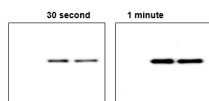


Product no **AS23 4970****Anti-Sumo tag****Product information**

Immunogen	Recombinant protein of Sumo-tag protein, expressed in <i>E.coli</i> .
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.

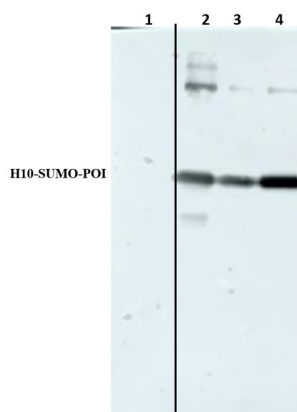
Application information

Recommended dilution	1 : 1000 (WB)
Expected apparent MW	Depending upon fusion partner
Confirmed reactivity	SUMO tag
Predicted reactivity	SUMO tag
Selected references	To be added when available. Antibody released in January 2025.



For conducted *in-vitro* assay, protein concentration of the purified protein was approximately 2.5 µg/well for the input in gel staining image; Proteins are expressed in Ecoli, Rosetta2 strain, and the protein origin is Arabidopsis thaliana. The sample buffer: 25mM HEPES and 100 mM NaCl and denatured using 5x Western (Protein) Loading dye to 1x, denatured at 70°C for 15 minutes. Samples were separated in the cold with 1xSDS running buffer, using BioRad mini protean apparatus with homemade 10% acryl SDS PAGE and blotted for 1h to Amersham Hybond P 0.45 µm pore size, PVDF membrane, using wet transfer (Tris-glycine buffer) with 5% MeOH at 4C, 18 Volt, overnight. Blot was blocked with 3% milk TBS-T for 1h/RT with agitation. The SUMO antibody was used at 1:2000 dilution in 1% milk TBS-T for 1h/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 matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1: 5 000 in for h/RT with agitation. The blot was washed as above and developed with a following chemiluminescent detection reagent and an image was captured after maximum 5 minutes.

Courtesy of M.Sc. Alaa Allahham, RWTH Aachen University, Germany



E. coli BL21 (DE3) Rosetta production of protein of interest (POI): H10-SUMO-POI.

Samples:

- 1- 15 µl of total fraction of cell lysate, before induction.
- 2- 15 µl of total fraction of cell lysate of IPTG (0.05mM) induced (ON, 16°C) BL21 (DE3) Rosetta.
- 3- 15 µl of soluble fraction of cell lysate of IPTG (0.05mM) induced (ON, 16°C) BL21 (DE3) Rosetta.

15 µl of protein extract from BL21 (DE3) Rosetta (all bacteria pellets were normalized to final DO of 6.5). Exact buffer components were: Tris-HCl 50mM, NaCl 500mM, 10% glycerol, pH:8 and denatured with 2X buffer (Tris-HCl 125mM, 20% glycerol, SDS 4%, BeOH, 2% and blue bromophenol 0.001%) at 95 °C 5min. Samples were separated in the cold on 10 % SDS-PAGE and blotted for 1h 30 minutes to nitrocellulose (0.45 µm), using: wet transfer in the cold. Blot was blocked with 5 % milk for: 2h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1000 for ON/4°C with agitation. The antibody solution was decanted, and the blot was rinsed briefly twice, then washed 3 times for 10 min in TBS-T at RT with agitation. Blot was incubated in matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1:10 000 in 5% fat milk TBT-T for 1 h/RT with agitation. The blot was washed as above and developed with a following chemiluminescent detection reagent. Exposure time was 40 seconds.

Courtesy of Dr. Pablo Cerdán, Fundación Instituto Leloir, Argentina