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Product no AS08 349 Anti-SUMO3 | Small ubiquitin-like modifier protein 3 (peptide antibody)

Product information

Immunogen	KLH-conjugated peptide derived from SUMO3 sequence of Arabidopsis thaliana, UniProt: Q9FLP5, TAIR: AT5G55170
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.

Application information

Recommended dilution	1 : 5000 for detection of recombinant SUMO3 (WB)
Confirmed reactivity	Arabidopsis thaliana
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Additional information	The antibody is recognizing recombinant SUMO3 and shows no cross reactivity to SUMO1/2
Selected references	Saleh et al. (2015). Posttranslational Modifications of the Master Transcriptional Regulator NPR1 Enable Dynamic but Tight Control of Plant Immune Responses. Cell Host Microbe. 2015 Aug 12;18(2):169-82. doi: 10.1016/j.chom.2015.07.005.

Application example

100-70-55-

Protein, tagged with a 6xhis-tag, was expressed from a strain of *E. coli* that reconstituted the sumoylation system with SUMO3 (described in Elrouby et al). Cells were harvested and lysated by sonication in buffer containing 6M urea. Proteins were used to load a Nickel column under denaturing conditions with buffers containing 6M urea. A pH gradient was applied to release the specifically bound proteins and 0.5 ml aliquots were collected. The presence of the desired protein in each aliquot was checked in SDS-PAGE by Comassie staining. 3 ul of the aliquot showing the most intense signal were separated on 10% SDS-PAGE and blotted 1h to PVDF using tank transfer. Blots were blocked with TBS-T (0.1%) and 5% dry-milk powder for 4 h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1: 5000 overnight at 4°C with agitation. The antibody solution was decanted and the blot was rinsed 4 times for 15 min in TBS-T (0.25%) at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, <u>AS09 602</u> from Agrisera) diluted to 1:50 000 in TBS-T (0.1%) and 5% dry-milk powder for 1 h at RT with agitation. The blot was washed as above and developed for 5 min with chemiluminescent detection reagent, according to the manufacturer's instructions. Exposure time was 15 second.

Courtesy of Dr. Concepción Almoguera, CSIC, Spain