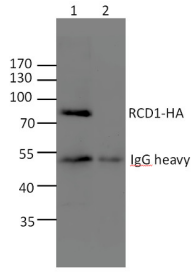

5 µl of anti-RCD1 antibody was used for immunoprecipiation. Samples: transgenic line expressing HA-tagged RCD1 (Jaspers et al., 2009, Plant J) **(1)**, rcd1-4 mutant **(2)**. Afterwards these samples were separated on 10% SDS-PAGE and blotted 1h to PVDF using tank transfer. Blots were blocked with 5% milk for 1h at room temperature (RT) with agitation. Blot was incubated in the anti-HA antibody (Roche) at a dilution of 1: 1 000 in 1% milk, TBS-T overnight at +4C 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-rat IgG horse radish peroxidase conjugated) diluted to 1:10 000 in 1% milk TBS-T for 1h at RT with agitation. The blot was washed as above and developed for 5 min with ECL Prime, GE Healthcare. Exposure time was 1 min.

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