product information

**Background**  

**Immunogen**  
KLH-conjugated peptide, chosen from Arabidopsis thaliana Bak1 sequence, TAIR: AT4G33430 UniProt: Q94F62

**Host**  
Rabbit

**Clonality**  
Polyclonal

**Purity**  
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 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 1859 | Anti-BRI1 | BRASSINOSTEROID INSENSITIVE 1, rabbit antibodies  
AS12 1857 | Anti-FLS2 | FLAGELLIN-SENSITIVE 2, 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**  
Antibodies bind to AtBAK1-Myc. They do not cross react with AtSOBIR.

**Application information**  
**Recommended dilution**  
2 µl/50 µl of Protein G agarose, 1 : 5000 (WB)

**Expected | apparent MW**  
68 | 70 kDa

**Confirmed reactivity**  
Arabidopsis thaliana, Solanum lycopersicum

**Predicted reactivity**  
Theungiella halophila

**Not reactive in**  
Hordeum vulgare, Nicotiana benthamiana, Oryza sativa

**Additional information**  
Extra Information on CE extraction buffer: CE buffer does not need to be made freshly everytime. Aliquots can be kept at -20°C. Na2MoO4 and NaF are phosphatase inhibitors, included to prevent lose phosphorylation form our protein of interest during extraction. EDTA chelates metal ions and thus inhibits many enzymes which need metal ions as co-factors and inhibits the action of proteases. Protease inhibitor coctail is Sigma product number P 9599 which is used in dilution 1 : 100.

Antibody is reconizing AtBAK1-Myc in total extracts.

**Selected references**
Hu et al. (2018). A group of receptor kinases are essential for CLAVATA signalling to maintain stem cell homeostasis. Nat Plants. 2018 Apr;4(4):205-211. doi: 10.1038/s41477-018-0123-z. (CoIP)

Application information

Plant material: Arabidopsis thaliana Col-0 (wild type) bak1-4 (knock-out mutant for BAK1, does not show any full-length BAK1 transcript, Kemmerling et al., 2007). Method: Two-week-old seedlings and leaf material of 5-week-old plants (grown under short day conditions, 8h light) were collected and frozen in liquid nitrogen. Approximately 100 mg of plant material were extracted in 0.2 ml of homogenization buffer (250 mM sucrose, 50 mM HEPES-KOH pH 7.5, 0.5% Triton X-100, 5% glycerol, 50 mM Na4P2O7, 1 mM Na2MoO4, 25 mM NaF, 2 mM DTT, Sigma plant protease inhibitor cocktail) with a glass piston and a small amount of sand in an 1.5ml Eppendorf tube. Another 0.8 ml of buffer were added and the extract was mixed thoroughly. Debris was pelleted by centrifugation in a table-top microcentrifuge at 13 000 rpm. The supernatant was mixed with 4x SDS loading buffer (200 mM TRIS-HCl pH 6.8, 400 mM DTT, 8% SDS, 40% glycerol, 0.1% bromophenol blue) and boiled at 95°C for 5min. 10µl were run on an 10% polyacrylamide gel and blotted onto a 0.45µm PVDF membrane (Carl Roth). The membrane was blocked for 1h in TBS-T (150mM NaCl, 10mM Tris-HCl pH8, 0.05% Tween-20) containing 5% skimmed milk powder. The primary antibody (Agrisera Rabbit anti-BAK1, AS12 1858, 1µg/µl) was diluted 1:5000 in TBS-T containing 5% milk powder and incubated on the membrane overnight at 4°C. Then the membrane was washed 5 times 15min with TBS-T containing 5% milk powder. The secondary antibody (Agrisera Goat anti-rabbit IgG (H&L) HRP conjugate, AS09 602, 0.91 µg/µl) was diluted 1:5000 in the same solution and incubated on the membrane at room temperature for 2h. The membrane was then washed 5 times 15min with TBS-T (no milk powder) and the blot was developed using chemiluminescent detection reagent. Short exposure: 1 minute. Long exposure: 10 minutes.

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