COSMO BIO CO., LTD.
Inspiration for Life Science

Agrisera
Antibodies for plant sciences

Product: Anti-AtpB (ATP synthase) Global antibody

Product no: AS03 030

Product Information

Antibody clonality: Polyclonal

Raised in: Hen

Purity: Total hen IgY purified by PEG precipitation method in PBS pH 7.4 with 0.02 % sodium azide

Quantity: 100 µl

Concentration: 25.3 µg/µl

Antibody form: Liquid.
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: 4°C. Do not freeze. Make aliquots instead.

Related products:
AS03 030S AtpB
Protein Standard for quantitation
AS05 085 Anti-AtpB Global antibody (rabbit)

Background
ATP synthase is the universal enzyme that synthesizes ATP from ADP and phosphate using the energy stored in a transmembrane ion gradient.

Immunogen: A peptide, which is highly conserved across the beta subunits of known F-type ATP Synthases from chloroplasts (CF1 Atp Synthase), mitochondria (F1 ATP) and most bacterial F-type Atp synthases.

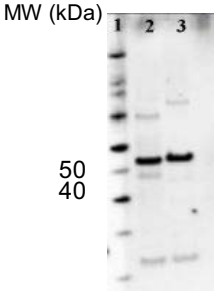
Peptide used for antibody production is located in a beta sheet, which is partly exposed near the surface of the AtpB protein.

Reactivity: AtpB subunit of the F1 (extrinistic) sub-complex of F-type ATP Synthases involved in both photosynthesis and respiration for a very wide range of species.
Anti-AtpB antibody will not cross-react with -V-type ATP Synthase, found mainly in Archaea.

References: Six et al. (2007) Contrasting photoacclimation strategies in oceanic and lagoon ecotypes of the eukayotic picoplankter Ostreococcus. accepted pending revision, Limnology and Oceanography, 18 May 2007

Application information:

Western Blot: 1: 50 000 (ECL Advance, GE Healthcare) 1: 2000 - 1: 5 000 with regular ECL
MW: 53.9 kDa *(Arabidopsis thaliana)*



From left to right: molecular weight markers (MagicMark XP, Invitrogen), whole cell extract of *Synechocystis* PCC6803 (size 51.7 kDa), isolated thylakoid fraction of spinach (size 53.7 kDa)
Note: Double bands detected can be a result of degradation or contamination.

Detailed experimental conditions are described on page 2

Immunolocalization: 1: 1000 Antibody will recognize a native protein and has been used in immonolocalization studies of the native enzyme using immunogold.
Experimental conditions:

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

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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 140 mM Tris base, 105mM Tris-HCl, 0.5mM ethylenediaminetetraacetic acid (EDTA), 2% Lithium dodecyl sulfate (LDS), 10% glycerol, 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 immediatly 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 4C. 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 (anti-hen IgY 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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