Product no AS12 1859
BRI1 | Brassinosteroid insensitive 1

Product information

**Background:** BRI1 (Protein BRASSINOSTEROID INSENSITIVE 1) is a receptor which binds brassinolide and has a dual specificity kinase activity acting on both serine/threonine- and tyrosine-containing substrates. Involved in a signaling cascade including expression of light- and stress-regulated genes, promotion of cell elongation, normal leaf and chloroplast senescence, and flowering. Alternative names: BRI1, BRASSINOSTEROID INSENSITIVE 1, CBB2, CABBAGE 2, DWARF 2, BIN1, BR INSENSITIVE 1, ATBRI1, Brassinosteroid LRR receptor kinase

**Immunogen:** KLH-conjugated synthetic peptide derived from Arabidopsis thaliana BRI1 protein, Uniprot: O22476, TAIR: AT4G39400

**Host:** Rabbit

**Clonality:** Polyclonal

**Purity:** Affinity purified serum in PBS, pH 7.4

**Format:** Lyophilized in PBS pH 7.4.

**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 tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.

**Tested applications:** Immunoprecipitation (IP), Western blot (WB)

**Related products:**
- AS12 1858 | Anti-BAK1 | Brassinosteroid insensitive 1-associated receptor kinase 1, rabbit antibodies
- AS16 3203 | Anti-BIN2 | brassinosteroid insensitive 2, rabbit antibodies
- AS16 3204 | Anti-SOBIR1 | supressor of BIR1, rabbit antibodies
- Plant protein extraction buffer
- Secondary antibodies

**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 rapa

**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...
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