Agrisera
Part of Olink® Group

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 AS13 2635**

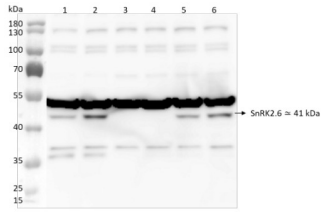
# SRK2E | Ser/Thr-protein kinase SnRK2,6

## Product information

| | |
|---|---|
| **Immunogen** | KLH-conjugated inique synthetic peptide derived from *Arabidopsis thaliana* SRK2E sequence UniProt: Q940H6, TAIR: AT4G33950 |
| **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

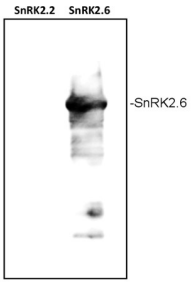
| | |
|---|---|
| **Recommended dilution** | 5 µg (IP), 1 : 1000 (WB) 10 µg (pull-down assay) |
| **Expected \| apparent MW** | 41 kDa |
| **Confirmed reactivity** | *Arabidopsis thaliana* |
| **Not reactive in** | No confirmed exceptions from predicted reactivity are currently known |
| **Selected references** | Wang et al. (2017). Reciprocal Regulation of the TOR Kinase and ABA Receptor Balances Plant Growth and Stress Response. Mol Cell. 2017 Dec 27. pii: S1097-2765(17)30930-9. doi: 10.1016/j.molcel.2017.12.002. |




Samples:
1 - 50 µg of *Arabidopsis thaliana* Col0 mock-treated (MG132 50 µM, 6 hours)
2 - 50 µg of *Arabidopsis thaliana* Col0 ABA-treated (MG132 50 µM + ABA 50 µM, 6 hours)
3 - 50 µg of *Arabidopsis thaliana* ost1(snrk2.6) mock-treated (MG132 50 µM, 6 hours)
4 - 50 µg of *Arabidopsis thaliana* ost1(snrk2.6) ABA-treated (MG132 50 µM + ABA 50µM 6, hours)
5 - 50 µg of *Arabidopsis thaliana* abi1-2 mock-treated (MG132 50 µM, 6 hours)
6 - 50 µg of *Arabidopsis thaliana* abi1-2 ABA-treated (MG132 50 µM + ABA 50 µM, 6 hours)
The ost1-3 (SALK_008068) and the abi1-2 (SALK_72009) mutants were used as controls.

50 µg/well of total protein extracted freshly from *Arabidopsis thaliana* roots with extraction buffer containing: 150 mM NaCl, 50 mM Tris-HCL pH 8, 1% Triton X-100, anti-proteases cocktail (Complete mini EDTA free, "ROCHE") (1 tablet for 10ml), 3 mM DTT, 50 mM MG132, or 50 mM ABA and denatured with exact buffer components at 95 °C/5 min. Samples were separated on 10% SDS-PAGE and blotted overnight (ON) to PVDF (Inmobilon®-FL) (pore size of 0.45 µm), using: wet transfer. Blot was blocked with 3% milk for: 6h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 10 000 in TBS-T 1X for ON/4°C with agitation. The antibody solution was decanted, and the blot was rinsed briefly twice, then washed 3 times for 5 min in TBS-T at RT with agitation. Blot was incubated in matching secondary antibody (Goat anti-rabbit IgG HRP conjugated, AS09 602, Agrisera) diluted to 1: 10000 in for 1h/RT with agitation. The blot was washed as above and developed with a following chemiluminescent detection reagent: AgriseraECL SuperBright (AS16 ECL-S-10, Agrisera). Exposure time was 30 seconds.

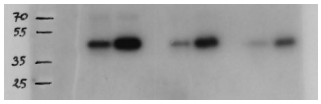
Courtesy of Drs. Javier Ocaña, Alberto Coego and Pedro L. Rodriguez, CSIC, Spain




Bacterial lysates were separated on 12% SDS-PAGE and blotted 1h to PVDF using semi-dry or tank transfer. Blots were blocked with 5 % milk for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 3 000 for 1h at RT 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 PBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, AS09 602 from Agrisera) diluted to 1:50 000 in for 30 min. at RT with agitation. The blot was washed as above and developed for 3 min with ECL according to the manufacturer's instructions. Exposure time was 30 seconds.

Courtesy of Dr. Agnieszka Ludwików, UAM, Poznań, Poland

---




Protein A agarose beads (40µl) where coated with 10µl (1µg/ul) antibodies and after incubation with amount of extract (10 mg/ml) indicated washed extensively and loaded on gel. In gel kinase assay was performed as described in Fujii, 2007.
Autoradiograph shows immunoprecipitated kinase from plant extracts. 1 beads with BSA 20 µl loaded on gel 2 beads with plant extract (WT) 20 µl loaded on gel 3 beeds with plant extract (mutant X) 20 µl loaded on gel 4 beads with BSA 10 µl loaded on gel 5 beads with plant extract (WT) 10 µl loaded on gel 6 beeds with plant extract (mutant X) 10 µl loaded on gel 7 beads with BSA 5 µl loaded on gel 8 beads with plant extract (WT) 5 µl loaded on gel 9 beeds with plant extract (mutant X) 5 µl loaded on gel

Courtesy of Dr. Szymon Świeżewski, Institute of Biochemistry and Biophysics, Polish Academy of Science, Warsaw, Poland