product AS10 700
RA | Rubisco activase

product information

Background  
RA - Ribulose bisphosphate carboxylase/oxygenase activase is an enzyme localized to chloroplasts which activates Rubisco by promoting ATP-dependent conformational changes. Alternative name: RuBisCo activase RCA.

Immunogen  
Purified, recombinant Rubisco activase from Gossypium hirsutum Q9AXG1

Host  
Rabbit

Clonality  
Polyclonal

Purity  
Serum

Format  
Lyophilized

Quantity  
50 µl

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  
Western blot (WB)

Related products  
Antibodies to Rubisco/Carbon metabolism
Plant and algal protein extraction buffer
Secondary antibodies

Additional information  
This product can be sold containing ProClin if requested

Application information

Recommended dilution  
1 : 5000-1 : 10 000 (WB)

Expected | apparent MW
Arabidopsis thaliana, Camelina sativa, Chlamydomonas reinhardtii, Hordeum spontaneum, Festuca pratensis, Glycine max, Gossypium hirsutum, Gossypium barbadense, Lollum perenne, Nannochloropsis oceanica, Nicotiana tabacum, Oryza sativa, Populus balsamifera, Rheoe discolor, Solanum lycopersicum, Zea mays, Thellungiella salsuginea, red sulfur bacterium Thiodictyon sp. Cad16 (isolated from Lake Cadagno)

Confirmed reactivity
47 and 42 kDa (maize, tobacco, Chlamydomonas)

Predicted reactivity
Glycine max, Gossypium mexicanum, Hordeum vulgare, Medicago sativa, Olea europaea, Picea sitchensis, Physcomitrella patens, Ricinus communis, Spinacia oleracea, Triticum aestivum, Zea mays, Vitis vinifera

Additional information  
There are two forms of activase (alpha and beta) in some species (for example Arabidopsis, camelina, spinach, rice) and only one form in other species (tobacco, maize, Chlamydomonas). Alpha is about 46-47 Kda, beta is about 42 kDa. Species that have only one form have the beta form.

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Selected references


Application example

3, 5 and 11 µg of total soluble protein from Arabidopsis thaliana (1), Oryza sativa (2) and Camelina sativa (3) extracted with 50 mM Tricine-NaOH, pH 8, 10 mM EDTA, 1% PVP-40, 20 mM -mercaptoethanol, 1 mM PMSF and 10 µM leupeptin were separated on 12% SDS-PAGE and blotted 1h to PVDF. Blots were blocked with 4% non-fat milk in TBS for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 10 000 over night with agitation. The antibody solution was decanted and the blot was rinsed briefly with H2O, then washed six times for 15 min in TBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG alkaline phosphatase conjugated) diluted to 1:3000 in 0.5% non-fat milk in TBS for 2h at RT with agitation. The blot was washed with four changes of TBS-T and developed for 5 min with NBT/BCIP according to the manufacturer’s instructions (Promega). There are two forms of activase (alpha and beta). Alpha is about 46-47 Kda, beta is about 42 kDa what is shown on the blot above.

5 µg of total protein from samples such as Arabidopsis thaliana leaf (1), Hordeum vulgare leaf (2), Zea mays leaf (3), Nicotiana tabacum (4), Chlamydomonas reinhardtii total cell (5), 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 kept on ice before loading. Protein samples were separated on 4-12% Bolt Plus gels, LDS-PAGE and blotted for 70 minutes to 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: 10 000 (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 AS10 1489, Agrisera) 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 (VersaDoc MP 4000) and Quantity One software (Bio-Rad). Exposure time was 30 seconds.