abcam

Product datasheet

Anti-Sumo 1 antibody [Y299] - BSA and Azide free ab219724



Recombinant

RabMAb

1 References 15 Images

Overview

Product name Anti-Sumo 1 antibody [Y299] - BSA and Azide free

Description Rabbit monoclonal [Y299] to Sumo 1 - BSA and Azide free

Host species Rabbit

Specificity This antibody recognises small ubiquitin-related modifier-1 (SUMO-1), also known as SMT3,

Sentrin, GMP1 UBL1 and PIC1.

Tested applications Suitable for: ICC/IF, Flow Cyt (Intra), IP, WB, IHC-P, ChIP

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 ab219724 is the carrier-free version of <u>ab32058</u>.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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

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- 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. Do Not Freeze.

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal

Clone number Y299
Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab219724 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
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 12 kDa (predicted molecular weight: 12 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ChIP		Use at an assay dependent concentration.

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.

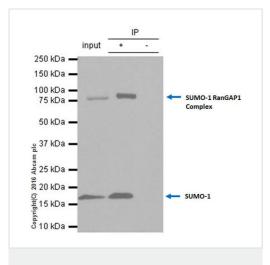
Post-translational modifications

Cleavage of precursor form by SENP1 or SENP2 is necessary for function.

Polymeric SUMO1 chains undergo polyubiquitination by RNF4.

Cellular localizationNucleus membrane. Nucleus speckle. Cytoplasm. Recruited by BCL11A into the nuclear body.

Images



Immunoprecipitation - Anti-Sumo 1 antibody [Y299] - BSA and Azide free (ab219724)

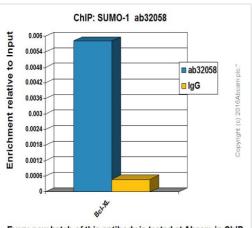
<u>ab32058</u> immunoprecipitating Sumo 1. 10μg of NIH/3T3 (Mouse embryonic fibroblast) cell lysate was incubated with primary antibody at a dilution of 1/20 and VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at a dilution of 1/1000.

Lane 1: NIH/3T3 whole cell lysate 10ug

Lane 2: ab32058 IP in NIH/3T3 whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab32058</u> in

NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate



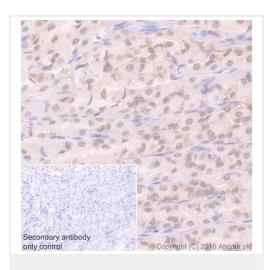
Every new batch of this antibody is tested at Abcam in ChIP.

ChIP - Anti-Sumo 1 antibody [Y299] - BSA and Azide free (ab219724)

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 (blue), and 20µl of Anti rabbit lgG sepharose beads. 5µg of rabbit normal lgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

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

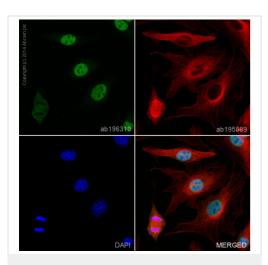
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32058).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sumo 1 antibody [Y299] - BSA and Azide free (ab219724)

ab32058 staining Sumo 1 in rat stomach tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded 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.



Immunocytochemistry/ Immunofluorescence - Anti-Sumo 1 antibody [Y299] - BSA and Azide free (ab219724)

ab196533 ab195887

Immunocytochemistry/ Immunofluorescence - Anti-Sumo 1 antibody [Y299] - BSA and Azide free (ab219724)

Clone Y299 (ab219724) has been successfully conjugated by Abcam. This image was generated using Anti-Sumo 1 antibody [Y299] (Alexa Fluor® 488). Please refer to **ab196310** for protocol details.

ab196310 staining Sumo 1 in NIH3T3 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilised in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab196310 at 1/50 dilution (shown in green) and ab195889, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor[®] 594, shown in red) at 1/167 dilution overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

This product gave a positive signal in 100% methanol (5 min) fixed NIH3T3 cells under the same testing conditions.

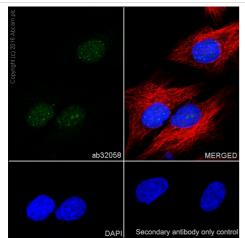
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Clone Y299 (ab219724) has been successfully conjugated by Abcam. This image was generated using Anti-Sumo 1 antibody [Y299] (Alexa Fluor® 647). Please refer to ab196533 for protocol details.

<u>ab196533</u> staining Sumo 1 in NIH3T3 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilised in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with <u>ab196533</u> at 1/50 dilution (shown in red) and <u>ab195887</u>, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor[®] 594, shown in green) at 1/167 dilution overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

This product gave a positive signal in 100% methanol (5 min) fixed NIH3T3 cells under the same testing conditions.

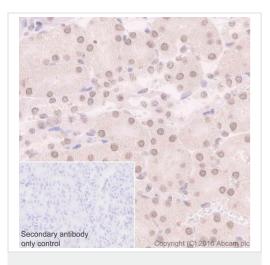
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-Sumo 1 antibody [Y299] - BSA and Azide free (ab219724)

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.

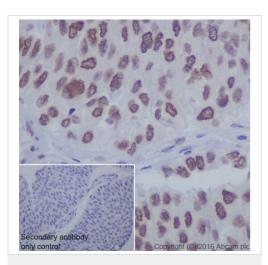
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32058).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sumo 1 antibody [Y299] - BSA and Azide free (ab219724)

ab32058 staining Sumo 1 in mouse kidney tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded 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.

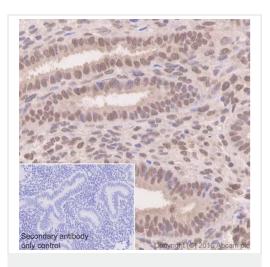


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sumo 1 antibody [Y299] - BSA and Azide free (ab219724)

<u>ab32058</u> staining Sumo 1 in human bladder carcinoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehydefixed, 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) <u>ab97051</u> was used as the secondary antibody at a dilution of 1/500.

Negative control 1: PBS in place of primary antibody.

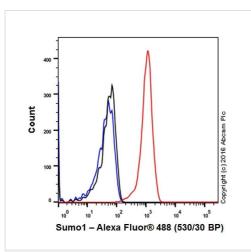
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32058).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sumo 1 antibody [Y299] - BSA and Azide free (ab219724)

ab32058 staining Sumo 1 in human endometrium tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded 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.

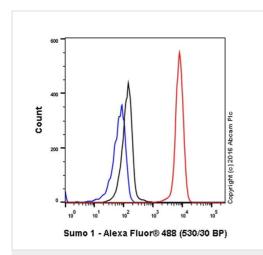


Flow Cytometry (Intracellular) - Anti-Sumo 1 antibody [Y299] - BSA and Azide free (ab219724) <u>ab32058</u> staining Sumo 1 in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with 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.

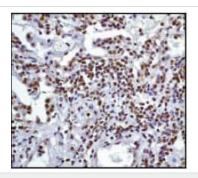
Isoytype control: Rabbit monoclonal IgG (Black)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32058).



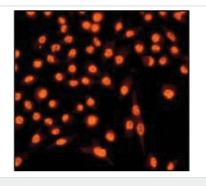
Flow Cytometry (Intracellular) - Anti-Sumo 1 antibody [Y299] - BSA and Azide free (ab219724) Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labelling Sumo 1 with **ab32058** at 1/20 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. An Alexa Fluor[®]488-conjugated goat anti-rabbit lgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal lgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sumo 1 antibody [Y299] - BSA and Azide free (ab219724)

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

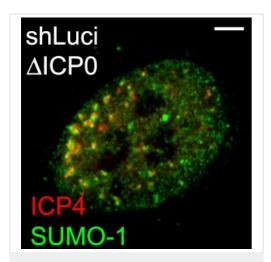
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32058).



Immunocytochemistry/ Immunofluorescence - Anti-Sumo 1 antibody [Y299] - BSA and Azide free (ab219724)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32058).



Immunocytochemistry/ Immunofluorescence - Anti-Sumo 1 antibody [Y299] - BSA and Azide free (ab219724)

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 <u>ab32058</u>. An AlexaFluor®-conjugated goat anti-rabbit lgG was used as the seconday antibody.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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