Agrisera
Part of Thermo Fisher Scientific

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

**contact: support@agrisera.com**

Agrisera AB | Box 57 | SE-91121 Vännäs | Sweden | +46 (0)935 33 000 | www.agrisera.com

**Product no AS15 2905**

# Anti-MKK2 | Mitogen-activated protein kinase kinase 2

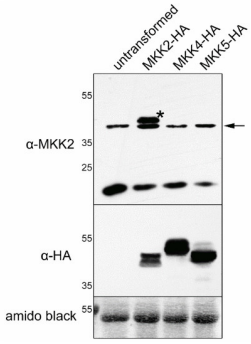
## Product information

| | |
|---|---|
| **Immunogen** | KLH-conjugated synthetic peptide derived from *Arabidopsis thaliana* sequence of MKK2, UniProt: Q9S7U9, TAIR: AT4G29810 |
| **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. |

## Application information

| | |
|---|---|
| **Recommended dilution** | 1 : 1000 (WB) |
| **Expected \| apparent MW** | 38.8 kDa |
| **Confirmed reactivity** | *Arabidopsis thaliana* (protoplasts) |
| **Not reactive in** | |
| **Additional information** | Reactivity on other tissues of *Arabidopsis thaliana* has not been confirmed as yet. |

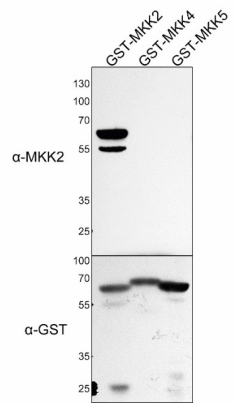
**Application example**




*Arabidopsis thaliana* mesophyll protoplasts (Col-0) were isolated and transformed according to Yoo et al. (2007). 300 µl of protoplasts (2*105 protoplasts/ml) expressing MKK2/MKK4/MKK5-HA (or untransformed protoplasts as control) were pelleted by centrifugation. The resulting pellets were resuspended in standard Laemmli sample buffer and then the samples were denatured at 95°C for 5 min. Samples were separated on 10% SDS-PAGE and blotted 1 h onto NCL membrane using semi-dry transfer. The blot was blocked with 5% skimmed milk in TBS-T for 1h at room temperature (RT) with agitation. The blot was then incubated with the primary antibody (anti-MKK2) at a dilution of 1:1000 for 1 h at RT with agitation in 3% skimmed milk in TBS-T. The antibody solution was decanted and the blot was washed 4 times for 10 min with TBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, Agrisera) diluted to 1:15000 in 3% skimmed milk in TBS-T for 1h at RT with agitation. The blot was washed as above and incubated for 5 min with highest sensitivity detection reagent. Arrow indicates endogenous MKK2, star indicates MKK2-HA. Exposure time was 2 minutes. As a control, the blot was re-probed with anti-HA after stripping.

This product is **for research use only** (not for diagnostic or therapeutic use) **contact: support@agrisera.com**

Agrisera AB | Box 57 | SE-91121 Vännäs | Sweden | +46 (0)935 33 000 | www.agrisera.com




Courtesy Dr. Lennart Eschen-Lippold, Leibniz Institute of Plant Biochemistry, Germany

Optical densities (OD600) of IPTG-induced *E. coli* expressing GST-MKK2/MKK4/MKK5 were determined and sample volumes based on the equation 1/OD600 were centrifuged to pellet bacteria. The resulting pellets were resuspended in 7 M urea, mixed with standard Laemmli sample buffer and then the samples were denatured at 95°C for 5 min. Samples were separated on 10% SDS-PAGE and blotted 1 h onto NCL membrane using semi-dry transfer. The blot was blocked with 5% skimmed milk in TBS-T for 1h at room temperature (RT) with agitation. The blot was then incubated with the primary antibody (anti-MKK2) at a dilution of 1:1000 for 1 h at RT with agitation in 3% skimmed milk in TBS-T. The antibody solution was decanted and the blot was washed 4 times for 10 min with TBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, Agrisera) diluted to 1:15000 in 3% skimmed milk in TBS-T for 1h at RT with agitation. The blot was washed as above and incubated for 5 min with highest sensitivity detection reagent. Exposure time was 2 minutes. As a control, the blot was re-probed with anti-GST after stripping.

Courtesy Dr. Lennart Eschen-Lippold, Leibniz Institute of Plant Biochemistry, Germany