

This product is for research use only (not for diagnostic or therapeutic use)

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Product no AS09 615 Anti-BiP | Lumenal-binding protein (goat antibody)

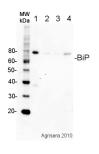
Product information

| Immunogen | KLH-conjugated synthetic peptide derived from Arabidopsis thaliana BiP proteins: BiP1 UniProt:Q9LKR3, TAIR: At5g28540, BiP2 UniProt: F4K007, TAIR: At5g42020, BiP3 UniProt:Q8H1B3, TAIR:At1g09080 |
|----------------|---|
| Host | Goat |
| Clonality | Polyclonal |
| Purity | Immunogen affinity purified serum in PBS pH 7.4. |
| Format | Lyophilized |
| Quantity | 100 μg |
| Reconstitution | For reconstitution add 100 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 : 2000 (WB) |
|---------------------------|---|
| Expected apparent MW | 73.5 80 kDa |
| Confirmed reactivity | Arabidopsis thaliana, Hordeum vulgare, Spinacia oleracea, Zea mays |
| Predicted reactivity | Hordeum vulgare, Nicotiana tabacum, Oryza sativa, Picea sitchensis, Populus trichocarpa, Physcomitrium patens, Spinacia oleracea, Zea mays Species of your interest not listed? <u>Contact us</u> |
| | Species of your interest not listed? Contact us |
| Not reactive in | No confirmed exceptions from predicted reactivity are currently known |
| Additional information | Protein or membrane sample should be treated at 70°C for 10 min before loading on the gel. |
| | Antibody has a reduced reactivity to monocots in western blot. |
| Selected references | Narusaka et al (2016). Leucine zipper motif in RRS1 is crucial for the regulation of Arabidopsis dual resistance protein complex RPS4/RRS1. Sci Rep. 2016 Jan 11;6:18702. doi: 10.1038/srep18702. |

Application example

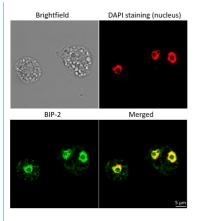


5 µg of total protein from *A.thaliana* (1), *H. vulgare* (2), *Z.mays* (3), S. oleracea (4), extracted with Agrisera PEB extraction buffer (AS08 300) were separated on 4-12% SDS-PAGE and blotted 1h to PVDF. Blots were blocked immediately following transfer in for 1h at room temperature with agitation. Blots were incubated in the primary antibody at a dilution of 1: 10 000 for 1h at room temperature 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 room temperature with agitation. Blots were incubated in secondary antibody (anti-goat IgG horse radish peroxidase conjugated, from Agrisera <u>AS09 605</u>) diluted to 1:50 000 for 1h at room temperature with agitation. The blots were washed as above and developed for 5 min with ECL detection reagent according to the manufacturers instructions. Exposure time was 5 seconds.

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Immunofluorescent localization of BiP in suspension cultures of *Arabidopsis thaliana* (Landsberg erecta cv. MM1) using goat anti-BiP polyclonal antibodies (AS09 615) and donkey anti-goat IgG DyLight®488 conjugated secondary antibodies (<u>AS10 1116</u>, Agrisera).

Material: suspension cultures of Arabidopsis thaliana ecotype Landsberg erecta cv. MM1) Fixation: Packed cell volume to fixer ratio: 250 µl : 5ml Fixer composition and buffer: 4% (w/v) paraformaldehyde (freshly prepared as 8% stock and 0.2 µm filtered) 0.01% (v/v) Triton-X100 in Phosphate Buffered Saline (PBS), pH 7.4 (2x stock, 0.2 µm filtered) Container and method: in 6 cm Petri dish, gentle shaking at room temperature (RT) Duration: 25 min. Hydrophilization: No Cell wall digestion: Yes Packed cell volume to enzyme ratio: 100 ul : 2ml Enzyme composition: 1% Cellulase (chromatically purified, powder, Worthington) 1% Pectinase (protease free, liquid, Sigma) Buffer: 0.5% (w/v) MES buffer, pH 5.6 Container and method: in 2 ml microfuge tube by rolling at room temperature (RT) Duration: 30 min. Membrane permeabilization: Triton-X100 (0.5%), 10 min/RT Antigen retrieval: No Blocking buffer: Fish gelatin (5% v/v) Washing buffer: PBS Primary antibody dilution and incubation time: 1:400, ON/4ºC Secondary antibody: donkey anti-goat IgG DyLight®488 conjugated secondary antibodies (AS10 1116, Agrisera), 1: 600, 1h/RT Co-staining of the nucleus (DAPI): Yes Cell wall and nucleus staining: 100 ng/ml DAPI

Courtesy of Dr. Ferhan Ayaydin, Hungarian Centre of Excellence for Molecular Medicine (HCEMM), Szeged, Hungary