Agrisera
Part of Olink® Group

This product is **for research use only** (not for diagnostic or therapeutic use) **contact: support@agrisera.com**

Agrisera AB | Box 57 | SE-91121 Vännäs | Sweden | +46 (0)935 33 000 | www.agrisera.com

Product no AS15 2998

Anti-GFP | Green Fluorescence Protein (total IgY)

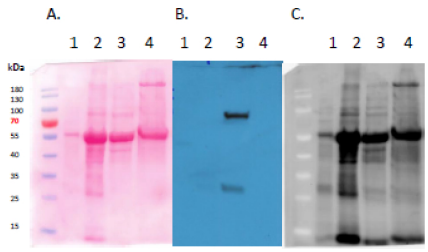
Product information

Immunogen	highly purified native GFP protein derived from *Aequorea victoria,* UniProt: P42212
Host	Chicken
Clonality	Polyclonal
Purity	Total IgY in 10 mM TRIS, 0,15 mM NaCl, pH 8. Contains 0.02 % sodium azide.
Format	Liquid
Quantity	100 µl (1 mg/ml)
Storage	Store at 4°C; make aliquots to avoid working with a stock. Or Store in small aliquots at -20°C, 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 : 25 000 (ELISA), 1 : 2000-1 : 10 000 (WB)
Confirmed reactivity	Native GFP, Recombinant GFP (E,coli), all variants of GFP
Additional information	Minimal cross-reactivity with *E.coli* proteins

Application examples



Samples
20 ug of *Arabidopsis thaliana* Columbia-0 overexpressing GRP8-GFP leaf protein extract **(1)**, 70 ug of *Arabidopsis thaliana* Columbia-0 overexpressing GFP-SnRK2.4 leaf protein extract **(2)**, 50 ug of *Nicotiana benthamiana***a** transiently expressing GFP-SnRK2.4 leaf protein extract **(3)**, 50 ug of *Nicotiana benthamiana* transiently expressing SnRK2.4-GFP leaf protein extract **(4)**. Mark: MW markers: PageRuler Prestain Protein Ladder from Thermo Fisher Scientific (#26616) The total protein extracts were freshly prepared from *A. thaliana* leaves with extraction buffer 1 (samples 1&2) or *N. behtamiana* leaves with extraction buffer 2 (samples 3&4). The extraction buffer 1 contained: 20 mM Tris-HCl pH 7.5; 2 mM EDTA; 2 mM EGTA; 50 mM -glycerophosphate; 250 mM sucrose; 10 mM Na3VO4; 1% Triton X-100; 150 mM NaCl; 10 mM DTT; 1 mM PMSF and 1 x Complete Protease Inhibitor Cocktail (Roche). The extraction buffer 2 contained: 100 mM HEPES, pH7.5; 5 mM EDTA; 5 mM EGTA; 10 mM DTT; 1 mM Na3VO4; 10 mM NaF; 50 mM b-glycerophosphate; 10 mM pyridoxal 5-phosphate; 10% glycerol and 1 x Complete protease inhibitors (EDTA-free, Roche). The extraction buffers were added to the powdered material in a 1:1 v:v ratio. Samples were incubated on a rotator for 30 min at 4°C and then centrifuged for 30 min, 12 000 rpm at 4°C. Supernatant was transferred into new tubes and protein concentration was measured using Bradford Protein Assay. Samples were then denatured with 3x Laemmli sample buffer for 5 min in 95°C. Next samples were separated on 10 % SDS-PAGE stain-free gels (Bio-Rad) and blotted overnight to PVDF membrane using wet transfer. Blot was blocked with 3% milk and 0,1% Tween 20 in TBS for 1h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 5 000 for 1,5h/RT with agitation in 3% milk and 0,1% Tween 20 in TBS solution. The antibody solution was decanted and the blot was rinsed briefly, then washed 5 times for 5 min in TBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (rabbit anti-chicken IgG horse radish peroxidase conjugated, AS10 1489, Agrisera) diluted to 1:10 000 in for 1h/RT with agitation. The blot was washed as above and developed for 1 min with Pierce ECL Western Blotting Substrate. Exposure time was 5 min (short exposure)/ 10 min (long exposure). Additionaly blot was briefly rinsed with water and developed for 1 min with chemiluminescent detection reagent. Exposure time was 1 min.

Courtesy of Katarzyna Szymańska, Institute of Biochemistry and Biophysics PAS > Plant Protein Phosphorylation Laboratory, Warsaw, Poland