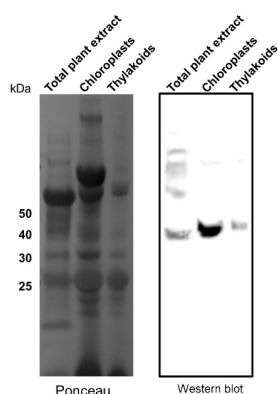


Product no **AS10 709****Tic40 | Inner envelope membrane translocon complex protein (chloroplast)****Product information**

Immunogen	KLH-conjugated synthetic peptide derived from available plant sequences of Tic40 including <i>Arabidopsis thaliana</i> UniProt: Q9FMD5 , TAIR: At5g16620
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 µl
Reconstitution	For reconstitution please 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.
Additional information	Cellular [compartment marker] of chloroplast membrane

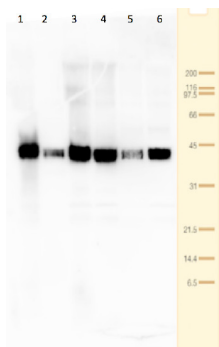
Application information

Recommended dilution	1 : 2500 (WB)
Expected apparent MW	48 45 kDa (<i>Arabidopsis thaliana</i>)
Confirmed reactivity	<i>Arabidopsis thaliana</i> , <i>Catharantus roseus</i> , <i>Nicotiana benthamiana</i> , <i>Nicotiana tabacum</i> , <i>Oryza sativa</i> , <i>Physcomitrium patens</i> , <i>Solanum lycopersicum</i> , <i>Triticum aestivum</i> , <i>Zea mays</i> , <i>Vitis vinifera</i>
Predicted reactivity	<i>Lactuca sativa</i> , <i>Picea sitchensis</i> , <i>Pisum sativum</i> , <i>Populus trichocarpa</i> , <i>Ricinus communis</i> Species of your interest not listed? Contact us
Not reactive in	<i>Chlamydomonas reinhardtii</i>
Additional information	This product can be sold containing ProClin if requested
Selected references	Loudya et al. (2021) Cellular and transcriptomic analyses reveal two-staged chloroplast biogenesis underpinning photosynthesis build-up in the wheat leaf. <i>Genome Biol.</i> 2021 May 11;22(1):151. doi: 10.1186/s13059-021-02366-3. PMID: 33975629; PMCID: PMC8111775. Koester et al. (2021) Transgenic insertion of the cyanobacterial membrane protein ictB increases grain yield in <i>Zea mays</i> through increased photosynthesis and carbohydrate production. <i>PLoS One.</i> 2021 Feb 4;16(2):e0246359. doi: 10.1371/journal.pone.0246359. PMID: 33539477; PMCID: PMC7861388. Wang et al. (2020) . Post-translational coordination of chlorophyll biosynthesis and breakdown by BCMs maintains chlorophyll homeostasis during leaf development. <i>Nat Commun.</i> 2020; 11: 1254. Van Gelder (2018) . Medium-Chain Polyprenols Influence Chloroplast Membrane Dynamics In <i>Solanum Lycopersicum</i> . <i>Plant Cell Physiol.</i> 2018 Sep 6. doi: 10.1093/pcp/pcy157. Fernández-San Millán et al. (2018) . Physiological Performance of Transplastomic Tobacco Plants Overexpressing Aquaporin AQP1 into Chloroplast Membranes. <i>J Exp. Bot.</i> ery148, https://doi.org/10.1093/jxb/ery148 .

Application example

10-15µg of chlorophyll from isolated total plant material (*Arabidopsis thaliana*), chloroplasts and thylakoids extracted with a buffer containing (25 mM Tricine-NaOH, pH 7.8, 330 mM sorbitol, 1 mM EDTA, 10 mM KCl, 0.15% [w/v] bovine serum albumin, 4 mM sodium ascorbate, and 7 mM L-Cys) were separated on 12 % SDS-PAGE and blotted 1h to PVDF using semi-dry transfer. Blots were blocked with 10% milk for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 2 000 overnight at 4 °C with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 5 min in TBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, from Agrisera [AS09_602](#)) diluted to 1:15 000 in TBS-T for 1h at RT with agitation. The blot was washed as above and developed for 60 seconds with a ImageQuant system from GE Healthcare, exposure time was 60 seconds.

Courtesy of Dr. Rikard Fristedt VU University Amsterdam Faculty of Sciences Department of Physics and Astronomy Biophysics of Photosynthesis, The Netherlands



Lanes 1-6: 20µg 21kxg pellet from whole *Physcomitrella patens* protonemata extract (1), 20µg 21kxg supernatant from whole protonemata extract (2), 20µg broken chloroplasts/thylakoids from 40% Percoll band (3), 20µg intact chloroplasts from 80% Percoll band (4), 10µg pellet material from Percoll gradient (including whole cells and enriched for nuclei) (5), 20µg whole protonemata extract (6)

Sample Preparation Up to 20µg of total protein/chloroplast material from *P. patens* was prepared by chopping the protonemata into an ice cold isotonic chloroplast buffer (0.3M D-sorbitol, 50mM Hepes-KOH pH8.0, 2mM EDTA, 1mM MgCl₂ with additives: 0.1% BSA and PVP-10 and added protease inhibitor tablet). The extract was filtered through a 70µm filter and chloroplasts were pelleted at 250 xg 4 °C 5 min then layered onto discontinuous 10, 40, 80% Percoll gradients (which employed the same buffer) and centrifuged at 9k xg 4 °C 1h. Broken chloroplasts/thylakoids and chloroplasts, respectively, were recovered from the 40 and 80% bands after centrifugation at 9k xg 4 °C 1h by centrifugation at 1k xg in four volumes of buffer (without additives). Samples were frozen at -20 °C. Samples were thawed on ice and were diluted in 50mM Tricine pH7.6, 1mM -mercaptoethanol, 1mM MgCl₂ with protease inhibitor tablet for protein concentration determinations and aliquots of up to 20 µg were prepared in 0.8 x Laemmli sample buffer and denatured at 95 °C 5 min and were separated on 10-20% Criterion stain free SDS-PAGE and blotted 7 min to PVDF using BioRAD mini Turbo blot semi-dry transfer. Blots were blocked O/N at 4 °C with agitation in 10% milk powder in PBS. Blot was rinsed in PBS-T 3 times for 5 min then incubated in the primary antibody at a dilution of 1: 1 000 for anti-Toc75 or 1 in 2000 for antiTic40 for 2h at RT with agitation in 0.5% milk powder in PBS-T. The antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 5 min in PBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, from an unknown source but labelled NIF824) diluted to 1:2,500 in for 1h at RT with agitation in 0.5% milk powder in PBS-T. The blot was washed as above and developed for 10min with chemiluminescent detection reagent. Exposure time was 30 min.

Dr. Amanda Dowson, University of Warwick, UK