

Product: Anti-GOGAT (glutamate synthase)

Product no: AS07 242

Antibodies for plant sciences

Product Information

Antibody clonality: Polyclonal

Raised in: Rabbit

Purity: Serum

Quantity: 200 µl

Antibody form: Lyophilized. For reconstitution please add 200 µl of sterile water. Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from liquid or lyophilized material adhering to the cap or sides of the tubes.

Storage instructions: -20°C or -80°C long Term storage (years). Please, avoid freezing and thawing of antibodies. Make aliquots instead.

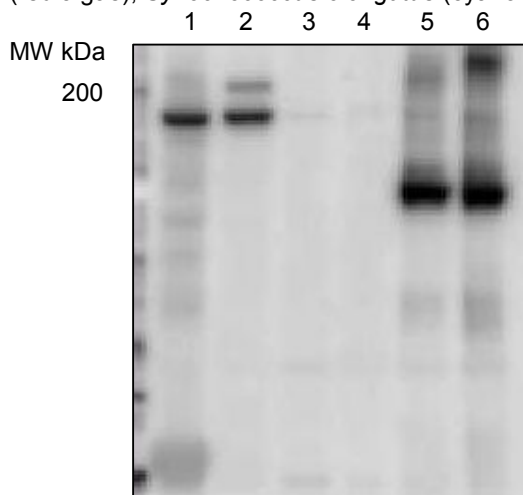
Application information:

Western Blot: 1: 1 000 with regular ECL

MW: 170-180 kDa depending upon the species.

Important note: Due to the MW of this protein we suggest to use a gradient gel for protein separation and a longer transfer time.

Reactivity: Predicted based on a sequence conservation: *Medicago truncatula* (legume), *Spinacea oleracea* (spinach), *Oryza sativa* (rice), *Zea mays* (corn), *Glycine max* (soybean), *Ostreococcus lucimarinus* (prasinophyte), *Leptolyngbya boryana* (cyanobacteria), *Porphyra purpurea* (red algae), *Gracilaria tenuistipitata* (red algae), *Synechococcus elongatus* (cyanobacteria).



Background

Glutamine oxoglutarate aminotransferase (abbreviated as GOGAT) is an enzyme involved in synthesis of glutamate from glutamine and α -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*). The protein products of *glu1* and *glu2* in *Arabidopsis thaliana* are highly conserved in terms of sequence. Additionally, Fd-GOGAT (both forms) is highly conserved among plants, red algae, and cyanobacteria.

Immunogen: Synthetic peptide well conserved in known Fd-GOGAT sequences from different species. Conjugated to KLH.

From left to right: *Arabidopsis thaliana* (1), *Phaseolus vulgaris* (2), *Zea mays* (3), *Spartina alterniflora* (4), *Spartina patens* (6).

(20 µg of total cellular protein was loaded per lane)

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.

Detailed experimental conditions are described on page 2

Antibodies are intended for the research use only not for diagnostic or therapeutic use.

Product support: inquiry@agrisera.com, <http://www.agrisera.com>

Experimental conditions:

Sample preparation: Leaf tissue was weighed and snap frozen in liquid nitrogen and stored at -80°C until processing. Frozen leaves were placed in a pre-chilled mortar and ground in liquid nitrogen with a pestle until a fine powder was obtained. Algal cultures were centrifuged to form a pellet and frozen at -80°C.

A single extraction buffer was used for disruption and solubilization of all species. Samples were suspended in Agrisera Protein Extraction Buffer (PEB) AS08 300 with added 0.1mg/mL PefaBloc SC (AEBSF) protease inhibitor (Roche). Leaf tissue was solubilized at 0.1 to 1.0 mg tissue per µL extraction buffer.

Samples suspended in extraction buffer were immediately refrozen in liquid nitrogen and then sonicated with a microtip attachment at a setting of 30%, until just thawed. To avoid heating, samples were then refrozen immediately in liquid nitrogen.

Following disruption, samples were centrifuged for 3 min at 10 000 x g to remove insoluble material and unbroken cells. Check for color in the pellet, as this is the best indicator of incomplete breakage. The protein content was assayed using the Bio-Rad DC Protein Assay using bovine gamma-globulin in extraction buffer as a standard.

Samples in lithium dodecyl sulphate extraction buffer were brought to 50 mM dithiothreitol (DTT) final concentration and the volume was adjusted with 1X sample buffer. Cellular extracts were then heated at 70°C for 5 min. Following heating, samples were pulsed briefly in a microfuge to collect all of the material at the bottom of the tube.

Gel electrophoresis: Proteins were separated by electrophoresis on 4-12% acrylamide gradient mini-gels (NuPAGE Bis-Tris gels, Invitrogen) in MES SDS running buffer (Invitrogen) in an XCell Sure-Lock Tank (Invitrogen). Gels were electrophoresed at 200V for 35 minutes. Following electrophoresis, the proteins were transferred to polyvinylidene difluoride (PVDF) membranes pre-wetted in methanol and equilibrated in 1X transfer buffer (Invitrogen) using the XCell blot module (Invitrogen) for 80 minutes at 30V.

Western Blot development: Blots were blocked immediately following transfer in 2% ECL Advance blocking reagent (GE Healthcare) 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 or overnight at 4°C. Primary and secondary antibodies were used at a dilution of 1:10 000 to 1:100 000 in 2% ECL Advance Blocking solution. Blots were incubated in the primary antibody solution 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 (goat anti-rabbit horse radish peroxidase conjugated, from Abcam) diluted to 1:50 000 in 2% ECL Advance blocking solution for 1h at room temperature with agitation. The blots were washed as above and developed for 5 min with ECL Advance 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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