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Product no AS20 4443

Anti-GFP | Green Fluorescence Protein

Product information

Immunogen	Recombinant GFP protein derived from *Aequorea victoria,* UniProt: P42212
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	50 µg
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 the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.

Application information

Recommended dilution	1: 5000 - 1 : 10 000 (WB)
Confirmed reactivity	Recombinant GFP overexpressed in *E.coli* from *Arabidopsis thaliana, Chlamydomonas reinhardii, Nicotiana tabacum*
Predicted reactivity	mClover3
Selected references	Dieren et al. (2024). Analysis of abiotic and biotic stress-induced Ca2+ transients in the crop species Solanum tuberosum. Sci Rep . 2024 Nov 11;14(1):27625. doi: 10.1038/s41598-024-79134-3.

19.4 µg/well of total protein extracted freshly from tobacco with buffer (50mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, 10% Glicerol, 1 mM DTT, 1X Pefablock) and denatured with SDS 2% and 0.1% Bromophenol blue (Laemmli buffer) at 95°C for 5 min. Separated on 12 % SDS-PAGE and blotted 2h to PVDF/nitrocellulose, using wet transfer. Blot was blocked with 5% milk for 1h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 10 000 in TBS-T ON/4°C with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed 3 times for 10 min in TBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, AS09 602) diluted to 1:20 000 in for 1h/RT with agitation. The blot was washed as above and developed for 2 min with Agrisera ECLSuperBright. Exposure time was 60 seconds.

Courtesy of Delfina Gagliardi Instituto de Agrobiotecnología del Litoral (IAL), Argentina

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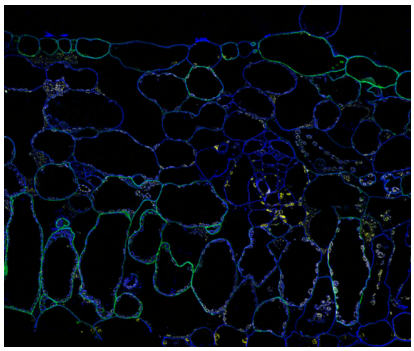

Super-resolution image of immunofluorescent localization of GFP-fused protein in *Nicotiana tabacum* (tobacco) leaf tissue using Agrisera anti-GFP antibodies (AS20 4443) and donkey anti-rabbit IgG DyLight 650 conjugated secondary antibodies (Green). Calcofluor White (Blue) and Tissue Autofluorescence (Yellow),

Method:
Fixation: 2% paraformaldehyde + 0.5% glutaraldehyde, dehydration in ethanol, embedment and UV polymerization in HM20 resin
Cell wall digestion: no
Membrane permeabilization: DMSO-IGEPA
Air drying; No
Antigen retrieval: No
Blocking buffer: 4% w/v non-fat milk powder in 1X Phosphate Buffered Saline buffer, prepared freshly, filtered with 0.2 µm filer
Washing buffer: 1X Phosphate Buffered Saline buffer Primary antibody dilution and incubation time: 1:100; 1 hour at room temperature
Secondary antibody dilution and incubation time and supplier: 1:200 Donkey anti-rabbit IgG (H&L) DyLight®650 (Agrisera, AS12 2254); 1 hour at room temperature
Co-staining of the nucleus (DAPI): No
Cell wall and nucleus staining: Calcofluor White
All material was freshly prepared. All incubation volumes were kept the same: 200 µL per 22x22 mm cover glass/sample

Courtesy of Dr. Kirk Czymmek, Donald Danforth Plant Science Centre, USA.