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product AS07 242

GOGAT | Glutamine oxoglutarate aminotransferase

product information

Background	**Glutamine oxoglutarate aminotransferase** (abbreviated as GOGAT) is an enzyme involved in synthesis of glutamate from glutamine and alpha-ketoglutarate. GOGAT has two forms in plants: ferredoxin-dependent GOGAT (Fd-GOGAT) and NADH-dependent GOGAT (NADH-GOGAT). 95% of GOGAT found in plants is the Fd-GOGAT type. Fd-GOGAT is encoded by two genes, glu1 and glu2 found on chromosomes 5 and 2 respectively (in Arabidopsis). Fd-GOGAT (both forms) is highly conserved among plants, red algae, and cyanobacteria.
Immunogen	KLH-conjugated synthetic peptide well conserved in known GOGAT sequences from different species including *Arabidopsis thaliana* Fd-GOGAT 1 Q9ZNZ7, At5g04140 and Fd-GOGAT 2 Q9T0P4, At2g41220
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	Collection of antibodies to proteins involved in nitrogen metabolism Plant and algal protein extraction buffer Secondary antibodies
Additional information	This antibody can be used as a plastidial marker.

Application information

Recommended dilution	1 : 1000 (WB)
Expected | apparent MW	177 | 170-180 kDa depending upon the species
Confirmed reactivity	*Arabidopsis thaliana, Miscanthus giganteus, Nicotiana tabacum, Oryza sativa, Phaseolus vulgaris, Populus tremula, Solanum lycopersicum, Spartina sp., Zea mays*
Predicted reactivity	*Arthrospira platensis* PCC 7345, *Beta vulgaris, Brachypodium distachyon, Burkholderia glumae, Camellia sinensis, Chloroflexi bacterium, Chlamydonas reinhardtii, Gracilaria tenustipitata, Cyanobacteria, Crocosphaera watsonii WH 8502, Cyanidioschyzon merolae (*strain 10D*), Galdieria sulphuraria , Glycine max, Helicosporidium sp.* ATCC 50920, *Hydrogenobaculum sp. HO, Lactococcus lactis subsp. lactis bv. diacetylactis str.* TIFN2, *Leptospira interrogans serovar Bataviae str.* HAI135, *Leptolyngbya boryana, Medicago trucutula, Microcystis panniformis, Monoraphidium neglectum, Morus notabilis , Nicotiana tabacum, Ostreococcus lucimarinus,Physcomitrella patens subsp. patens, Porphyra purpurea, Sutterella wadsworthensis, Sulfurihydrogenibium yellowstonense, Veillonella atypica*
Not reactive in	No confirmed exceptions from predicted reactivity are currently known.
Additional information	A 40 kDa band present in *A. thaliana* sample is not competed away during antibody neutralization assay. In this assay free peptide used for antibody production is incubated together with anti-GOGAT antibodies. Due to the MW of this protein we suggest to use a gradient gel for protein separation and a longer transfer time.
Selected references	

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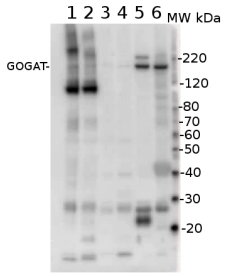
Application example



20 µg of total protein from (**1**) *Spartina patens* total cell extracted with **P**rotein **E**xtration **B**uffer, PEB (**AS08 300**), (**2**) *Spartina alterniflora* total cell, extracted with PEB, (**3**) Miscanthus giganteus total cell extracted with PEB, (**4**) Zea mays total cell extracted with PEB, (**5**) Phaseolus vulgaris total cell extracted with PEB, (**6**) *Arabidopsis thaliana* total cell extracted with PEB, were separated on **4-12%** NuPage (Invitrogen) **LDS-PAGE** and blotted 1h to **PVDF**. Blots were blocked immediately following transfer in 2% blocking reagent 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 for 1h at room temperature 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 TBS-T at room temperature with agitation. Blots were incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1:50 000 in 2% blocking solution for 1h at room temperature with agitation. The blots were washed as above and 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).

Approximately 40 µg of total protein from *Arabidopsis thaliana* wild-type (WT) and gls1-103 mutant (M) leaves, the latter of which showed reduced GOGAT levels, was extracted with a protein extraction solution containing 50 mM Imidazole/HCl (pH 7.0 at 4ºC), 20% glycerol, 5mM 6-aminocaproic acid, 1mM EDTA. Protein extracts were then separated on 6% SDS-PAGE and blotted overnight at 4ºC to PVDF using the tank transfer method. Blots were blocked with PBS-T containing 5% skim milk for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 20 000 in PBS-T containing 1% (w/v) BSA 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) diluted to 1:20 000 in PBS-T containing 1% (w/v) BSA for 1h at RT with agitation. The blot was washed as above and developed for 1 min with chemiluminescent detection reagent, according to the manufacturer's

instructions. Exposure time was 5 min.

Courtesy Dr. Atsushi Takabayashi, Institute of Low Temperature Science, Hokkaido University, Japan