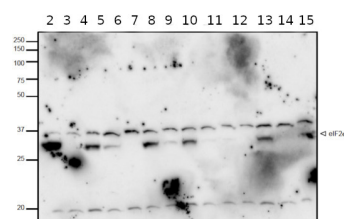


Product no **AS20 4447****Anti-eIF2-alpha-P | Phosphorylated Eukaryotic translation initiation factor 2 subunit alpha****Product information**

Immunogen	Immunogen <u>KLH</u> -conjugated, phosphorylated peptide derived from <i>Arabidopsis thaliana</i> eIF2-alpha, UniProt: <u>Q9FE78</u> , TAIR: <u>AT5G05470</u>
Host	Rabbit
Clonality	Polyclonal
Purity	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.

Application information

Recommended dilution	1 : 1000 (WB)
Expected apparent MW	38.8 kDa
Confirmed reactivity	<i>Arabidopsis thaliana</i>
Predicted reactivity	<i>Oryza sativa</i>
	Species of your interest not listed? Contact us
Selected references	To be added when available, antibody available in September 2021.

**Samples:**

Lane 1 (not shown, marker does not appear in chemiluminescence image): Bio-Rad Precision Plus All Blue prestained protein ladder
 Lane 2: *Arabidopsis thaliana* Ler wt seedlings treated with glyphosate for an unknown time (positive control for eIF2alpha phosphorylation)
 Lane 3: *Arabidopsis thaliana* Ler gcn2^{-/-} seedlings treated with glyphosate for an unknown time (negative control for eIF2alpha phosphorylation)
 Lanes 4-9 are the C24 ecotype, with lane 4 being the control C24 seedlings and lanes 5-9 being stressed Lanes 10-15 are the Col-0 ecotype, with lane 10 being the control Col-0 seedlings and lanes 11-15 being stressed Lane 5-15: *Arabidopsis thaliana* C24 wt seedlings treated with various stresses

Five *Arabidopsis thaliana* seedlings (6 days old) per sample were collected & stored at -80 until use. Each sample was ground in liquid nitrogen, and total protein was extracted using 1x SDS-PAGE sample buffer. Samples were denatured at 70°C for 10 minutes and separated on a 10% gel using SDS-PAGE. Transfer to PVDF membrane was done at 10V for 110 minutes using semi-dry transfer. Blot was blocked with Bio-Rad EveryBlot blocking buffer for 2 hours at room temperature. Blot was incubated with primary antibody (AgriSera #AS20 4447) at 1:1000 in EveryBlot buffer at 4°C overnight, then washed for 3 x 20 minutes with 1x TBS-T with agitation. Blot was incubated with secondary antibody (AgriSera #AS09 602) at 1:10,000 in EveryBlot buffer at room temperature for 2 hours, then washed for 3 x 20 minutes with 1x TBS-T with agitation. Blot was developed for 5 minutes with AgriSera ECLBright and exposed for 2 minutes on the Bio-Rad ChemiDoc imaging system.

Courtesy of Dr. Anna Nelson Dittrich, Boyce Thompson Institute, USA