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Product no AS08 351

Toc75 | Protein TOC75-3, chloroplastic, POTRA domain 3

Product information

Immunogen	*Pisum sativum* TOC75; Predicted POTRA Domain #3; Expressed and purified in *E. coli* using the Impact System from NEB. Peptide confirmed by MALDI. UniProt: Q43715
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 µl
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 : 500 (Flow cyt). 1 : 100 (IL), 1 : 2000-1 : 100 000 (WB)
Expected | apparent MW	88 | 75 kDa (ocassionally a processing intermediate at 78 kDa is observed)
Confirmed reactivity	*Arabidopsis thaliana, Nicotiana tabacum, Physcomitrella patens, Pisum sativum, Spinacia oleracea*, *Thellungiella salsuginea*, some cross-reactivity was observed for cyanobacteria including: *Synechocystis, Synechococcus and Thermosynechococcus* sp.
Predicted reactivity	*Aegilops tauschii, Ananas comosus, Anthurium amnicola, Capsicum annuum, Catalpa bungei, Cicer arietinum, Cucumis melo, Glycine soja, Gossypium arboreum, Medicago truncatula, Morus notabilis, Nelumbo nucifera, Nicotiana sylvestris, Nicotiana tabacum, Noccaea caerulescens, Populus trichocarpa, Ricinus communis, Sorghum bicolor, Theobroma cacao, Zostera marina, Vigna radiata, Vitis vinifera* Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known.
Additional information	Antibody detects Toc75 POTRA domain 3 as purified protein, in chloroplast fraction and in crude envelope fraction.For high resolution images, please visit the specific product page at www.agrisera.com
Selected references	Sasaki and Yamamoto (2015). Arabidopsis LAZY1 is a peripheral membrane protein of which the carboxy-terminal fragment potentially interacts with microtubules. Copyright © 2015 The Japanese Society for Plant Cell and Molecular Biology, Plant Biotechnology, 32, 1–6 (2015) DOI: 10.5511/plantbiotechnology.15.0116a Vera-Estrella et al. (2014). Comparative 2D-DIGE analysis of salinity responsive microsomal proteins from leaves of salt-sensitive Arabidopsis thaliana and salt-tolerant Thellungiella salsuginea. J Proteomics. 2014 Jun 2. pii: S1874-3919(14)00288-7. doi: 10.1016/j.jprot.2014.05.018. Hsueh et al. (2014). The chloroplast outer envelope protein P39 in Arabidopsis thaliana belongs to the Omp85 protein family. Proteins. 2014 Nov 17. doi: 10.1002/prot.24725.

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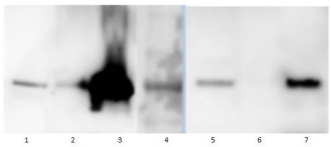
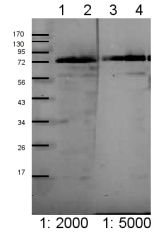
Application example



Samples of crude *Arabidopsis thaliana* chloroplast preparation from 250xg pellet **(1)**, unknown sample **(2)**, *Arabidopsis thaliana* 100kDa membrane preparation **(3)**, prestained marker 80kDa band (NEB Broad Range) **(4)**, *Physcomitrella patens* 'broken' chloroplasts from above 40% on Percoll gradient **(5)**, unknown sample **(6)**, *Physcomitrella patens* intact chloroplasts from above 80% on Percoll gradient **(7)** were blotted to PVDF membrane. Blot was incubated overnight at 4°C in 5% milk powder in Tris Buffered Saline (TBS), rinsed twice and then washed for 3 x 15 min in TBS+ 0.1%Tween20 (TBS-T). Incubation in anti-Toc75 POTRA3 antibody was done for 2h/RT using 8µl antibody per 10 ml 3% milk powder in TBS-T. Washes: 3 x 15 min in TBS+ 0.1%Tween20 (TBS-T). Incubation in goat anti-rabbit peroxidase secondary antibody was for 1h at room temperature and used 4µl antibody per 10ml 3% milk powder in TBS-T. Washes: 3 x 15 min in TBS+ 0.1%Tween20 (TBS-T) followed by incubation with chemiluminescence detection reagent and exposure for 10 min or 2min in an ImageQuant LAS4000 (lanes 1-3 and 5-7, respectively).

Courtesy Dr. Amanda Dowson, Warwick University, UK



580 ng of Chl of *Pisum sativum* plants (10 day old) **(2, 4)** and 10 µg of combined envelopes of *Pisum sativum* 10 day-old **(1,3)** were separated on 15% SDS-PAGE and blotted 2h to PVDF. PVDF was blocked 1h with 3% non-fat milk powder in TBS-T (0.1% TWEEN 20) and probed with anti-Toc75 POTRA domain 3 antibodies AS08 351 (1:2000 and 1: 5000, 1h) and secondary donkey-anti-rabbit (1:20000, 1 h) antibody (HRP conjugated) in TBS-T containing 3% non fat milk powder. Antibody incubations were followed by washings in TBS-T. All steps were performed at RT with agitation. Blots were developed for 5 min with chemiluminescence detection reagent according to the manufacturers instructions. Exposure time was 600 seconds.

Ashita Dave and Barry D. Bruce (UTK), USA