Agrisera
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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 AS06 128A

LOX | Lipoxygenase (Affinity purified)

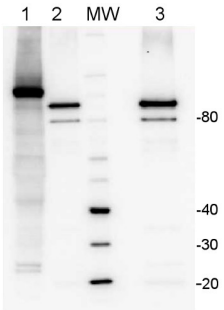
Product information

Immunogen	native lipoxygenase, type I-B, purified from *Glycine max* (Sigma, product number L7395) UniProt: P08170
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	50 µg
Reconstitution	For reconstitution of lyophilized unit please 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 : 5000 (WB)
Expected | apparent MW	54 (subunit), 108 (native enzyme)
Confirmed reactivity	*Arabidopsis thaliana, Glycine max, Solanum tuberosum*
Predicted reactivity	*Cucumis sativus, Lathyrus undulatus, Malus x domestica* Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known

Application example



10 µg of total protein from *Arabidopsis thaliana* **(1)**, native LOX protein from *Glycine max* **(2,3)** total protein from all the samples were extracted with Protein Extraction Buffer PEB (AS08 300). Samples were diluted with 1X sample buffer (NuPAGE LDS sample buffer (Invitrogen) supplemented with 50 mM DTT and heat at 70°C for 5 min and keep on ice before loading. Protein samples were separated on NuPAGE 4-12% Tris-Bis gel (Invitrogen) LDS-PAGE and blotted for 1h to 1.5h on PVDF using tank transfer. Blots were blocked immediately following transfer in 2% blocking reagent or 5% non-fat milk dissolved in 20 mM Tris, 137 mM sodium chloride pH 7.6 with 0.1% (v/v) Tween-20 (TBS-T) for 1h at room temperature with agitation. Blots were incubated in the primary antibody at a dilution of 1: 5 000 (in blocking reagent) for 1h at room temperature with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, and then washed 1x15 min and 3x5 min with TBS-T at room temperature with agitation. Blots were incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, recommended secondary antibody AS09 602) diluted to 1:25 000 in blocking reagent for 1h at room temperature with agitation. The blots were washed as above. The blot was developed for 5 min with chemiluminescent detection reagent according the manufacturers instructions. Images of the blots were obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad).

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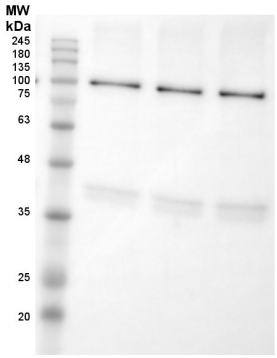

Proteins were extracted from tuber flesh of *Solanum tuberosum* cv. Russet Burbank with 0.1 M Tris HCl (pH=8.0), 5% sucrose (m/v), 2% (m/v) SDS, protease inhibitors (PMSF 1mM). Samples were heated 95°C 5 min, and 10 µg of total protein was resolved in 12% SDS PAGE and blotted to PVDF membrane for 1h-1.5h using tank transfer. Blots were blocked with a skimmed milk 4% (m/v) in T-TBS (1.5h) at RT with agitation. Primary antibodies (AS06 128A) were applied overnight +4°C in dilution 1:5000 with agitation. After washing with T-TBS 2 times, membrane was incubated with secondary antibodies (Goat Anti-Rabbit HRP conjugated) 1:10 000 for 1 hour at RT. Blot was washed as above and developed with chemiluminescence detection reagent, for 5 – 10 minutes. Exposure time – 7.000 seconds.

Courtesy of Msc. Iauhenia Isayenka, Laboratory of Nathalie Beaudoin, University of Sherbrooke, Canada