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Product no AS21 4690 ASY1 | Asynapsis 1

Product information

Immunogen Recombinant ASY1 protein from *Hordeum vulgare*, UniProt: <u>A0A8I6YI54</u>

Host Rabbit

Clonality Polyclonal

Purity Antigen affinity purified serum, in PBS pH 7.4

Format Lyophilized

Quantity 50 μg

Reconstitution For reconstitution add 50 μl, of sterile or deionized water.

Storage Store lyophilized/reconstituted at -20 °C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from lyophilized

material adhering to the cap or sides of the tubes.

Application information

Recommended dilution 1:1000 (WB)

Expected | apparent

66.3 kDa

Confirmed reactivity | Hordeum vulgare

- Toracam valgar

Predicted reactivity Arabidopsis thaliana, Hordeum vulgare, Oryza sativa, Triticum aestivum, Zea mays

Species of your interest not listed? Contact us

Not reactive in No confirmed exceptions from predicted reactivity are currently known

Selected references To be added when available, antibody available in September 2022.



0.25 µg/well of total overexpressed *Hordeum vulgare* ASY1 protein extracted the previous day from Rosetta 2 pLysS cells with BugBuster master mix and Complete EDTA free protease inhibitor, captured with NiNTA, digested at 4°C overnight with ProTEV protease to remove the affinity tag, and run through a second round of NiNTA capture to remove the cleaved affinity tag. Each lane in the gel also includes His-tagged ASY1 protein. Denaturation was performed with LDS buffer, DTT, and Urea at 90°C for 10 min. Samples were separated on 4-12% NuPAGE Bis-Tris SDS-PAGE at 200v for 1h in MOPS buffer with 0.5 ml NuPAGE antioxidant then transferred to PVDF (pore size of 45 µm) at 11 v overnight in NuPAGE transfer buffer with 0.5 ml NuPAGE antioxidant. The blot was fixed in methanol, washed in SDW then blocked with 5 % milk 1h/RT with agitation. The blot was incubated in the primary antibody at a dilution of 1:5000 for 2h/RT with agitation in TBS-T. 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. The blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, <u>AS09 602</u>) diluted to 1:25000 in for 1h/RT with agitation. The blot was washed as above and developed for 3 min with <u>Agrisera ECLSuperBright</u>. The exposure time was 37 seconds.

Courtesy Jamie Orr, The James Hutton Institute, United Kindgdom