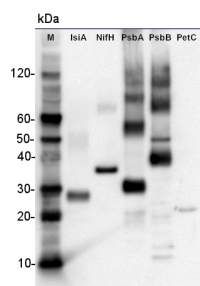
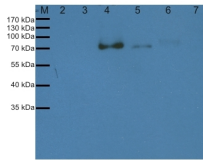


Product no **AS11 1771****His-tag | 6xHis (clone HIS,H8 / EH158)****Product information****Immunogen** | KLH-conjugated synthetic peptide 6xHis**Host** | Mouse**Clonality** | Monoclonal**Subclass/isotype** | IgG2b**Purity** | Total IgG fraction. Protein A purified.**Format** | Liquid**Quantity** | 50 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 7.2His-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** polyacrylamide 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.



20 µl of media from *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