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Product no AS12 1859

BRI1 | Brassinosteroid insensitive 1

Product information

Immunogen	KLH-conjugated synthetic peptide derived from *Arabidopsis thaliana* BRI1 protein, Uniprot: O22476, TAIR: AT4G39400
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	50 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.
Additional information	This product can be sold containing proclin if requested

Application information

Recommended dilution	1 : 5000 (WB)
Expected | apparent MW	above 130 kDa (due to N-glycosylation)
Confirmed reactivity	*Arabidopsis thaliana*
Predicted reactivity	*Brassica napus, Brassica rapa* Species of your interest not listed? Contact us
Not reactive in	*Hordeum vulgare, Oryza sativa, Solanum lycopersicum*
Additional information	Antibody was tested on bri1-1 and bri1-5 mutants. Bri1-1 is a point mutation in the kinase domain that renders the protein non-functional and plants compensate for that by over-accumulating the non-functional receptor. Bri1-5 is a mutant in the extracellular domain and the bri1-5 protein is retained in the ER. The bri1-5 plants contain less protein than the wild type and show an intermediate brassinosteroid deficient phenotype. Also BRI1-5 migrates higher than wild type BRI1 in SDS-PAGE, because it carries ER-type high mannose N-glycans. For IP: 15 µl GFP-trap beads was used for 200 mg plant material to precipitate GFP-tagged protein followed by detection with Co-IPed BRI1 on Western with 1:5000 diluted anti-BRI1 antibody. Protein extraction has to be done efficiently as this step is crucial, recommended material to buffer ratio: 15 µl/µg or less.
Selected references	Jing et al. (2024). Copine proteins are required for brassinosteroid signaling in maize and Arabidopsis. Nat Commun. 2024 Mar 8;15(1):2028.doi: 10.1038/s41467-024-46289-6. Lee et al (2021). Chaperone-like protein DAY plays critical roles in photomorphogenesis. Nat Commun. 2021 Jul 7;12(1):4194. doi: 10.1038/s41467-021-24446-5. PMID: 34234144; PMCID: PMC8263706. Chen et al. (2019). BES1 is activated by EMS1-TPD1-SERK1/2-mediated signaling to control tapetum development in Arabidopsis thaliana. Nat Commun. 2019 Sep 13;10(1):4164. doi: 10.1038/s41467-019-12118-4. Hou et al. (2019). Less Conserved LRRs Is Important for BRI1 Folding. Front Plant Sci. 2019 May 21;10:634. doi: 10.3389/fpls.2019.00634. eCollection 2019. Chen et al. (2019). BZR1 Family Transcription Factors Function Redundantly and Indispensably in BR Signaling but Exhibit BRI1-Independent Function in Regulating Anther Development in Arabidopsis. Mol Plant. 2019 Jun 20. pii: S1674-2052(19)30207-2. doi: 10.1016/j.molp.2019.06.006.

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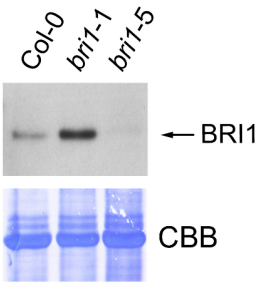
Application example



15 µg of total protein from leaf material of 5 week-old plants of *Arabidopsis thaliana*, were extracted with homogenization buffer (250 mM sucrose, 50 mM HEPES-KOH pH 7.5, 5% glycerol, 0.5% Triton X-100, 50 mM Na4P2O7, 1 mM Na2MoO4, 25 mM NaF, 2 mM DTT, Sigma plant protease inhibitor cocktail). 3 parts of protein extract were mixed with 1 part of standard SDS loading buffer (200 mM TRIS pH=6.8, 400 mM DTT, 8% SDS, 40% glycerol, 0.1% bromophenol blue). Protein denaturation was done at 90°C/5 min. Proteins were separated on a 10 % SDS-PAGE and blotted using BioRad Tank Blot device onto a PVDF membrane at 100 V for 1 h 15 using following blotting buffer: 50 mM TRIS-base, 50 mM boric acid of pH of 8.3. Blots were blocked with TBS-T (150mM NaCl, 10mM Tris-HCl pH8, 0.05% Tween-20) containing 5% skimmed milk powder for 1h at room temperature (RT) with agitation. The blot was incubated in the primary antibody at a dilution of 1: 5 000 in TBS-T with milk powder overnight at 4°C with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed 5 times for 15 min in TBS-T (with milk powder) at RT with agitation. The blot was then incubated in secondary antibody (Agrisera Goat anti-rabbit IgG (H&L) HRP conjugate, AS09 602) diluted to 1:5000 in TBS-T (with milk powder) for 2h at RT with agitation. The blot was washed 5 times for 15 min in TBS-T (without milk powder) and developed using chemiluminescent detection. Exposure time was 3 minutes.

Courtesy of Dr. Elena Petusching, Georg-August-University Goettingen, Germany