Agrisera

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product AS08 295

GLN1 GLN2 | GS1 GS2 glutamine synthetase global antibody

product information

Background	**Glutamine synthetase** (GLN or GS) is one of the key enzymes involved in nitrogen metabolism of plants. It catalyses the synthesis of glutamine from glutamate and ammonia in an ATP-dependent reaction. There are two general classes of glutamine synthetase in plants: GLN1, a cytosolic form and GLN2, a chloroplastic form. GLN1 is highly abundant in the vascular elements of roots nodules, flowers and fruits, functioning in the assimilation of ammonium and the biosynthesis of glutamine for nitrogen transport. GLN2 is encoded by a single gene and is highly abundant in leaf mesophyll chloroplasts. Here GLN functions in the assimilation of ammonia produced from photorespiration and the reduction of nitrate in the chloroplasts
Immunogen	KLH-conjugated synthetic peptide derived from a wide range of available sequences including all isoforms *of Arabidopsis thaliana* GLN1-1,1-2,1-3 and 1-4, (At5g37600, At1g66200, At3g17820, At5g16570)
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	AS08 295PRE | GLN1 GLN2 | GS1 glutamine synthetase, pre-immune serum AS08 296 GLN | GS2, chloroplastic form of glutamine synthetase collection of antibodies to proteins involved in nitrogen metabolism plant and algal protein extraction buffer secondary antibodies
Additional information	The antibody will recognize both, cytoplasmic and chloroplastic forms of the GS enzyme.

Application information

Recommended dilution	1 : 10 000 (WB)
Expected | apparent MW	39-40 kDa (GLN1,cytoplasmic form) , 44-45 kDa (GLN2, chloroplastic form)
Confirmed reactivity	*Arabidopsis thaliana, Eragrostis tef, Gracilaria gracilis* (red algae)*, Gracilaria lemaneiformis, Medicago truncatula, Physcomitrella patens, Pinus strobus, Spinacia oleracea, Solanum lycopersicum, Triticum aestivum, Zea mays*
Predicted reactivity	*Brachypodium distachyon, Brassica napus, Camellia sinensis, Citrus clementina, Cucumis melo, Daphnia magna, Datisca glomerata, Emiliania huxleyi, Eucalyptus grandis, Gazania splendens, Genlisea aurea, Glycine max, Helianthus annuus, Hordeum vulgare, Oryza sativa, Panax quinquefolius, Phaseolus angularis, Phytophthora cinnamomi, Populus trichocarpa, Saccharum officinarum, Securigera parviflora, Solanum lycopersicum, Solanum tuberosum, Stevia rebaudiana, Theobroma cacao, Zea mays, Vitis labrusca* GLN1 dicots including: *Brassica napus*, *Phaseolus vulgaris*, monocots including: *Hordeum vulgare, Oryza sativa*, trees: *Pinus sylvestris, Populus* sp., *Zosteria marina*

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GLN2 dicots including: *Brassica napus, Glycine max, Phaseolus vulgaris,* monocots including: *Triticum aestivum, Oryza sativa*

GLN3: *Zea mays,*

GLN1 in algae: *Chlamydomonas reinhardii*

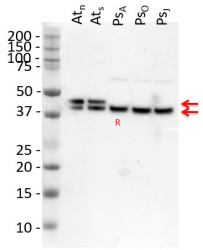
Not reactive in

No confirmed exceptions from predicted reactivity are currently known.

Selected references

Silva et al. (2019). Characterization of plant glutamine synthetase S-nitrosation. Nitric Oxide. 2019 Apr 23;88:73-86. doi: 10.1016/j.niox.2019.04.006.
Wang et al. (2018). Response of Gracilaria lemaneiformis to nitrogen deprivation. Algal Research Volume 34, September 2018, Pages 82-96.
Witzel et al. (2017). Temporal impact of the vascular wilt pathogen Verticillium dahliae on tomato root proteome. J Proteomics. 2017 Oct 3;169:215-224. doi: 10.1016/j.jprot.2017.04.008.
Silva et al. (2015). Possible role of glutamine synthetase of the prokaryotic type (GSI-like) in nitrogen signaling in Medicago truncatula. Volume 240, November 2015, Pages 98–108.
Podgórska et al. (2013). Long-term ammonium nutrition of Arabidopsis increases the extrachloroplastic NAD(P)H/NAD(P)+ ratio and mitochondrial reactive oxygen species level in leaves but does not impair photosynthetic capacity. Plant Cell Environ. April 10.
Brouwer et al. (2011) TheImpact ofLightIntensity onShade-InducedLeaf Senescence. Plant Cell Environ. Dec. 15 (ahead of print).
Lang et al. (2011).Simultaneous isolation of pure and intact chloroplasts and mitochondria from moss as the basis for sub-cellular proteomics. Plant Cell Rep. Feb;30(2):205-15. (reactivity confirmed for *Physcomitrella patens*).

Application example



10 µg of total protein extracted freshly from *Arabidopsis thaliana* wt leaf tissue (At_n non-senescent leaves), *Arabidopsis thaliana* wt leaf tissue (Ats_senescent leaves), *Pinus strobus* needle tissue (PS_{A-J}) with 1 M Tris-HCl, pH 6.8, 10 % SDS, 15 % sucrose, 0.5 DTT and denatured at 75°C for 5 min. were separated on 10 % Bis-Tris Nupage Novex gel (120 V/45 min. using MES buffer system) and blotted 30 min. to PVDF. Blot was blocked with 5 % non-fat milk 45 min./RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 10 000 for 1h/RT with agitation in TBS with 2 % non-fat milk or ON/4°C with agitation. The antibody solution was decanted and the blot was rinsed briefly twice for 10 min. in TBS at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, AS09 602) diluted to 1:75 000 in for 1h/RT with agitation. The blot was washed as above and developed using chemiluminescent detection. Exposure time was 26.5 seconds.

Courtesy of Dr. Christine Yao-Yun Chang and the Ensminger lab, University of Toronto, Canada

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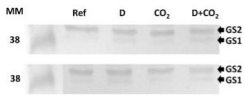

The detection of GS1 and GS2 proteins was performed using the crude extract of soluble proteins from *Oryza sativa* plants: Ref: Reference (control); D: Drought; CO_2: High CO_2 D+CO_2: Drought + High CO_2. Fresh leaves samples were ground until obtaining a fine powder in presence of liquid N_2, ice-cold 100 mM K-phosphate buffer (pH 7.0) containing 1 mM EDTA and 2 mM ascorbic acid. After centrifugation at 14,000 x g for 30 min, the supernatant was collected and used as protein extract. All extraction stages were carried out at 4°C. The total soluble protein was

measured according to the Bradford's method. Leaf protein extracts were first separated by SDS-PAGE (Laemmli 1970). Equal amounts of protein (20 µg) were electrophoretically transferred to a nitrocellulose membrane (Towbin et al. 1979). Polypeptide detection was performed using specific polyclonal antibodies against GS1 and GS2 (AS08 295, Agrisera, Sweden). Membranes were blocked for 3 hours with 5% non-fat milk in saline Tris-HCl buffer (100 mM Tris-HCl, pH 7.6, 150 mM NaCl), incubated with GS antibody overnight and after with alkaline phosphatase-conjugated secondary antibody by 6 hours. The protein detection was developed using NBT/BCIP (Sigma-Aldrich©, USA) by adding 1 tablet to 10 mL dH_2O, until bands were visualized.

Courtesy of Dr. Ana Karla Lobo, Laboratory of Plant Metabolism, Federal University of Ceara, Brazil