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Product no AS22 4825

Anti-RbcS | Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (Algal)

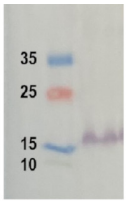
Product information

Immunogen	KLH-conjugated peptide derived from *Chlamydomonas reinhardti*i RbcS, UniProt: P00873, P08475, Q31NB2
Host	Rabbit
Clonality	Polyclonal
Purity	Antigen affinity purified serum, in PBS pH 7.4
Format	Lyophilized
Quantity	50 µg
Reconstitution	For reconstitution, add 50 µl, of sterile or deionized 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.

Application information

Recommended dilution	1 : 1000 (WB)
Expected | apparent MW	20 | 15 kDa
Confirmed reactivity	*Arabidopsis thaliana, Chlamydomonas reinhardtii, Synechococcus* sp. PCC7002
Predicted reactivity	*Cucurbita maxima, Cucurbita pepto, Hordeum vulgare, Pisum sativum, Zea mays* Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Selected references	To be added when available, antibody available in October 2023.

5 µg/well of total protein extracted freshly from *Arabidopsis thaliana* and *Zea mays* denatured with 4XLDS at 70°C for 5 min. Protein were separated on NuPAGE Bis-Tris SDS gel and blotted 1h to Invitrogen PVDF (pore size of 0.2 um), using wet transfer. Blot was blocked with 5% milk 4°C/ON with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1 000 for 1h/RT with agitation in TBS-T Blocking. The antibody solution was decanted, and the blot was rinsed briefly, then washed 3 times for 5 min in TBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (AS09 607) diluted to 1:2500 in TBS-T Blocking for 30min/RT with agitation. The blot was washed as above and developed for 2 min with Agrisera BCIP/NBT plus.



5 µg of total protein from *C.reinhardtii* was denatured with Invitrogen LDS sample buffer (4X) at 70°C/5 min. Samples were separated on Invitrogen NuPage Bis-Tris 4-12% SDS-PAGE 60 min and blotted for 1 h to Invitrogen PVDF (pore size of 0.45 µm), using wet transfer. Blot was blocked with 5% milk in TBS-T for 1h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1000 for 1h/RT with TBS-T Blocking. The antibody solution was decanted, and the blot was rinsed briefly twice, then washed once for 10 min and 2 times for 5 min in TBS-T at RT with agitation. Blot was incubated in matching secondary antibody (anti-rabbit IgG ALP conjugated, AS09 607 lot 2302) diluted to 1: 5 000 in TBS-T Blocking for 0.5h/RT with agitation. The blot was washed as above and developed with AS19 BCIP-NBT for 30 seconds. Image was captured after 1h.

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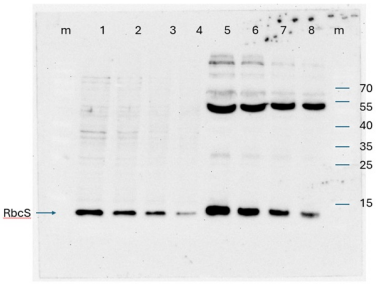

Samples:
1-4 *Arabidopsis thaliana* leaf tissue
5-8 *Arabidopsis thaliana* soluble extract from leaf tissue

40 mg of fresh *Arabidopsis thaliana* leaf tissue was used for extraction of the protein. To 40 mg of the tissue was resuspended in 400 µl of extraction buffer. Extraction buffer consists of following components: 100mM EDTA pH8.0, 120mM Tris-HCl pH 6.8, 4%w/v SDS, 10% v/v ß-Mercaptoethanol, 5% v/v glycerol, 0,005% w/v Bromphenol blue. Leaf tissue was ground in mortar and pestle in the Protein extraction Buffer. Boiled for 5 minutes at 95°C, span 5 min at top spin of table-top centrifuge (21.000g) supernatant used for PAGE. From the extract prepared from wt Col-0 a dilution series was prepared and loaded onto the gel. 5 µl, 2,5µl, 1,25µl and 0,62µl were used for the Antibody test. Next to the total protein extract soluble fraction of the cells extracted under native conditions in following buffer were used: 450 mM solbitol, 20mM Tricine-KOH pH8.4, 10mMEDTA pH8.0, and 0,1%BSA. From that extract concentration 3µg/µl a dilution series was loaded as following: 5µl, 2,5µl, 1,25µl, and 0,62µl. The lowest concentration used for detection range is 1,86µg of soluble protein. Samples were separated at RT PAGE on 12% SDS-PAGE and blotted with semidry blotting machine (BioRad) for 30 min to nitrocellulose membrane (Merck). Blot was blocked in 4%Milk for 1h at RT with agitation. Blot was incubated ON in cold in the primary antibody diluted 1:2000 with agitation in 2%Milk TBS-T solution. The antibody solution was decanted, and the blot was rinsed 4 times a 5 to 10 minutes in 1xTBS-T at RT with agitation. Blot was incubated in matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated antibody) which was diluted 1:10.000. The incubation was done for 1h at RT with agitation. The blot was washed as above and developed with self-made developing solution. Exposure time was 3 minutes.