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Product no AS11 1771

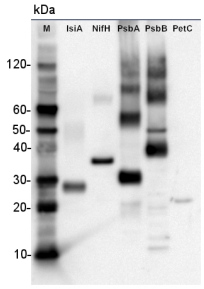
His-tag | 6xHis (clone HIS,H8 / EH158)

Product information

Immunogen	KLH-conjugated synthetic peptide 6xHis
Host	Mouse
Clonality	Monoclonal
Subclass/isotype	IgG2b
Purity	Total IgG fraction. Protein A purified.
Format	Liquid
Quantity	50 µg
Storage	Store at -20°C.
Additional information	Working dilution for ELISA, IL and IP needs to be determined experimentally

Application information

Recommended dilution	1 : 1000 (WB)
Confirmed reactivity	6xHis
Predicted reactivity	6xHis
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Additional information	Antibody is present in 10 mM PBS, pH 7.2 His-tag (6,8,10xHis) needs to be properly exposed to allow detection. To prevent target protein folding, extraction should be performed with 6 to 8 M urea or using TCA-acetone precipitation method.
Selected references	De Brasi-Velasco et al. (2021). Autophagy Is Involved in the Viability of Overexpressing Thioredoxin o1 Tobacco BY-2 Cells under Oxidative Conditions. Antioxidants. 2021; 10(12):1884. https://doi.org/10.3390/antiox10121884 Tan et al. (2020). Salicylic Acid Targets Protein Phosphatase 2A to Attenuate Growth in Plants. Curr Biol. 2020 Feb 3;30(3):381-395.e8. doi: 10.1016/j.cub.2019.11.058. López-Vidal et al. (2020). Is Autophagy Involved in Pepper Fruit Ripening? Cells, 9 (1) , DOI: 10.3390/cells9010106 Häggmark-Månberg et al. (2016). Autoantibody targets in vaccine-associated narcolepsy. Autoimmunity. 2016 Sep;49(6):421-433. Epub 2016 May 20.



500 femtomoles of His-tagged proteins IsiA, NifH, PsbA, PsbB and PetC were loaded per gel well in Agrisera PEB extraction buffer. Proteins were separated on **4-12 % NuPAGE PAGE** Bis-Tris polycacrylamide gel (Invitrogen) and blotted 1h to **PVDF**. Blots were blocked with for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1 000 for 1h at RT 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 (goat, anti-mouse IgG horse radish peroxidase conjugated, from Agrisera AS11 1772) diluted to 1:25 000 in 2 % ECL Advance blocking reagent for 1h at RT with agitation. The blot was washed as above and developed for 5 min with ECL according to the manufacturers instructions. Exposure time was 5 seconds.

Apparent molecular weight of recombinant proteins: IsiA - 27 kda, NifH - 34 kDa, PsbA - 30-37 kDa, PsbB - 40 kDa, PetC - 23 kDa.

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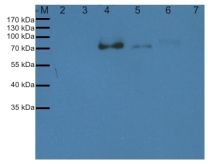

20 µl of media form *Pichia pastoris* culture overexpressed His-Tegged proteins were separated on 12 % SDS-PAGE and blotted 1h to PVDF. Blots were blocked with 5% skim milk for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1 000 for 1h at RT 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 (goat anti-rabbit IgG horse radish peroxidase conjugated, from Bio-Rad) diluted to 1:15 000 in for 1h at RT with agitation. The blot was washed as above and developed for 5 min with ECL (GE Heltcare) according to the manufacturer's instructions. Exposure time was 15 seconds.

M- protein ladder (Fermentas)
2- 20ul of medium before induction (PcGCE protein)
3-20ul of medium before induction (PcGCE S217N protein)
4-20ul of medium after 48h of induction (PcGCE protein)
5-20ul of medium after 48h of induction (PcGCE S217N protein)
6-20ul of medium after 96h of induction (PcGCE protein)
7-20ul of medium after 96h of induction (PcGCE S217N protein)

Courtesy of Dr. Marta Derba-Maceluch, UPSC, Umeå