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Datasheet for 200-401-428S

SUMO Antibody

Overview

Description:	Anti-SUMO (Yeast) (RABBIT) Antibody - 200-401-428S
Item No.:	200-401-428S
Size:	25 µL
Applications:	ELISA, WB, ChIP, IP
Reactivity:	Broad
Host Species:	Rabbit

Product Details

Background:

Anti SUMO Antibody recognizes SUMO. Covalent modification of cellular proteins by the ubiquitin-like modifier SUMO (small ubiquitin-like modifier) regulates various cellular processes, such as nuclear transport, signal transduction, stress responses and cell cycle progression. But, in contrast to ubiquination, sumoylation does not tag proteins for degradation by the 26S proteasome, but rather seems to enhance stability or modulate their subcellular compartmentalization. Ubiquitin-like proteins fall into two classes: the first class, ubiquitin-like modifiers (UBLs) function as modifiers in a manner analogous to that of ubiquitin. Examples of UBLs are SUMO, Rub1 (also called Nedd8), Apg8 and Apg12. Proteins of the second class include parkin, RAD23 and DSK2, are designated ubiquitin-domain proteins (UDPs). These proteins contain domains that are related to ubiquitin but are otherwise unrelated to each other. In contrast to UBLs, UDPs are not conjugated to other proteins. Once covalently attached to cellular targets, SUMO regulates protein:protein and protein:DNA interactions, as well as localization and stability of the target protein. Sumoylation occurs in most eukaryotic systems, and SUMO is highly conserved from yeast to humans. Where invertebrates have only a single SUMO gene termed SMT3, three members of the SUMO family have been identified in vertebrates: SUMO-1 and the close homologues SUMO-2 and SUMO-3. SUMO has been called SMT3 (yeast), sentrin, PIC1, GMP1 and UBL1. SUMO has been shown to bind and regulate mammalian SP-RINGs (such as Mdm2, PIAS and PML), RanGAP1, RanBP2, p53, p73, HIPK2, TEL, c-Jun, Fas, Daxx, TNFRI, Topo-I, Topo-II, WRN, Sp100, IkB-a, Androgen receptor (AR), GLUT1/4, Drosophila Ttk69, Dorsal, CaMK, yeast Septins, and viral CMV-IE1/2, EBV-BZLF1, HPV/BPV-E1. These bindings implicate SUMO in the stabilization of the target proteins and/or their localization to subcellular complexes. SUMO has an apparent molecular weight of ~12kDa and human SUMO-1 (a 101 amino acid polypeptide) shares 50% sequence identity with SUMO-2 and SUMO-3 and with yeast SMT3. SUMO and ubiquitin only show about 18% homology, but both possess a common three-dimensional structure characterized by a tightly packed globular fold with b-sheets wrapped around an a-helix.

Synonyms:	rabbit anti-SUMO Antibody, Ubiquitin-like protein SMT3 antibody, SMT3 antibody, Ubiquitin like protein of the SUMO family antibody, SMT3_YEAST antibody, DmSUMO 1 antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	SMT3
Reactivity:	Broad
Immunogen Type:	Recombinant Protein
Immunogen:	This purified antibody was prepared from rabbit serum after repeated immunizations with recombinant yeast SUMO protein.
Purity/Specificity:	Anti-Sumo Antibody is an IgG fraction antibody purified from monospecific antiserum by a multi-step process which includes delipidation, salt fractionation and ion exchange chromatography followed by extensive dialysis against the buffer stated above. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum.
Relevant Links:	• UniProtKB - Q12306 • GeneID - 852122 • NCBI - 6320718

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	ChIP, IP (Based on references)
Application Note:	Anti-Sumo purified polyclonal antibody reacts yeast SUMO tested by western blot and ELISA. Although not tested, this antibody is likely functional in immunohistochemistry and immunoprecipitation. For immunoblotting a 1:1,000 dilution is recommended. A 12 kDa band corresponding to yeast SUMO is detected. Most yeast cell lysates can be used as a positive control without induction or stimulation. For ELISA a 1:1,000 to 1:5,000 dilution is recommended. Researchers should determine optimal titers for other applications.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ChIP:	User Optimized
ELISA:	1:5,000 - 1:25,000

IP:	User Optimized
WB:	1:500 - 1:3,000

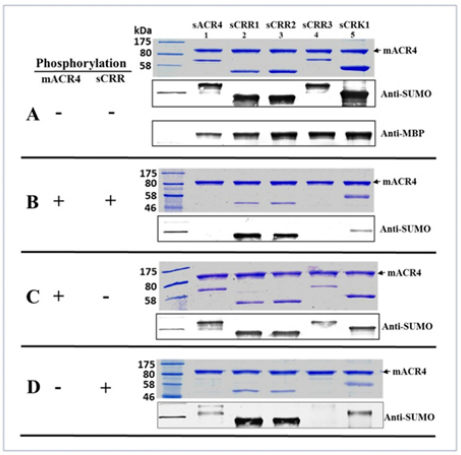
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

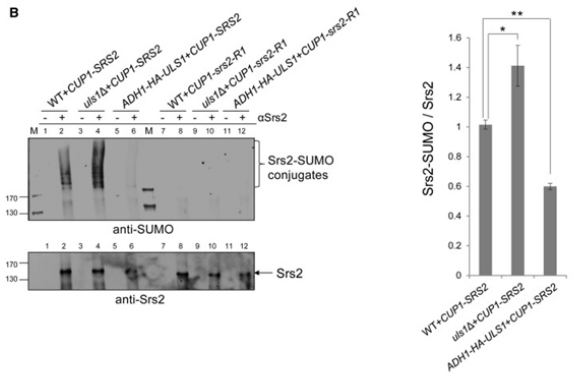
Images



Western Blot

Pull-down assays were performed to determine the effects of phosphorylation on interactions between the ICDs of ACR4 and the CRRs. The autophosphorylation status of each protein was varied for individual experiments, (+) denotes autophosphorylated protein, (-) denotes unphosphorylated protein. In (A), mACR4 (-) and sCRRs (-), (B) mACR4 (+) and sCRRs (+), (C) mACR4 (+) and sCRRs (-), (D) mACR4 (-) and sCRRs (+). In A-D, proteins were separated by 12% SDS-PAGE (top panels) and the presence of interacting proteins was determined by anti-SUMO (p/n 200-401-428) western blot (lower panels). In the bottom panel of A, anti-MBP (p/n 200-401-385) western blot demonstrates the presence of mACR4 in each reaction.

Fig 2. PMID: 25756623

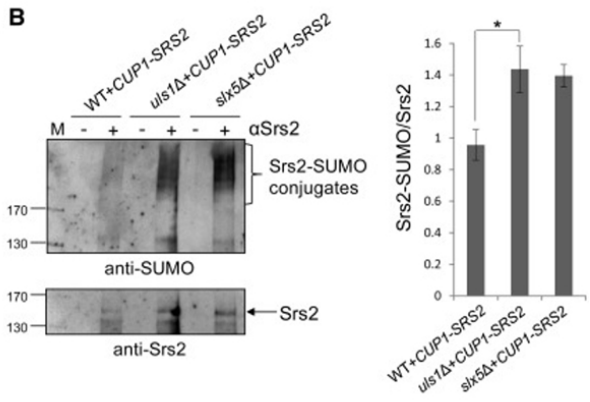


Western Blot

Srs2 is a target of Uls1 activity. (B) SUMOylation status of Srs2 is affected by activity of Uls1. Aliquots of protein extracts obtained in (A) were used for IP reaction with anti-Srs2 antibody. Immunoprecipitates were analyzed by immunoblotting with anti-Srs2 and anti-SUMO antibodies. Srs2-SUMO levels vs. total immunoprecipitated Srs2 from two experiments were quantified. SDs are shown and Student's t-test was used to calculate the P-value (* 0.01 < P-value ≤ 0.05, ** 0.001 < P-value ≤ 0.01).

Figure 2.

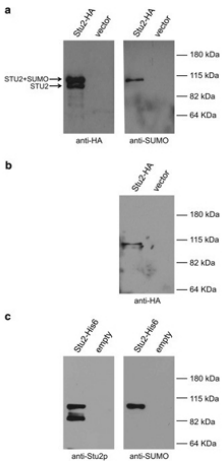
PMID: 28341648



Western Blot

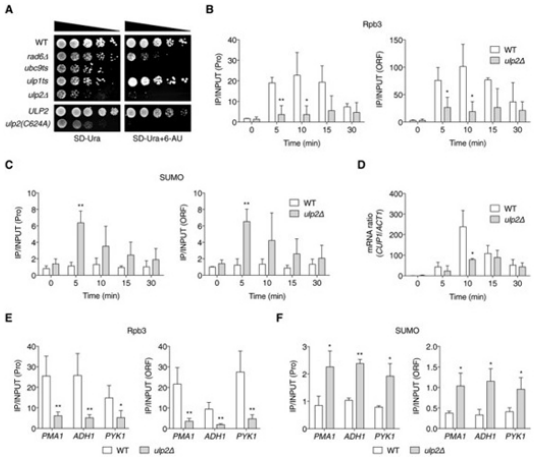
Comparison of the effect of SLX5 and ULS1 deletion on the accumulation of SUMOylated Srs2. (B) IP of Srs2 from extracts obtained in (A) and visualization of SUMOylated Srs2 using anti-Srs2 and anti-SUMO antibody. Srs2-SUMO levels vs. total immunoprecipitated Srs2 from two experiments were quantified. Figure 3.

PMID: 28341648



Western Blot

(a) SUMO co-IPs with Stu2p. Plasmid expressing Stu2-HA (pRM2119) or vector (pRM2200) where transformed into a ulp1-TS strain (yRM8139). Whole-cell lysates were prepared from cultures grown overnight to saturation, as described in Materials and Methods. Stu2-HA was immunoprecipitated using mouse anti-HA agarose beads (Sigma-Aldrich). The western blot was probed with rabbit anti-HA (Sigma-Aldrich). (b) Stu2p co-IPs with SUMO. SUMO was immunoprecipitated with anti-SUMO (Rockland, Inc. Gilbertsville, PA) from a ulp1-TS strain (yRM8139) containing Stu2-HA (pRM2119), or an empty vector, as described in Materials and Methods. The blot was probed with mouse anti-HA (Sigma-Aldrich). Saturated overnight cultures were used to prepare whole-cell extracts. (c) Stu2-his6 co-isolates with SUMO under denaturing conditions. Yeast whole-cell extracts containing Stu2-his6 (yRM9417) where incubated with Ni-NTA agarose in the presence of 8M urea. To analyze bands for co-reactivity, identical blots where probed with rabbit anti-Stu2p and anti-SUMO.
Figure 6. PMID: 29729126



ChIP

(A) Sensitivity of the indicated mutants to 6-AU. All strains carried a URA3 plasmid, pRS316, and were spotted on SD-Ura with or without 6-AU (100 µg/ml); plates were incubated for 2-4 days at 30°C. ULP2 and ulp2(C624A) represent ulp2Δ::HIS3 cells containing either pRS314-ULP2-FLAG or pRS314-ulp2(C624A)-FLAG.

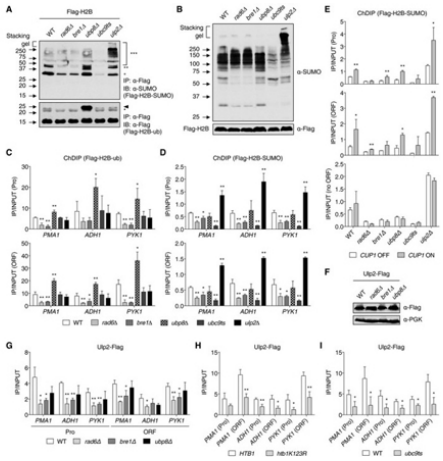
(B, C) ChIP analyses using anti-Rpb3 (B) and anti-SUMO (C) antibodies in ulp2Δ cells as in Fig 1F. Error bars indicate the SD from three independent experiments.

(D) qRT–PCR analysis of CUP1 mRNA levels in ulp2Δ cells. Data were normalized to ACT1 mRNA levels. CUP1 gene induction was performed as in Fig 1F. Error bars represent the SD from three RNA samples.

(E, F) ChIP assays using anti-Rpb3 (E) and anti-SUMO (F) antibodies in ulp2Δ cells as done in Fig 1E. “Pro” denotes the #1 PCR product of PMA1, ADH1, and PYK1 genes described in Fig 1D, while “ORF” indicates the #3 products of PMA1 and #2 of ADH1 and PYK1; these are used in all ensuing figures except where specified. Error bars indicate the SD from four (E) and three (F) independent assays.

Data information: Asterisks indicate statistically significant differences determined by pairwise comparisons between WT and ulp2Δ using a two-tailed Student's t-test (*P < 0.05; **P < 0.01). See Dataset EV2 for qPCR raw data.

Figure 3. PMID: 31313851



Immunoprecipitation

(A) Immunoblot analysis of immunoprecipitated Flag-tagged histone H2B using anti-Flag or anti-SUMO antibodies in the indicated mutants, as described in Fig 4A. The upper and lower panels show Flag-H2B-SUMO and Flag-H2B-ub, respectively. One, two, or three asterisks indicate mono-, di-, and polysumoylated histones (upper panel), respectively, and an arrowhead and bullet point represent mono-ubiquitylated H2B, and a non-specific band, respectively.

(B) Immunoblot assay of sumoylated proteins in extracts prepared from the strains in (A). Anti-Flag blotting for Flag-H2B was used to verify the similar loading.

(C) Chromatin double immunoprecipitation (ChDIP) of Flag-H2B-ub in the indicated strains expressing Flag-H2B. The first ChIP was performed with anti-Flag agarose, and the eluted samples were immunoprecipitated with an antibody against ubiquitin. PCR signals from the indicated genes were normalized to an internal control and the input DNA. The error bars indicate the SD from three independent chromatin samples.

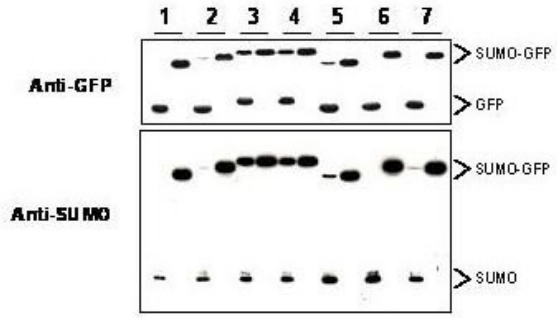
(D) ChDIP of Flag-H2B-SUMO in the indicated strains expressing both Flag-H2B and HA-SUMO. Chromatin samples were sequentially immunoprecipitated with anti-HA agarose followed by anti-Flag agarose. PCR signals were normalized to the input DNA. The error bars indicate the SD from three independent experiments.

(E) ChDIP of Flag-H2B-SUMO in the indicated mutant strains during CUP1 gene induction, analyzed as in (D).
The CUP1 induction was performed as in Fig 1F. CUP1 OFF and ON indicate uninduced and induced (5 min) conditions, respectively. The error bars represent the SD from three independent ChDIP assays.

(F) Immunoblot assay of Flag-tagged Ulp2 in the indicated strains. Anti-PGK blotting shows similar loading.

(G–I) ChIP assays in the indicated strains expressing Flag-tagged Ulp2 as in Fig 1E. The error bars indicate the SD from three experiments.

Data information: Asterisks indicate statistically significant differences compared with WT in (C, D, G, H, I) and significant differences between uninduced and induced cells in (E) (*P < 0.05; **P < 0.01). See Dataset EV2 for qPCR raw data.Source data are available online for this figure.
Figure 5.
PMID: 31313851



Western Blot
Western blot of SUMO-GFP fusion proteins cleaved by insect cell protein extracts. Anti-SUMO antibody, generated by immunization with recombinant yeast SUMO, was tested by western blot against several constructs of SUMO-GFP fusion proteins after cleavage by proteases in insect cell protein extracts. These constructs contained various linkers between the SUMO and GFP portion of the fusion proteins. Each sample was run twice. The left lanes each contain 2 µg E. coli expressed and purified SUMO-GFP fusion proteins after incubation with lysed cells (50 µg total protein) for 1 h. The right lanes contain the same fusion proteins incubated with the lysate in the presence of 2% SDS. After probing with Anti-GFP antibodies the membranes were stripped of antibody using SDS-DTT solution for 30 m at 60°C and were then re-probed using the Anti-SUMO antibody at a 1:1000 dilution incubated overnight at 4°C in 5% non-fat dry milk in TTBS. Detection occurred using a 1:2000 dilution of HRP-labeled Donkey Anti-Rabbit IgG (code # 611-703-127) for 1 hour at room temperature. A chemiluminescence system was used for signal detection (Roche). Other detection systems will yield similar results.

References

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