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Product no AS21 4696

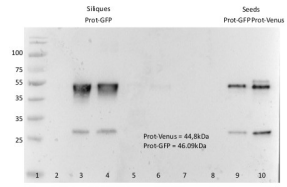
Anti-GFP | Green Fluorescence Protein, monoclonal (clone 2G4:F2)

Product information

Immunogen	Recombinant GFP protein derived from *Aequorea victoria,* UniProt: P42212
Host	Mouse
Clonality	Monoclonal
Subclass/isotype	IgG2a kappa-chains
Purity	Purified mouse IgG 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 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

Application information

Recommended dilution	1: 1000 - 1 : 30 000 (WB)
Expected | apparent MW	Depends upon fusion partner
Confirmed reactivity	GFP-tagged proteins
Selected references	To be added when available. Antibody released in March 2023.



Samples

1. Ladder (PageRuler Prestained, 10 à 180 kDa - Thermo)
2. 20 µg of total protein Col-0 (leaves, control)
3. 20 µg of total Prot-GFP siliques (extract before purification)
4. Purified Prot-GFP siliques - siliques (with Mylteny beads)
5. Purified Elution Prot-GFP siliques (with Mylteny beads) –SDS-buffer
6. Purified Prot-GFP - siliques (with ProteinTech beads)
7. Purified Elution Prot-GFP siliques 6M Urea (with ProteinTech beads)
8. Purified Elution Prot-GFP - SDS-buffer siliques (with ProteinTech beads)
9. 20 µg of total Seeds *of A. thaliana* Prot-GFP
10. 20 µg of total Seeds of *A. thaliana* Prot-Venus

20 µg/well of total protein extracted freshly from siliques (stade 20dap, 50mg of powder) and imbibed seeds (50 mg starting material). Exact buffer components were Cell Culture Lysis 1X Reagent (Promega - E153A + inhibitor protease cocktail) and denatured with Laemmli buffer (375 mM Tris.HCl, 9% SDS, 50% Glycerol, 0.03% Bromophenol blue) at 95 °C/5 min. Samples were separated on 12 % SDS-PAGE and blotted for 1 h to nitrocellulose (pore size of 0,45 µm), using wet transfer. Blot was blocked with 5 % milk for 2h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1000 in 5 % milk TBS-T ON/4°C with agitation. The antibody solution was decanted, and the blot was washed 4 times for 5 min in TBS-T at RT with agitation. Blot was incubated with secondary antibody (AS09 627-trial, Rabbit anti-mouse IgG, HRP conjugated, Agrisera) diluted to 1: 10 000 in 5 % milk for 1h/RT with agitation. The blot was washed as above and developed with a following chemiluminescent detection reagent AS16 ECL-S-10, AgriseraSuperBright. Exposure time was 20 seconds.

Courtesy of Dr. Victoria Gomez Roldan,French National Centre for Scientific Research, France

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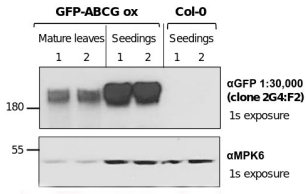

Total proteins were isolated from 4 leaves (5-week-old plants) of *Arabidopsis thaliana* were ground in 400 µL homogenization buffer or from 15 seedlings (7-day-old) ground in 100 µL homogenization buffer as described by LaMontagne et al (2016). Plant lines were Arabidopsis thaliana Col-0 (ecotype, wild-type) and an overexpressing GFP-tagged ABCG-protein (GFP-ABCG ox). 15 µl of total proteins were denatured at 37°C for 5 min, separated on 8% SDS-PAGE and transferred for 70 min at 55V using a tank transfer system to nitrocellulose membrane (0.45 µm). Blots were blocked with PBS+0.1% Tween 20 (PBS-T)+5 % milk at room temperature (RT) with agitation for 2 hours. Monoclonal primary anti-GFP-antibody (AS21 4696, Agrisera; clone 2G4:F2) was diluted to 1:30,000 and incubated with membrane portion overnight at 4°C with agitation in PBS-T+5 % milk. The primary antibody solution was decanted, and blots were washed 4 times (6 minutes each) in PBS-T at RT with agitation. Blot was incubated with secondary rabbit anti-mouse IgG (H&L) HRP conjugated (AS09 627-trial, Agrisera) diluted to 1: 25,000 for 2 hours, washed as described above (4 times with PBS-T at RT), and developed with chemiluminescent detection reagent ECL Bright (AS16 ECL-N, Agrisera) according to manufacture's recommendations Exposure time was 1 second (1s) on X-ray films. Ponceau Stain of the total proteins and anti-MPK6 detection served as loading control.

Courtesy of Nga Nguyen and Dr. Antje Heese at the University of Missouri-Columbia, Div. of Biochemistry, Interdisciplinary Plant Group (IPG), Columbia MO (USA).

Reference: LaMontagne ED, Collins CA, Peck SC, Heese A. Isolation of Microsomal Membrane Proteins from Arabidopsis thaliana. Curr Protoc Plant Biol. 2016 May;1(1):217-234. doi: 10.1002/cppb.20020. PMID: 31725992.