abcam

Product datasheet

Anti-Sumo 1 antibody [Y299] - ChIP Grade ab32058

KO VALIDATED Recombinant RabMAb

10 Abreviews 90 References 16 Images

Overview

Product name	Anti-Sumo 1 antibody [Y299] - ChIP Grade
Description	Rabbit monoclonal [Y299] to Sumo 1 - ChIP Grade
Host species	Rabbit
Specificity	ab32058 recognises small ubiquitin-related modifier-1 (SUMO-1), also known as SMT3, Sentrin, GMP1 UBL1 and PIC1.
Tested applications	**Suitable for:** Flow Cyt (Intra), ChIP, WB, IHC-P, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, A549, C6 and NIH/3T3 cell lysates. Wild-type HAP1 whole cell lysate. IHC-P: Human endometrium, lung carcinoma and bladder carcinoma tissue. Rat stomach tissue. Mouse kidney tissue. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells. ChIP: Chromatin prepared from SK-OV-3 cells. IP: NIH/3T3 cell lysate.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	Y299
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32058 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/20 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ChIP	★★★★☆ (1)	Use 5 µg for 25 µg of chromatin.
WB	★★★★☆ (4)	1/1000 - 1/5000. Detects a band of approximately 12 kDa (predicted molecular weight: 12 kDa).
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	★★★★★ (3)	1/250 - 1/500.
IP		1/20.

Target

Function Ubiquitin-like protein that can be covalently attached to proteins as a monomer or a lysine-linked polymer. Covalent attachment via an isopeptide bond to its substrates requires prior activation by the E1 complex SAE1-SAE2 and linkage to the E2 enzyme UBE2I, and can be promoted by E3 ligases such as PIAS1-4, RANBP2 or CBX4. This post-translational modification on lysine residues of proteins plays a crucial role in a number of cellular processes such as nuclear transport, DNA replication and repair, mitosis and signal transduction. Involved for instance in targeting RANGAP1 to the nuclear pore complex protein RANBP2. Polymeric SUMO1 chains are also susceptible to polyubiquitination which functions as a signal for proteasomal degradation of modified proteins. May also regulate a network of genes involved in palate development.

Involvement in disease Defects in SUMO1 are the cause of non-syndromic orofacial cleft type 10 (OFC10) [MIM:613705]; also called non-syndromic cleft lip with or without cleft palate 10. OFC10 is a birth defect consisting of cleft lips with or without cleft palate. Cleft lips are associated with cleft palate in two-third of cases. A cleft lip can occur on one or both sides and range in severity from a simple notch in the upper lip to a complete opening in the lip extending into the floor of the nostril and involving the upper gum. Note=A chromosomal aberation involving SUMO1 is the cause of OFC10. Translocation t(2;8)(q33.1;q24.3). The breakpoint occurred in the SUMO1 gene and resulted in haploinsufficiency confirmed by protein assays.

Sequence similarities Belongs to the ubiquitin family. SUMO subfamily.
Contains 1 ubiquitin-like domain.

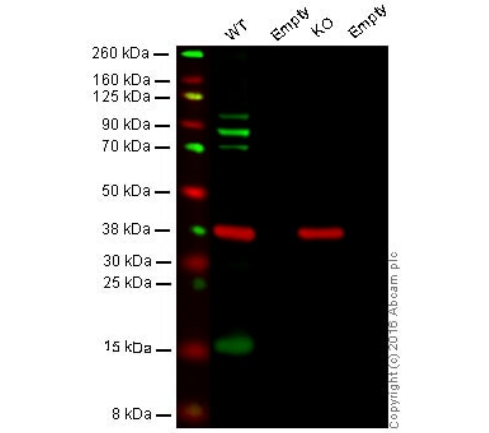
Post-translational modifications	Cleavage of precursor form by SENP1 or SENP2 is necessary for function. Polymeric SUMO1 chains undergo polyubiquitination by RNF4.
Cellular localization	Nucleus membrane. Nucleus speckle. Cytoplasm. Recruited by BCL11A into the nuclear body.

Images

ChIP - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

Chromatin was prepared from SK-OV-3 (Human ovarian cancer cell line) cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 5µg of ab32058 (red, and 20µl of Anti rabbit IgG sepharose beads. 5µg of rabbit normal IgG was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

ChIP was performed according to the literature (PMID: 23770046).

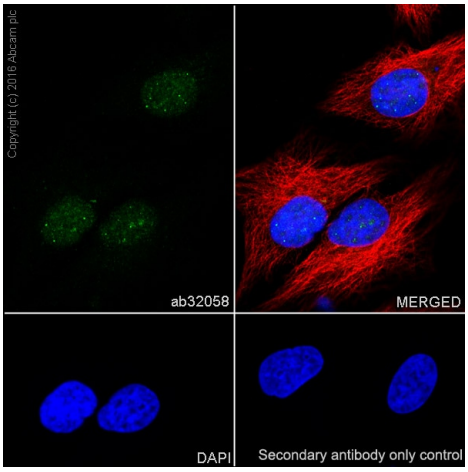


Western blot - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: Empty
Lane 3: Sumo 1 knockout HAP1 whole cell lysate (20 µg)
Lane 4: Empty

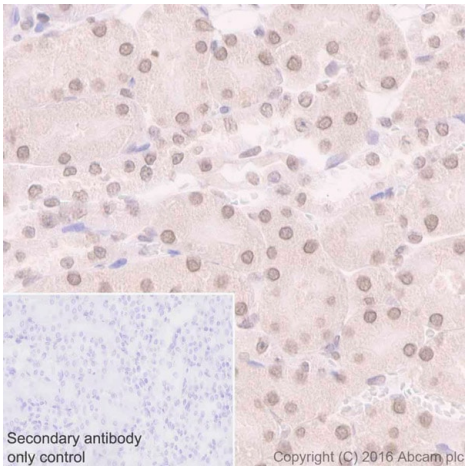
Lanes 1 - 4: Merged signal (red and green). Green - ab32058 observed at 16 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32058 was shown to react with Sumo 1 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when Sumo 1 knockout samples were used. Wild-type and Sumo 1 knockout samples were subjected to SDS-PAGE. Samples were incubated with ab32058 and **ab8245** (Mouse anti GAPDH loading control) overnight at 4°C at a 1/1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

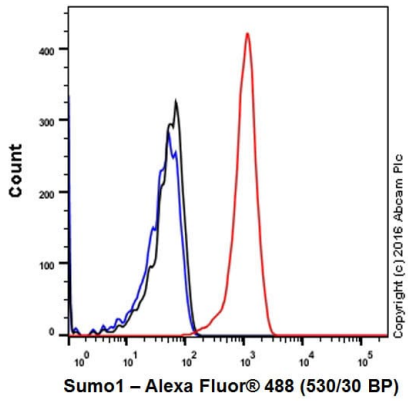
ab32058 staining Sumo 1 in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/500. A goat anti rabbit IgG (Alexa Fluor® 488) (**ab150077**) was used as the secondary antibody at a dilution of 1/1000. **ab195889** was used as a counterstain for primary antibody **ab133645** at 1/200. DAPI was used as a nuclear counterstain and PBS as a negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

ab32058 staining Sumo 1 in mouse kidney tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/250. A goat anti-rabbit IgG H&L (HRP) **ab97051** was used as the secondary antibody at a dilution of 1/500.

Negative control 1: PBS in place of primary antibody.

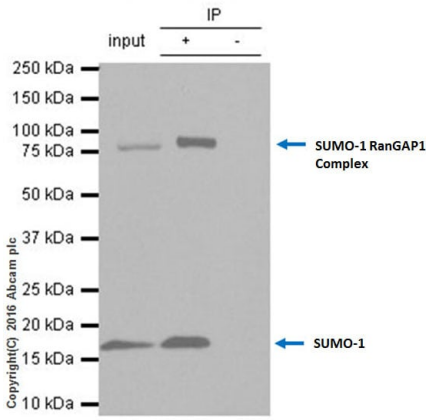


Flow Cytometry (Intracellular) - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

ab32058 staining Sumo 1 in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/20. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

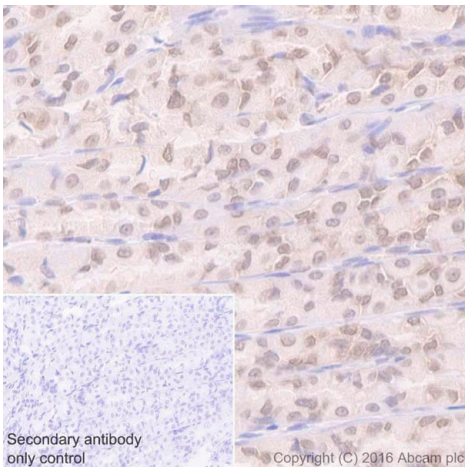
Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Immunoprecipitation - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

ab32058 immunoprecipitating Sumo 1. 10µg of NIH/3T3 (Mouse embryonic fibroblast) cell lysate was incubated with primary antibody at a dilution of 1/20 and VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at a dilution of 1/1000.

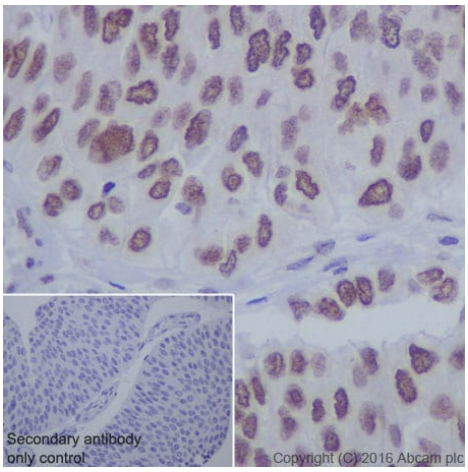
Lane 1: NIH/3T3 whole cell lysate 10ug
Lane 2: ab32058 IP in NIH/3T3 whole cell lysate
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab32058 in NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

ab32058 staining Sumo 1 in rat stomach tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/250. A goat anti-rabbit IgG H&L (HRP) **ab97051** was used as the secondary antibody at a dilution of 1/500.

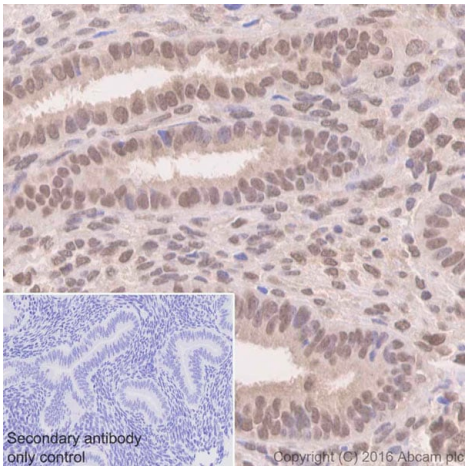
Negative control 1: PBS in place of primary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

ab32058 staining Sumo 1 in human bladder carcinoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/250. A goat anti-rabbit IgG H&L (HRP) **ab97051** was used as the secondary antibody at a dilution of 1/500.

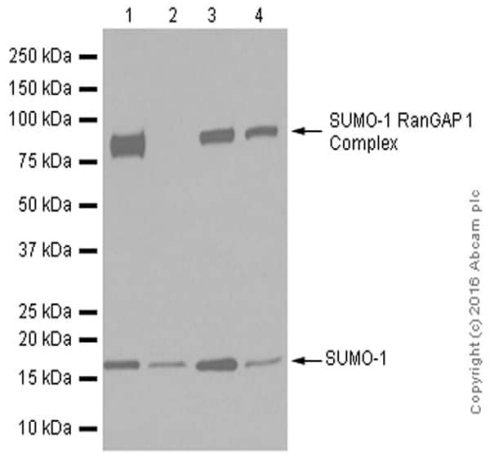
Negative control 1: PBS in place of primary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

ab32058 staining Sumo 1 in human endometrium tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/250. A goat anti-rabbit IgG H&L (HRP) **ab97051** was used as the secondary antibody at a dilution of 1/500.

Negative control 1: PBS in place of primary antibody.



Western blot - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

All lanes : Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058) at 1/5000 dilution

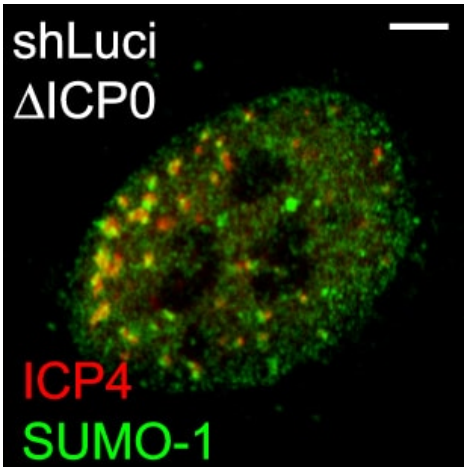
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : A549 (Human lung carcinoma epithelial cell) whole cell lysate
Lane 3 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate
Lane 4 : C6 (Rat glial tumor glial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 12 kDa

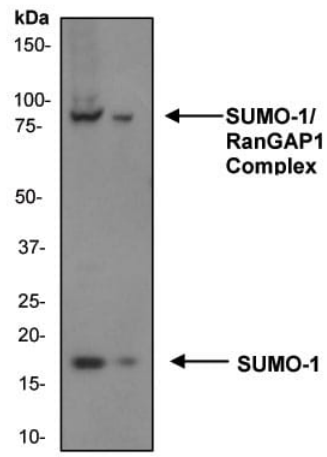
Blocking and diluting buffer: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

Image from Cuchet-Lourenço D et al., PLoS Pathog. 2011 Jul;7(7):e1002123. Epub 2011 Jul 14. Fig 9.; doi:10.1371/journal.ppat.1002123; July 14, 2011, PLoS Pathog 7(7): e1002123.

Immunofluorescence analysis of ICP0-null mutant HSV-1 infected HepaRG cells, staining Sumo1 (green) with ab32058. An AlexaFluor®-conjugated goat anti-rabbit IgG was used as the seconday antibody.

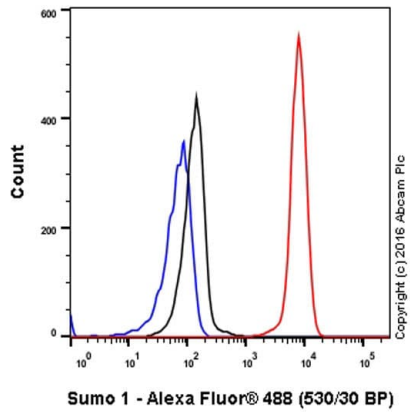


Western blot - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

All lanes : Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058) at 1/1000 dilution

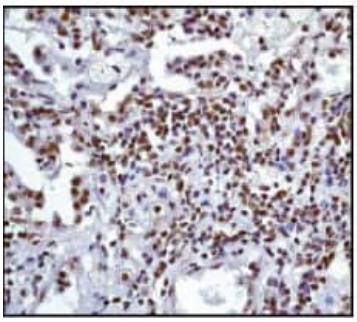
Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 2 : NIH/3T3 (Mouse embryo fibroblast cell line) cell lysate

Predicted band size: 12 kDa
Observed band size: 12,80 kDa



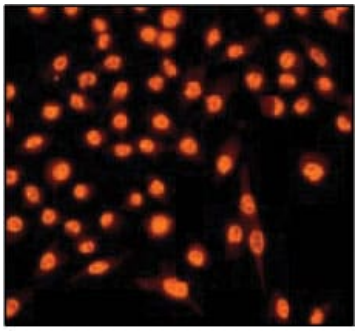
Flow Cytometry (Intracellular) - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labelling Sumo 1 with ab32058 at 1/20 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. An Alexa Fluorr®488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

IHC of paraffin-embedded human lung carcinoma using anti-SUMO 1 (ab32058) diluted 1:250



Immunocytochemistry/ Immunofluorescence - Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

Immunofluorescent staining of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells using anti-SUMO 1 (ab32058) diluted 1/250.

Anti-Sumo 1 antibody [Y299] - ChIP Grade (ab32058)

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