abcam

# Product datasheet

# Anti-SIRT6 antibody [EPR18463] ab191385

KO VALIDATED Recombinant RabMAb

★★★★★ 2 Abreviews 16 References 11 Images

## Overview

| | |
|---|---|
| **Product name** | Anti-SIRT6 antibody [EPR18463] |
| **Description** | Rabbit monoclonal [EPR18463] to SIRT6 - ChIP Grade |
| **Host species** | Rabbit |
| **Tested applications** | **Suitable for:** ICC/IF, IP, WB, ChIP-sequencing |
| **Species reactivity** | **Reacts with:** Mouse, Rat, Human |
| **Immunogen** | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. |
| **Positive control** | WB: HeLa, Jurkat, NIH/3T3, C6, RAW 264.7 and PC-12 cell lysates; HeLa nuclear lysate; rat brain and spleen lysates. ICC/IF: HeLa and HCT 116 cells. IP: Jurkat whole cell lysate. ChIP-seq: HeLa cells. |
| **General notes** | This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information see here.<br>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 long term. Avoid freeze / thaw cycle. |
| **Storage buffer** | pH: 7.2<br>Preservative: 0.01% Sodium azide<br>Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | EPR18463 |

**Isotype** IgG

## Applications

**The Abpromise guarantee** Our Abpromise guarantee covers the use of ab191385 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** | | 1/1000. |
| **IP** | | 1/40. |
| **WB** | ★★★★★ (1) | 1/2000. Detects a band of approximately 42 kDa (predicted molecular weight: 39 kDa). |
| **ChIP-sequencing** | | Use 8µg for $10^7$ cells. |

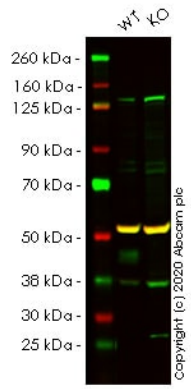
## Target

**Function** NAD-dependent protein deacetylase. Has deacetylase activity towards histone H3K9Ac and H3K56Ac. Modulates acetylation of histone H3 in telomeric chromatin during the S-phase of the cell cycle. Deacetylates histone H3K9Ac at NF-kappa-B target promoters and may down-regulate the expression of a subset of NF-kappa-B target genes. Acts as a corepressor of the transcription factor HIF1A to control the expression of multiple glycolytic genes to regulate glucose homeostasis. Required for genomic stability. Regulates the production of TNF protein. Has a role in the regulation of life span (By similarity). Deacetylation of nucleosomes interferes with RELA binding to target DNA. May be required for the association of WRN with telomeres during S-phase and for normal telomere maintenance. Required for genomic stability. Required for normal IGF1 serum levels and normal glucose homeostasis. Modulates cellular senescence and apoptosis. On DNA damage, promotes DNA end resection via deacetylation of RBBP8. Has very weak deacetylase activity and can bind NAD(+) in the absence of acetylated substrate.

**Sequence similarities** Belongs to the sirtuin family. Class IV subfamily.
Contains 1 deacetylase sirtuin-type domain.

**Cellular localization** Nucleus, nucleoplasm. Predominantly nuclear. Associated with telomeric heterochromatin regions.

## Images




Western blot - Anti-SIRT6 antibody [EPR18463] (ab191385)

**All lanes :** Anti-SIRT6 antibody [EPR18463] (ab191385) at 1/1000 dilution

**Lane 1 :** Wild-type HeLa cell lysate
**Lane 2 :** SIRT6 knockout HeLa cell lysate

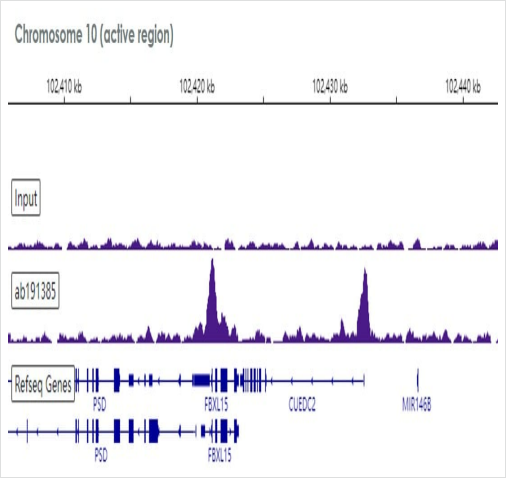
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 39 kDa
**Observed band size:** 42 kDa

**Lanes 1-2:** Merged signal (red and green). Green - ab191385 observed at 40 kDa. Red - loading control ab7291 observed at 50 kDa.

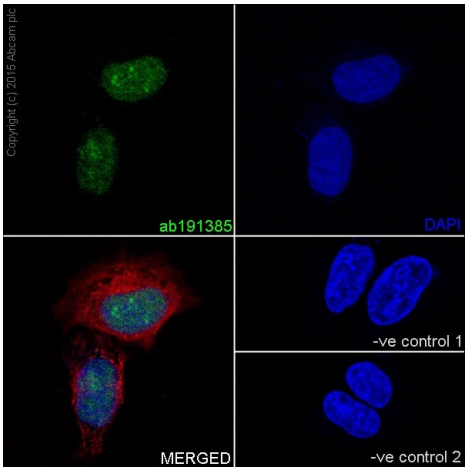
ab191385 Anti-SIRT6 antibody [EPR18463] was shown to specifically react with SIRT6 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265054 (knockout cell lysate ab257673) was used. Wild-type and SIRT6 knockout samples were subjected to SDS-PAGE. ab191385 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) were incubated overnight at 4°C 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.




ChIP-sequencing - Anti-SIRT6 antibody [EPR18463] - ChIP Grade (ab191385)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with $10^7$ HeLa cells and 8 µg of ab191385 [EPR18463]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded here.




Immunocytochemistry/ Immunofluorescence - Anti-SIRT6 antibody [EPR18463] (ab191385)

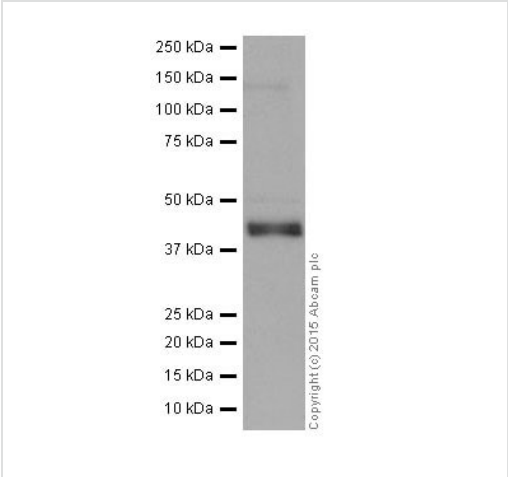
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling SIRT6 with ab191385 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab191385 at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.




Western blot - Anti-SIRT6 antibody [EPR18463] (ab191385)

Anti-SIRT6 antibody [EPR18463] (ab191385) at 1/10000 dilution + HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 20 µg

**Secondary**

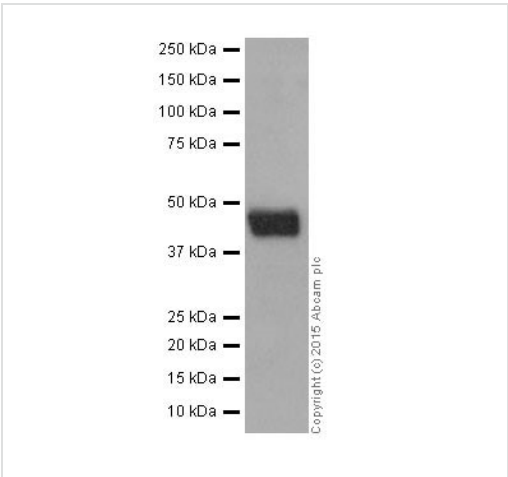
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

**Predicted band size:** 39 kDa
**Observed band size:** 42 kDa

**Exposure time:** 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The observed MW and doublets are consistent with what has been described in the literature. Two bands run closely together as doublets representing distinct isoforms; see UniProt annotation and PMID 24169447.




Western blot - Anti-SIRT6 antibody [EPR18463] (ab191385)

Anti-SIRT6 antibody [EPR18463] (ab191385) at 1/10000 dilution + Jurkat (Human T cell leukemia cell line from peripheral blood) cell lysate at 20 µg

**Secondary**

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

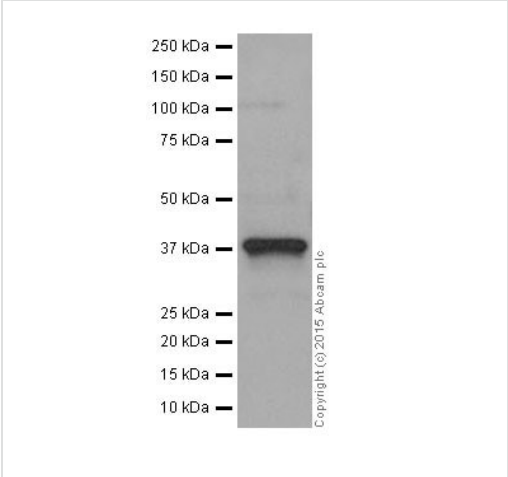
**Predicted band size:** 39 kDa
**Observed band size:** 42 kDa

**Exposure time:** 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The observed MW and doublets are consistent with what has been described in the literature. Two bands run closely together as doublets representing distinct isoforms; see UniProt annotation and

PMID 24169447.




Western blot - Anti-SIRT6 antibody [EPR18463] (ab191385)

Anti-SIRT6 antibody [EPR18463] (ab191385) at 1/10000 dilution + NIH/3T3 (Mouse embryonic fibroblast cell line) cell lysate at 20 µg

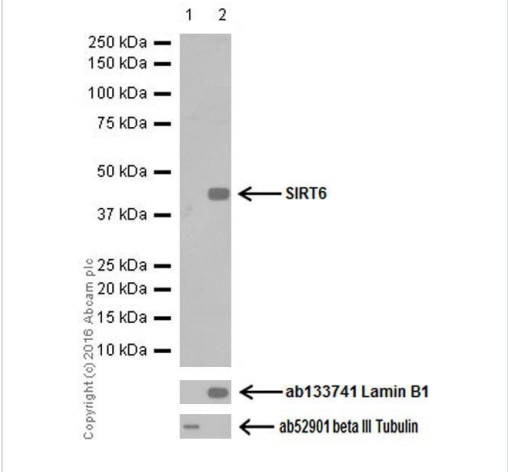
**Secondary**
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

**Predicted band size:** 39 kDa
**Observed band size:** 39 kDa

**Exposure time:** 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The observed MW and doublets are consistent with what has been described in the literature. Two bands run closely together as doublets representing distinct isoforms; see UniProt annotation and PMID 24169447.




Western blot - Anti-SIRT6 antibody [EPR18463] (ab191385)

**All lanes :** Anti-SIRT6 antibody [EPR18463] (ab191385) at 1/5000 dilution

**Lane 1 :** HeLa (Human epithelial cell line from cervix adenocarcinoma) cytoplasmic lysate
**Lane 2 :** HeLa (Human epithelial cell line from cervix adenocarcinoma) nuclear lysate

Lysates/proteins at 10 µg per lane.

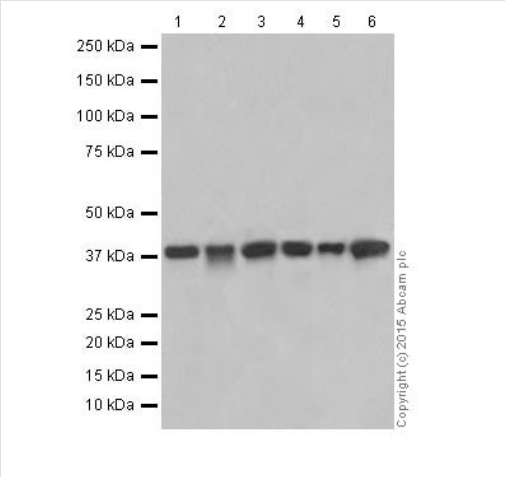
**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

**Predicted band size:** 39 kDa
**Observed band size:** 42 kDa

**Exposure time:** 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

SIRT6 is detected in nuclear fractions.




Western blot - Anti-SIRT6 antibody [EPR18463] (ab191385)

**All lanes :** Anti-SIRT6 antibody [EPR18463] (ab191385) at 1/2000 dilution

**Lane 1 :** Rat brain lysate
**Lane 2 :** Rat spleen lysate
**Lane 3 :** C6 (Rat glial tumor cell line) cell lysate
**Lane 4 :** RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cell lysate
**Lane 5 :** PC-12 (Rat adrenal gland pheochromocytoma cell line) cell lysate
**Lane 6 :** NIH/3T3 (Mouse embryonic fibroblast cell line) cell lysate

Lysates/proteins at 10 µg per lane.

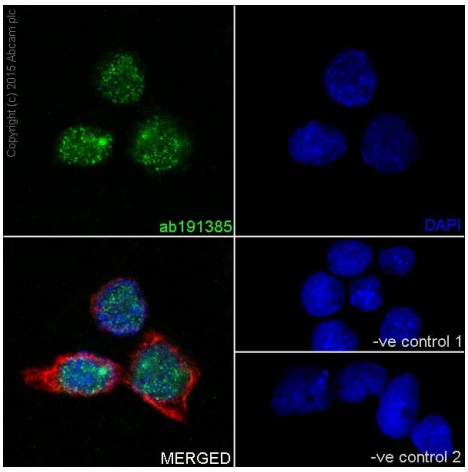
**Secondary**
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

**Predicted band size:** 39 kDa
**Observed band size:** 39 kDa

**Exposure time:** 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The observed MW is consistent with what has been described in the literature (PMID 24169447).




Immunocytochemistry/ Immunofluorescence - Anti-SIRT6 antibody [EPR18463] (ab191385)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HCT 116 (Human colorectal carcinoma cell line) cells labeling SIRT6 with ab191385 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear staining on HCT 116 cell line.

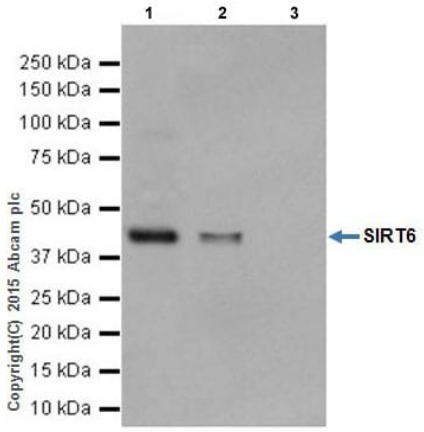
The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab191385 at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.




Immunoprecipitation - Anti-SIRT6 antibody [EPR18463] (ab191385)

SIRT6 was immunoprecipitated from 1 mg of Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate with ab191385 at 1/40 dilution.

Western blot was performed from the immunoprecipitate using ab191385 at 1/2000 dilution.

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: Jurkat whole cell lysate 10µg (Input).

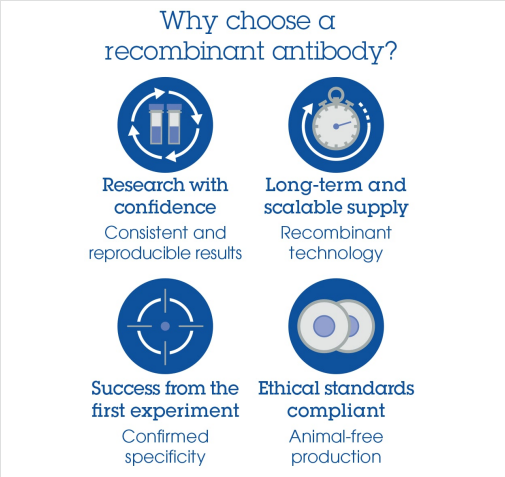
Lane 2: ab191385 IP in Jurkat whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) IP instead of ab191385 in Jurkat whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 seconds.

The observed MW and doublets are consistent with what has been described in the literature. Two bands run closely together as doublets representing distinct isoforms; see UniProt annotation and PMID 24169447.




Anti-SIRT6 antibody [EPR18463] (ab191385)

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