abcam

Product datasheet

Anti-SUFU antibody ab28083

KO VALIDATED

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Overview

Product name	Anti-SUFU antibody
Description	Rabbit polyclonal to SUFU
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat, Chicken, Dog
Immunogen	Synthetic peptide corresponding to Human SUFU aa 450 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. (Peptide available as **ab28932**, **ab28933**)
Positive control	WB: HeLa, LNCaP, HEK293T, Jurkat, A431 cell lysates; Recombinant human SUFU protein (**ab113584**); Mouse testis tissue lysate. ICC/IF: HeLa cell lysate.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab28083 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 a concentration of 5 µg/ml.
WB	★★★★★ (2)	Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 54 kDa (predicted molecular weight: 54 kDa).

Target

Function Negative regulator in the hedgehog signaling pathway. Down-regulates GLI1-mediated transactivation of target genes (PubMed:15367681, PubMed:24311597, PubMed:24217340). Down-regulates GLI2-mediated transactivation of target genes (PubMed:24311597, PubMed:24217340). Part of a corepressor complex that acts on DNA-bound GLI1. May also act by linking GLI1 to BTRC and thereby targeting GLI1 to degradation by the proteasome. Sequesters GLI1, GLI2 and GLI3 in the cytoplasm, this effect is overcome by binding of STK36 to both SUFU and a GLI protein (PubMed:10806483, PubMed:24217340). Negative regulator of beta-catenin signaling. Regulates the formation of either the repressor form (GLI3R) or the activator form (GLI3A) of the full length form of GLI3 (GLI3FL). GLI3FL is complexed with SUFU in the cytoplasm and is maintained in a neutral state. Without the Hh signal, the SUFU-GLI3 complex is recruited to cilia, leading to the efficient processing of GLI3FL into GLI3R. When Hh signaling is initiated, SUFU dissociates from GLI3FL and the latter translocates to the nucleus, where it is phosphorylated, destabilized, and converted to a transcriptional activator (GLI3A). Required for normal embryonic development. Required for the proper formation of hair follicles and the control of epidermal differentiation.

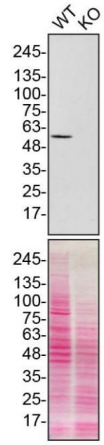
Tissue specificity Ubiquitous in adult tissues. Detected in osteoblasts of the perichondrium in the developing limb of 12-week old embryos. Isoform 1 is detected in fetal brain, lung, kidney and testis. Isoform 2 is detected in fetal testis, and at much lower levels in fetal brain, lung and kidney.

Involvement in disease Medulloblastoma

Sequence similarities Belongs to the SUFU family.

Cellular localization Cytoplasm. Nucleus.

Images



Western blot - Anti-SUFU antibody (ab28083)

All lanes : Anti-SUFU antibody (ab28083) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : sufU knockout HEK293T cell lysate

Lysates/proteins at 20 µg per lane.

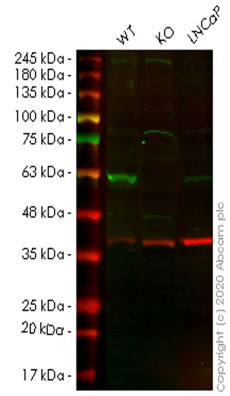
Secondary
All lanes : goat anti-rabbit HRP at 0.2 µg/ml

Performed under reducing conditions.

Predicted band size: 54 kDa

ab28083 was shown to react with sufU in wild-type HEK293T cells in Western blot with loss of signal observed in sufU knockout cell line **ab267282** (sufU knockout cell lysate **ab257718**). Wild-type HEK293T and sufU knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab28083 overnight at 4 °C at a 1/1000 dilution. Blots were incubated with goat anti-rabbit HRP secondary antibodies at 0.2µg/mL before imaging.

These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.



Western blot - Anti-SUFU antibody (ab28083)

All lanes : Anti-SUFU antibody (ab28083) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : SUFU knockout HEK293T cell lysate
Lane 3 : LNCaP cell lysate

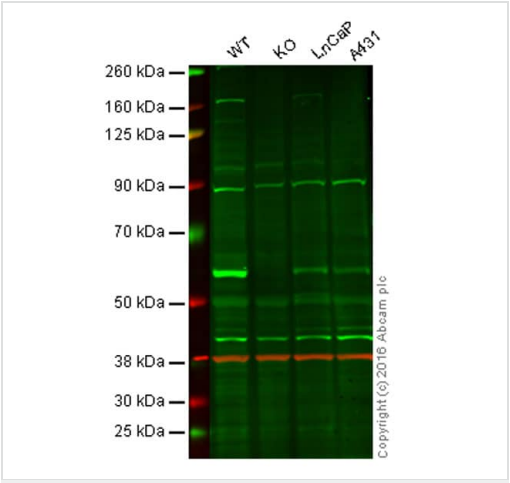
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 54 kDa
Observed band size: 58 kDa

Lanes 1-3: Merged signal (red and green). Green - ab28083 observed at 58 kDa. Red - loading control **ab8245** observed at 36 kDa.

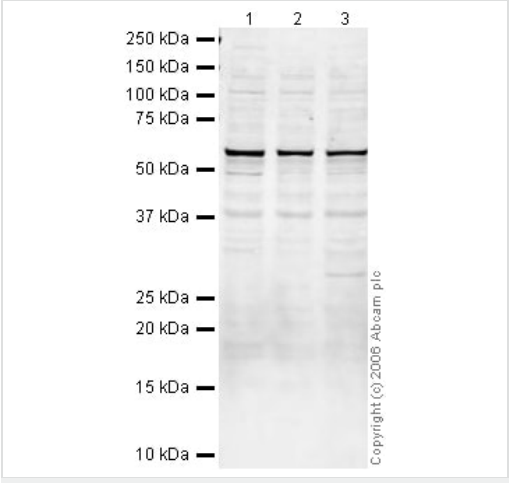
ab28083 Anti-SUFU antibody was shown to specifically react with SUFU in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab267282** (knockout cell lysate **ab257718**) was used. Wild-type and SUFU knockout samples were subjected to SDS-PAGE. ab28083 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2. 5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-SUFU antibody (ab28083)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: SUFU knockout HAP1 cell lysate (20 µg)
Lane 3: LnCaP cell lysate (20 µg)
Lane 4: A431 cell lysate (20 µg)
Lanes 1 to 4: Merged signal (red and green). Green - ab28083 observed at 58 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab28083 was shown to recognize SUFU when SUFU knockout samples were used, along with additional cross-reactive bands. Wild-type and SUFU knockout samples were subjected to SDS-PAGE. ab28083 and **ab8245** (loading control to GAPDH) were both diluted at 1 µg/ml and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-SUFU antibody (ab28083)

All lanes : Anti-SUFU antibody (ab28083) at 1 µg/ml

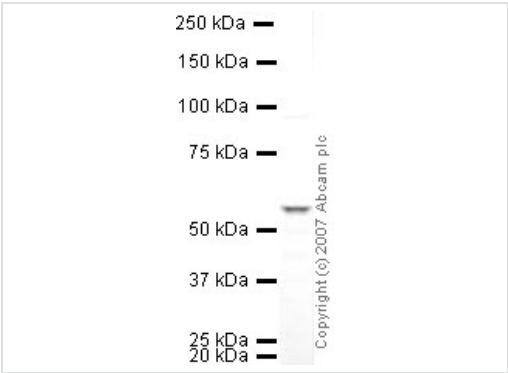
Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : Jurkat whole cell lysate (**ab7899**)
Lane 3 : A-431 whole cell lysate (**ab7909**)

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat polyclonal to Rabbit IgG (Alexa Fluor® 680) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 54 kDa
Observed band size: 54 kDa
Additional bands at: 37 kDa (possible cleavage fragment), 37 kDa (possible cross reactivity)



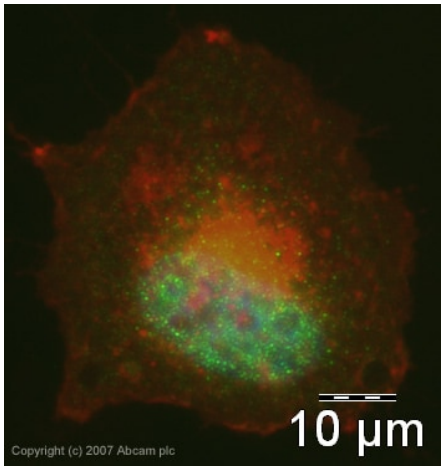
Western blot - Anti-SUFU antibody (ab28083)

Anti-SUFU antibody (ab28083) at 1 µg/ml + Testis (Mouse) Tissue Lysate - normal tissue at 10 µg

Secondary
IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 54 kDa
Observed band size: 54 kDa



Immunocytochemistry/ Immunofluorescence - Anti-SUFU antibody (ab28083)

ICC/IF image of ab28083 stained human HeLa cells. The cells were PFA fixed (10 min), permabilised in TBS-T (20 min) and incubated with the antibody (ab28083, 5µg/ml) for 1h at room temperature. 1%BSA / 10% normal goat serum / 0.3M glycine was used to quench autofluorescence and block non-specific protein-protein interactions. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red). DAPI was used to stain the cell nuclei (blue).

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