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Product no AS09 458 Anti-PEPC | Phosphoenolpyruvate carboxylase

Product information

Immunogen	<u>KLH</u> -conjugated synthetic peptide well conserved PEPC1 and sequences from different plant species including <i>Arabidopsis thaliana</i> <u>Q9MAH0</u> , <u>At1g53310</u> (PEPC 1), <u>Q84VW9</u> , <u>At3g14940</u> (PEPC 3). The peptide chosen to elicit this antibody is also perfectly conserved in bacterial type of this enzyme <u>NP 177043.2</u> (PEPC 4). For <i>Zea mays</i> , the peptide is converved in PEP1 and PEP4 isoforms.
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum 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 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. Please do not re-use this primary antibody solution. In case of cyanobacterial samples there will be no signal in your second incubation.
Additional information	PEPC is especially prone to proteolysis. To avoid artifacts, we use chymotrypsin, as recommended by <u>Plaxton</u> (2019), in addition to the Roche protease inhibitor cocktail.

Application information

Recommended dilution	1 : 500 (IL), 1: 1000 - : 10 000 (WB)
Expected apparent MW	110 105 kDa
Confirmed reactivity	Ananas comosus, Arabidopsis thaliana, Cenchrus ciliaris, Chenopodium quinoa, Chloris gayana, Chromera velia, Cyanthobasis fruticulosa, Glycine max, Hordeum vulgare, Jatropha curcas, Kochia prostrata, Leptochloa fusca, Lupinus sp., Megathyrsus maximus, Mesembryanthemum crystallinum, Nicotiana tabacum, Oryza sativa, Panicum antidotale, Panicum coloratum, Petrosimonia nigdeensis, Pinus strobus, Saccharum spp. hybrid clone C91-301, Salsola lanata, Salsola laricifolia,Salsola grandis, Salsola tragus Sorghum bicolor, Synechocystis PCC 6803, Phaeodactylum tricornutum (strain CCAP 1055/1), Pinus strobus, Thalassiosira weissfloggi, Zea mays, Zostera muelleri
Predicted reactivity	Brassica napus, Cucumis sativus (PEPC1, PEPC2, PEPC3), Flaveria bidentis, Flaveria trinervia, Lupinus albus, Mammillaria thornberi, Manihot esculenta, Manihot obovata, Medicago sativa, Morinda citrifolia, Nannochloropsis gaditana CCMP526, Nopalea gaumeri, Opuntia macbridei, Pachycereus pringlei, Pachycereus hollianus, Pisum sativa, Phaseolus vulgaris, Phragmites australis, Populus sp.,Saccharum spp, Solanum tuberosum, Spinacia oleracea, Streptanthus tortuosus,Triticum aestivum, algae, diatoms: Thalassiosira pseudonana, other species: Salmonella sp., Schiedea hookeri, Shigella sp. Schiedea sarmentosa, Solanum lycopersicum, Streptanthus farnsworthianus, Tacinga saxatilis,Yersinia sp. Vibrio sp., Quercus sp.
Not reactive in	Methanothermobacter thermautotrophicus
Additional information	Antibody can be also used following 2D gel electrophoresis
Selected references	 <u>Fang</u> et al. (2024). Subfunctionalisation and self-repression of duplicated E1 homologues finetunes soybean flowering and adaptation. Nat Commun. 2024 Jul 23;15(1):6184. doi: 10.1038/s41467-024-50623-3. <u>Nguyen</u> et al.(2024). The processed C-terminus of AvrRps4 effector suppresses plant immunity via targeting multiple WRKYs.J Integr Plant Biol. 2024 Jun 13.doi: 10.1111/jipb.13710. <u>Cruz</u> et al.(2023). Variation of photosynthesis along the canopy profile of sugarcane and energy canes. Research Square; DOI: 10.21203/rs.3.rs-3124093/v1 <u>Luo</u>, et al. (2023) Deubiquitinating enzymes UBP12 and UBP13 stabilize the brassinosteroid receptor BRI1. EMBO Rep. 2022;23(4):e53354. doi:10.15252/embr.202153355 <u>Durall</u> et al. (2021). Production of succinate by engineered strains of Synechocystis PCC 6803 overexpressing phosphoenolpyruvate carboxylase and a glyoxylate shunt. Microb Cell Fact. 2021 Feb 8;20(1):39. doi: 10.1186/s12934-021-01529-y. PMID: 33557832; PMCID: PMC7871529. <u>Wang</u> et al. (2021). Brassinosteroids inhibit miRNA-mediated translational repression by decreasing AGO1 on the endoplasmic reticulum. J Integr Plant Biol. 2021 May 21. doi: 10.1111/jipb.13139. Epub ahead of print. PMID: 34020507. <u>Bakhmankulova</u> et al. (2021) Possible Activation of ?3 Photosynthesis in ?4 Halophyte Kochia prostrata Exposed to an

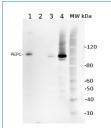


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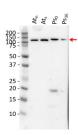
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Elevated Concentration of ??2. Russ J Plant Physiol 68, 1107–1114 (2021). https://doi.org/10.1134/S1021443721060169 Durall et al. (2020). Increased ethylene production by overexpressing phosphoenolpyruvate carboxylase in the cyanobacterium Synechocystis PCC 6803. Biotechnol Biofuels. 2020 Jan 28;13:16. doi: 10.1186/s13068-020-1653-y.



5 µg of total protein from (1) *Arabidopsis thaliana* leaf extracted with Protein Extration Buffer, PEB (AS08 300), (2) *Spinacia oleracea* total cell, extracted with PEB, (3) *Hordeum vulgare* total cell extracted with PEB, (4) Zea mays total cell extracted with PEB, were separated on **4-12%** NuPage (Invitrogen) **LDS-PAGE** and blotted 1h to **PVDF**. Blots were blocked immediately following transfer in 2% blocking reagent (GE Healthcare) in 20 mM Tris, 137 mM sodium chloride pH 7.6 with 0.1% (v/v) Tween-20 (TBS-T) for 1h at room temperature with agitation. Blots were incubated in the primary antibody at a dilution of 1: 10 000 for 1h at room temperature 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 room temperature with agitation. Blots were incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1:50 000 in 2% blocking solution for 1h at room temperature with agitation. The blots were washed as above and developed for 5 min with chemiluminescent detection reagent according the manufacturers instructions. Images of the blots were obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad).

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10 µg of total protein extracted freshly from *Arabidopsis thaliana* wt leaf tissue (At_n non-senescent leaves), *Arabidopsis thaliana* wt leaf tissue (At_s senescent leaves), *Pinus strobus* needle tissue (PS₀, PS₃₆) with 1 M Tris-HCI, pH 6.8, 10 % SDS, 15 % sucrose, 0.5 DTT and denatured at 75 °C for 5 min. were separated on 10 % Bis-Tris Nupage Novex gel (120 V/45 min. using MES buffer system) and blotted 30 min. to PVDF. Blot was blocked with 5 % non-fat milk 45 min./RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1000 for 1h/RT with agitation in TBS with 2 % non-fat milk or ON/4 °C with agitation. The antibody solution was decanted and the blot was rinsed briefly twice for 10 min. in TBS at RT with agitation. Blot was uncubated in Agrisera matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, <u>AS09 602</u>) diluted to 1:75 000 in for 1h/RT with agitation. The blot was washed as above and developed using chemiluminescent detection. Exposure time was 40 seconds.

Courtesy of Dr. Christine Yao-Yun Chang and the Ensminger lab, University of Toronto, Canada