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Product no AS23 4983

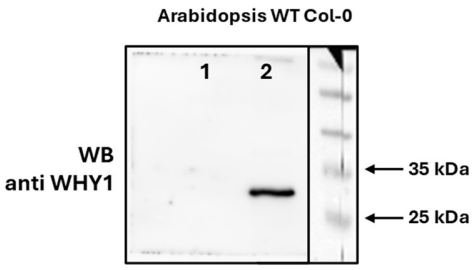
Anti-WHY1 | Whirly1

Product information

Immunogen	KLH-conjugated peptide derived from *Arabidopsis thaliana* WHY1 protein sequence, UniProt:A0A654E9P6 TAIR: AT1G14410
Host	Rabbit
Clonality	Polyclonal
Purity	Antigen affinity purified serum, in PBS pH 7.4
Format	Lyophilized
Quantity	50 µg
Reconstitution	For reconstitution, add 50 µl, of sterile or deionized 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 (WB)
Expected | apparent MW	29 kDa
Confirmed reactivity	*Arabidopsis thaliana*
Predicted reactivity	Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Additional information	The peptide used to elicit this antibody is not found in: Whirly2 (At1g71260) and Whirly3 (At2g02740).
Selected references	To be added when available. Antibody released in January 2025.



Samples:

1. Soluble chloroplast proteins extract of *Arabidopsis thaliana*
2. Insoluble chloroplast protein extract *Arabidopsis thaliana*

Chloroplasts were isolated from 4-week-old *Arabidopsis thaliana* rosette leaves according to the protocol by Klinkenberg, J. (2014). Intact chloroplasts were resuspended in 50 µl of protein extraction buffer (10% glycerol, 50 mM EDTA, 10 mM DTT, 100 mM Hepes pH 7.2, and protease inhibitor cocktail). After centrifugation, the soluble protein fraction was collected and transferred to a fresh tube, while the pellet (considered as insoluble proteins) was resuspended in 50 µl of protein extraction buffer. Ten microliters from each of the two fractions were combined with loading buffer, denatured at 98 °C for 10 minutes, separated on a 10% SDS-PAGE (custom-prepared gel), and blotted onto a PVDF membrane (pore size of 0.22 µm) using semi-dry transfer. The blot was blocked with 5% skim milk for 1 hour at room temperature (RT) with agitation. It was then incubated with the primary antibody at a dilution of 1:1000 for 1 hour at RT with agitation in PBS-T. After decanting the antibody solution, the blot was washed 3 times for 5 minutes in PBS-T at RT with agitation. The blot was then incubated with goat anti-rabbit HRP conjugate diluted to 1:25000 for 1 hour at RT with agitation. The blot was washed again as above and developed using chemiluminescent detection reagent. Images were captured using a Bio-Rad ChemiDoc system with an exposure time of approximately 2 minutes.
Soluble and insoluble chloroplast proteins were isolated using the following protocol: Klinkenberg, J., 2014. Extraction of chloroplast proteins from transiently transformed *Nicotiana benthamiana* leaves. Bio-protocol, 4(18), pp.e1238-e1238.

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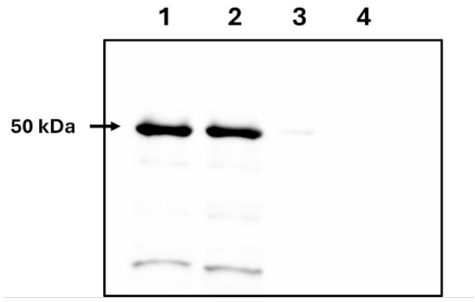
Courtesy of Dr. Agata Cieśla, UAM, Poznań, Poland



Samples:

1 – GST-WHY1
2 – GST-WHY1K241R (mutated protein with substitution of lysine (K) in position 241 to arginine (R))
3 – GST-WHY2
4 – GST-WHY3

5 µg/welll of recombinant GST-WHY1, GST-WHY1K241R, GST-WHY2 and GST-WHY3 were purified from bacterial cells through affinity chromatography. Samples were denatured in 98°C for 10 min. Samples were separated on 10 % SDS-PAGE and blotted to PVDF (pore size of 0,22 µm), using semi-dry transfer. Blot was blocked with 5 % skim milk for: 1h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1:1000 for 1h/RT with agitation in PBS-T The antibody solution was decanted, and the blot washed 3 times for 5 min in PBS-T at RT with agitation. The blot was incubated anti-rabbit IgG horse radish peroxidase conjugated diluted to 1: 25 000 for h/RT with agitation. The blot was washed as above and developed with chemiluminescent detection reagent. Exposure time was about 1 minute.

Courtesy of Dr. Agata Cieśla, UAM, Poznań, Poland