Agrisera
Part of Olink® Group

This product is **for research use only** (not for diagnostic or therapeutic use) **contact: support@agrisera.com**

Agrisera AB | Box 57 | SE-91121 Vännäs | Sweden | +46 (0)935 33 000 | www.agrisera.com

Product no AS12 2112

PIF5 | Phytochrome interacting factor 5 (rabbit antibody)

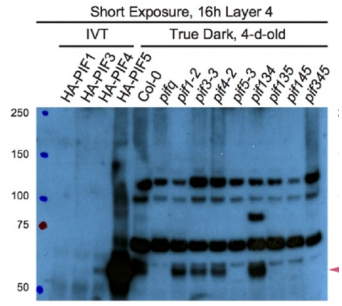
Product information

Immunogen	KLH-conjugated synthetic peptide derived from *Arabidopsis thaliana* PIF5, UniProt: Q84LH8, TAIR: AT3G59060
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.
Additional information	PIF proteins are not that stable, therefore special precautions should be taken during extraction and whole procedure should be performed in as little light as possible (light green light). Extraction of PIF proteins is described in Shen et al. (2007).

Application information

Recommended dilution	1 : 1000 (WB)
Expected | apparent MW	49.3 | 62 kDa
Confirmed reactivity	*Arabidopsis thaliana*
Not reactive in	*Physcomitrella patens, Solanum lycopersicum*
Additional information	For detection of PIF5 please use the most sensitive detection reagent
Selected references	Pham et al. (2018). Dynamic regulation of PIF5 by COP1-SPA complex to optimize photomorphogenesis in Arabidopsis. Plant J. 2018 Aug 25. doi: 10.1111/tpj.14074.

Application example



Total protein from *Arabidopsis thaliana* (10-20 µg) of 4-d-old dark grown Col-0, pif single, triple and quadruple mutants were extracted with the buffer described in Qiu et al. 2015 and denatured at 95°C for 5 min. were separated on 8 % Bis-Tris SDS-PAGE, and subsequently blotted to Nitrocellulose membrane using wet tank transfer. Blots were blocked with TBS containing 2% non-fat milk at room temperature for 1h. Primary anti-PIF5 antibodies were used in dilution of 1:1000 to TBS containing 2% milk. The incubation time was 14-20 hours at 4°C. After washing

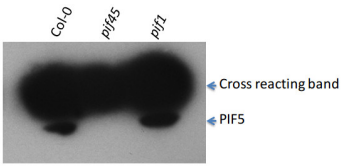
Agrisera
Part of Olink® Group

This product is **for research use only** (not for diagnostic or therapeutic use) **contact: support@agrisera.com**

Agrisera AB | Box 57 | SE-91121 Vännäs | Sweden | +46 (0)935 33 000 | www.agrisera.com

4x5 min. with TBST at room temperature, the blots were incubated with HRP-conjugated secondary antibodies (1:5000) at room temperature for 1h. The blots were washed 4x10 min with TBST again and the signal was detected using highest sensitivity chemiluminescent substrate.
PIF5 signal is marked by red.

Courtesy od Dr. Yongjian Qiu, University of California-Riverside, USA



10 µg of total protein from 4 day old dark grown *Arabidopsis thaliana* seedligns extracted with hot buffer (Shen et al., Plant Cell. 2008;20(6):1586-602) were separated on 8 % SDS-PAGE and blotted 1h to PVDF using semi-dry. Blots were blocked with the blocking buffer from Protein Detector LumiGLO western blotting kit (KPL, Cat: 54-12-50) for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1 000 for overnight at 4 degree with agitation. The antibody solution was decanted and the blot was for 3 times for 5 min in the washing buffer from the same kit at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, (AS09 602 from Agrisera) diluted to 1:25 000 in for 2h at RT with agitation. The blot was washed as above and developed for 5 min with high sensitivity chemiluminescent substrate according to the manufacturer's instructions. Exposure time was 2 minute.

Higher % of a gel can separate better cross-reacting band and PIF5 protein.

Courtesy of Dr. Ling Zhu, University of Texas, USA