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Product datasheet

Anti-SIRT1 antibody [19A7AB4] ab110304

★★★★★ 26 Abreviews 257 References 画像数 9

製品の概要

製品名	Anti-SIRT1 antibody [19A7AB4]
製品の詳細	Mouse monoclonal [19A7AB4] to SIRT1
由来種	Mouse
特異性	Expression levels of the target protein vary with sample type and some optimisation may be required. For western blotting, more concentrated lysates may be required when using tissues samples.
アプリケーション	**適用あり:** WB, Flow Cyt, ICC/IF, IHC-P
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Recombinant Human SIRT1
ポジティブ・コントロール	WB: HEK293, HeLa, MDA-MB-231, HepG2, H9C2 and MEF cell lysates. ICC: HDFn cells. Flow Cyt: HL60 cells. IHC-P: Human normal colon FFPE tissue.
特記事項	**Western blot protocol advice:** For best results in Western blot using this antibody, we recommend the following: **1) Using a gradient gel (such as 10-20% Tris-Glycine gel).** **2) CAPS transfer buffer with 10% isopropyl alcohol.** **3) PVDF membrane.** **4) Blocking solution: PBS + 5% milk incubated overnight at 4°C.** **5) Antibody solution: PBS + 1% milk + 0.05% Tween + Ab incubated for 2 hours.** **6) Washing solution: PBS + 0.05% Tween incubated for 5 mins (repeat 3 times). PBS only for the final wash.** **7) HRP detection methods (such as ECL Prime).** Our Technical team (technical@abcam.com) will be happy to provide further information and advice. This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com. 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

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C. Do Not Freeze.
バッファー	pH: 7.4 Preservative: 0.02% Sodium azide Constituent: HEPES buffered saline
精製度	Affinity purified
特記事項（精製）	ab110304 was produced in vitro using hybridomas grown in serum-free medium. Purity: >95% by SDS-PAGE.
ポリ/モノ	モノクローナル
クローン名	19A7AB4
アイソタイプ	IgG1
軽鎖の種類	kappa

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab110304の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB	★★★★☆ (14)	Use a concentration of 0.125 - 1 µg/ml. Predicted molecular weight: 81 kDa. Detects a band of approximately 110 kDa (110-121 kDa) which is likely to be due to post translational glycosylation. SIRT1 is known to bind to several other proteins, and the 121 kDa band could also be due to the presence of one of these complexes (ensure samples are adequately reduced and denatured).
Flow Cyt	★★★★★ (1)	Use a concentration of 1 µg/ml. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (3)	Use a concentration of 0.5 µg/ml.
IHC-P	★★★★★ (3)	Use a concentration of 5 µg/ml.

ターゲット情報

機能	NAD-dependent protein deacetylase that links transcriptional regulation directly to intracellular energetics and participates in the coordination of several separated cellular functions such as cell cycle, response to DNA damage, metobolism, apoptosis and autophagy. Can modulate chromatin function through deacetylation of histones and can promote alterations in the methylation of histones and DNA, leading to transcriptional repression. Deacetylates a broad

range of transcription factors and coregulators, thereby regulating target gene expression positively and negatively. Serves as a sensor of the cytosolic ratio of NAD(+)/NADH which is altered by glucose deprivation and metabolic changes associated with caloric restriction. Is essential in skeletal muscle cell differentiation and in response to low nutrients mediates the inhibitory effect on skeletal myoblast differentiation which also involves 5'-AMP-activated protein kinase (AMPK) and nicotinamide phosphoribosyltransferase (NAMPT). Component of the eNoSC (energy-dependent nucleolar silencing) complex, a complex that mediates silencing of rDNA in response to intracellular energy status and acts by recruiting histone-modifying enzymes. The eNoSC complex is able to sense the energy status of cell: upon glucose starvation, elevation of NAD(+)/NADP(+) ratio activates SIRT1, leading to histone H3 deacetylation followed by dimethylation of H3 at 'Lys-9' (H3K9me2) by SUV39H1 and the formation of silent chromatin in the rDNA locus. Deacetylates 'Lys-266' of SUV39H1, leading to its activation. Inhibits skeletal muscle differentiation by deacetylating PCAF and MYOD1. Deacetylates H2A and 'Lys-26' of HIST1H1E. Deacetylates 'Lys-16' of histone H4 (in vitro). Involved in NR0B2/SHP corepression function through chromatin remodeling: Recruited to LRH1 target gene promoters by NR0B2/SHP thereby stimulating histone H3 and H4 deacetylation leading to transcriptional repression. Proposed to contribute to genomic integrity via positive regulation of telomere length; however, reports on localization to pericentromeric heterochromatin are conflicting. Proposed to play a role in constitutive heterochromatin (CH) formation and/or maintenance through regulation of the available pool of nuclear SUV39H1. Upon oxidative/metabolic stress decreases SUV39H1 degradation by inhibiting SUV39H1 polyubiquitination by MDM2. This increase in SUV39H1 levels enhances SUV39H1 turnover in CH, which in turn seems to accelerate renewal of the heterochromatin which correlates with greater genomic integrity during stress response. Deacetylates 'Lys-382' of p53/TP53 and impairs its ability to induce transcription-dependent proapoptotic program and modulate cell senescence. Deacetylates TAF1B and thereby represses rDNA transcription by the RNA polymerase I. Deacetylates MYC, promotes the association of MYC with MAX and decreases MYC stability leading to compromised transformational capability. Deacetylates FOXO3 in response to oxidative stress thereby increasing its ability to induce cell cycle arrest and resistance to oxidative stress but inhibiting FOXO3-mediated induction of apoptosis transcriptional activity; also leading to FOXO3 ubiquitination and protesomal degradation. Appears to have a similar effect on MLLT7/FOXO4 in regulation of transcriptional activity and apoptosis. Deacetylates DNMT1; thereby impairs DNMT1 methyltransferase-independent transcription repressor activity, modulates DNMT1 cell cycle regulatory function and DNMT1-mediated gene silencing. Deacetylates RELA/NF-kappa-B p65 thereby inhibiting its transactivating potential and augments apoptosis in response to TNF-alpha. Deacetylates HIF1A, KAT5/TIP60, RB1 and HIC1. Deacetylates FOXO1 resulting in its nuclear retention and enhancement of its transcriptional activity leading to increased gluconeogenesis in liver. Inhibits E2F1 transcriptional activity and apoptotic function, possibly by deacetylation. Involved in HES1- and HEY2-mediated transcriptional repression. In cooperation with MYCN seems to be involved in transcriptional repression of DUSP6/MAPK3 leading to MYCN stabilization by phosphorylation at 'Ser-62'. Deacetylates MEF2D. Required for antagonist-mediated transcription suppression of AR-dependent genes which may be linked to local deacetylation of histone H3. Represses HNF1A-mediated transcription. Required for the repression of ESRRG by CREBZF. Modulates AP-1 transcription factor activity. Deacetylates NR1H3 AND NR1H2 and deacetylation of NR1H3 at 'Lys-434' positively regulates transcription of NR1H3:RXR target genes, promotes NR1H3 proteosomal degradation and results in cholesterol efflux; a promoter clearing mechanism after reach round of transcription is proposed. Involved in lipid metabolism. Implicated in regulation of adipogenesis and fat mobilization in white adipocytes by repression of PPARG which probably involves association with NCOR1 and SMRT/NCOR2. Deacetylates ACSS2 leading to its activation, and HMGCS1. Involved in liver and muscle metabolism. Through deacteylation and activation of PPARGC1A is required to activate fatty acid oxidation in skeletel muscle under low-glucose conditions and is involved in glucose homeostasis.

Involved in regulation of PPARA and fatty acid beta-oxidation in liver. Involved in positive regulation of insulin secretion in pancreatic beta cells in response to glucose; the function seems to imply transcriptional repression of UCP2. Proposed to deacetylate IRS2 thereby facilitating its insulin-induced tyrosine phosphorylation. Deacetylates SREBF1 isoform SREBP-1C thereby decreasing its stability and transactivation in lipogenic gene expression. Involved in DNA damage response by repressing genes which are involved in DNA repair, such as XPC and TP73, deacetylating XRCC6/Ku70, and faciliting recruitment of additional factors to sites of damaged DNA, such as SIRT1-deacetylated NBN can recruit ATM to initiate DNA repair and SIRT1-deacetylated XPA interacts with RPA2. Also involved in DNA repair of DNA double-strand breaks by homologous recombination and specifically single-strand annealing independently of XRCC6/Ku70 and NBN. Transcriptional suppression of XPC probably involves an E2F4:RBL2 suppressor complex and protein kinase B (AKT) signaling. Transcriptional suppression of TP73 probably involves E2F4 and PCAF. Deacetylates WRN thereby regulating its helicase and exonuclease activities and regulates WRN nuclear translocation in response to DNA damage. Deacetylates APEX1 at 'Lys-6' and 'Lys-7' and stimulates cellular AP endonuclease activity by promoting the association of APEX1 to XRCC1. Increases p53/TP53-mediated transcription-independent apoptosis by blocking nuclear translocation of cytoplasmic p53/TP53 and probably redirecting it to mitochondria. Deacetylates XRCC6/Ku70 at 'Lys-539' and 'Lys-542' causing it to sequester BAX away from mitochondria thereby inhibiting stress-induced apoptosis. Is involved in autophagy, presumably by deacetylating ATG5, ATG7 and MAP1LC3B/ATG8. Deacetylates AKT1 which leads to enhanced binding of AKT1 and PDK1 to PIP3 and promotes their activation. Proposed to play role in regulation of STK11/LBK1-dependent AMPK signaling pathways implicated in cellular senescence which seems to involve the regulation of the acetylation status of STK11/LBK1. Can deacetylate STK11/LBK1 and thereby increase its activity, cytoplasmic localization and association with STRAD; however, the relevance of such activity in normal cells is unclear. In endothelial cells is shown to inhibit STK11/LBK1 activity and to promote its degradation. Deacetylates SMAD7 at 'Lys-64' and 'Lys-70' thereby promoting its degradation. Deacetylates CIITA and augments its MHC class II transactivation and contributes to its stability. Deacteylates MECOM/EVI1. Deacetylates PML at 'Lys-487' and this deacetylation promotes PML control of PER2 nuclear localization. During the neurogenic transition, repress selective NOTCH1-target genes throug

Isoform 2: Isoform 2 is shown to deacetylate 'Lys-382' of p53/TP53, however with lower activity than isoform 1. In combination, the two isoforms exert an additive effect. Isoform 2 regulates p53/TP53 expression and cellular stress response and is in turn repressed by p53/TP53 presenting a SIRT1 isoform-dependent auto-regulatory loop.

(Microbial infection) In case of HIV-1 infection, interacts with and deacetylates the viral Tat protein. The viral Tat protein inhibits SIRT1 deacetylation activity toward RELA/NF-kappa-B p65, thereby potentiates its transcriptional activity and SIRT1 is proposed to contribute to T-cell hyperactivation during infection.

SirtT1 75 kDa fragment: catalytically inactive 75SirT1 may be involved in regulation of apoptosis. May be involved in protecting chondrocytes from apoptotic death by associating with cytochrome C and interfering with apoptosome assembly.

組織特異性

Widely expressed.

配列類似性

Belongs to the sirtuin family. Class I subfamily.

Contains 1 deacetylase sirtuin-type domain.

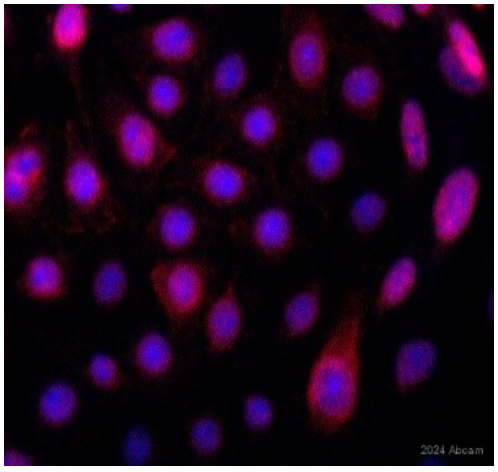
翻訳後修飾

Methylated on multiple lysine residues; methylation is enhanced after DNA damage and is dispensable for deacetylase activity toward p53/TP53.

Phosphorylated. Phosphorylated by STK4/MST1, resulting in inhibition of SIRT1-mediated p53/TP53 deacetylation. Phosphorylation by MAPK8/JNK1 at Ser-27, Ser-47, and Thr-530 leads to increased nuclear localization and enzymatic activity. Phosphorylation at Thr-530 by DYRK1A and DYRK3 activates deacetylase activity and promotes cell survival. Phosphorylation by

	mammalian target of rapamycin complex 1 (mTORC1) at Ser-47 inhibits deacetylation activity. Phosphorylated by CaMK2, leading to increased p53/TP53 and NF-kappa-B p65/RELA deacetylation activity (By similarity). Phosphorylation at Ser-27 implicating MAPK9 is linked to protein stability. There is some ambiguity for some phosphosites: Ser-159/Ser-162 and Thr-544/Ser-545. Proteolytically cleaved by cathepsin B upon TNF-alpha treatment to yield catalytic inactive but stable SirtT1 75 kDa fragment (75SirT1). S-nitrosylated by GAPDH, leading to inhibit the NAD-dependent protein deacetylase activity.
細胞内局在	Cytoplasm. Mitochondrion and Nucleus, PML body. Cytoplasm. Nucleus. Recruited to the nuclear bodies via its interaction with PML (PubMed:12006491). Colocalized with APEX1 in the nucleus (PubMed:19934257). May be found in nucleolus, nuclear euchromatin, heterochromatin and inner membrane (PubMed:15469825). Shuttles between nucleus and cytoplasm (By similarity). Colocalizes in the nucleus with XBP1 isoform 2 (PubMed:20955178).

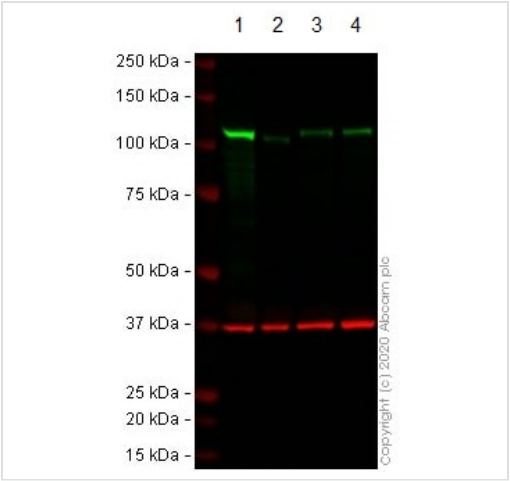
画像



Immunocytochemistry - Anti-SIRT1 antibody [19A7AB4] (ab110304)

This image is courtesy of an anonymous Abreview

Immunocytochemistry analysis of acetone-fixed human cervix cancer (HT3) cells staining with ab110304 at 1/400 dilution. Secondary antibody was PE Rat anti-mouse IgG at 1/300 dilution. Samples were incubated with the primary antibody with PBS for 1 hour at 21°C. Blocking was done using 5% serum for 30 minutes at 21°C.



Western blot - Anti-SIRT1 antibody [19A7AB4] (ab110304)

All lanes : Anti-SIRT1 antibody [19A7AB4] (ab110304) at 1 µg/ml

Lane 1 : Wild-type HEK-293 cell lysate
Lane 2 : SIRT1 CRISPR/Cas9 edited HEK-293 cell lysate
Lane 3 : MDA-MB-231 cell lysate
Lane 4 : HeLa cell lysate

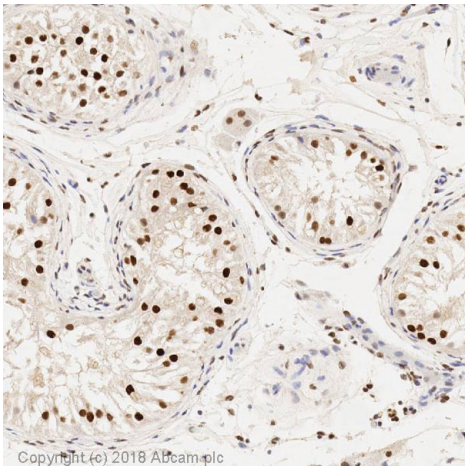
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 81 kDa
Observed band size: 110 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab110304 observed at 110 kDa. Red - loading control, **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) observed at 37kDa.

ab110304 was shown to react with SIRT1 in western blot. The band observed in the CRISPR/Cas9 edited lysate lane below 110kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab110304 and **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) overnight at 4°C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

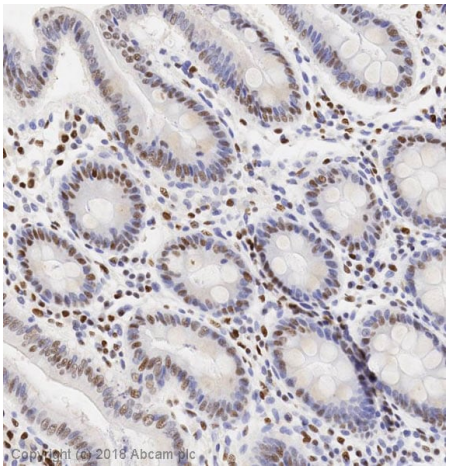


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SIRT1 antibody [19A7AB4] (ab110304)

IHC image of SIRT1 staining in a section of formalin-fixed paraffin-embedded normal human testis* performed on a Leica BOND™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab110304, 1ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

**Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre*



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SIRT1 antibody [19A7AB4] (ab110304)

IHC image of SIRT1 staining in a section of formalin-fixed paraffin-embedded normal human colon* performed on a Leica BOND™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab110304, 5ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

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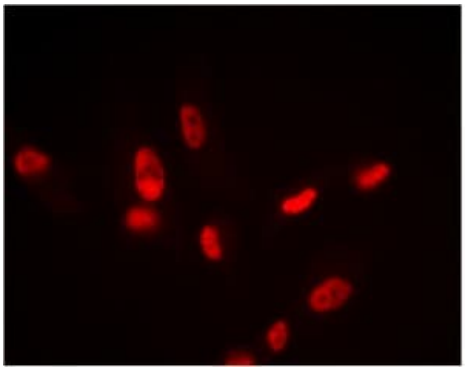
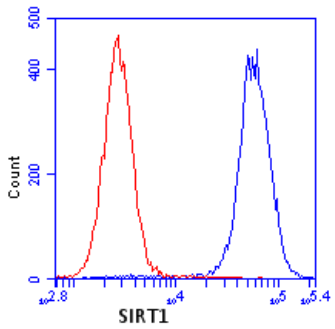


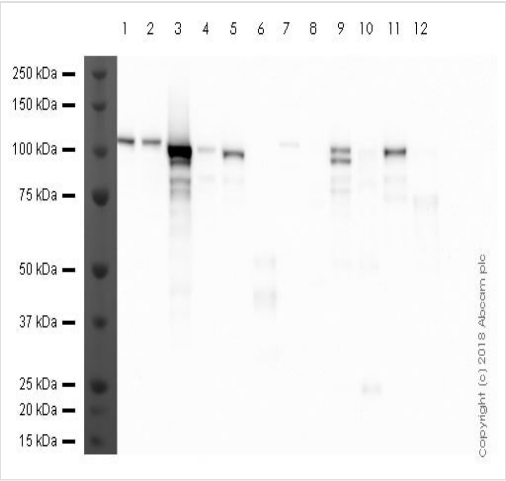
Immunocytochemistry/ Immunofluorescence - Anti-SIRT1 antibody [19A7AB4] (ab110304)

Immunocytochemistry/Immunofluorescence analysis using ab110304 at 0.5µg/ml staining SIRT1 in HDFn cells (paraformaldehyde fixed and Triton X-100 permeabilized). The secondary antibody was (Red) Alexa Fluor® 594 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. 10% Goat serum was used as the blocking agent for all blocking steps.



Flow Cytometry - Anti-SIRT1 antibody [19A7AB4] (ab110304)

HL-60 cells were stained with 1 µg/mL SIRT1 antibody ab110304(blue) or an equal amount of an isotype control antibody (red) and analyzed by flow cytometry.



Western blot - Anti-SIRT1 antibody [19A7AB4] (ab110304)

All lanes : Anti-SIRT1 antibody [19A7AB4] (ab110304) at 1 µg/ml

Lane 1 : HeLa whole cell lysate
Lane 2 : HepG2 whole cell lysate
Lane 3 : F9 whole cell lysate
Lane 4 : MEF1 whole cell lysate
Lane 5 : PC-12 whole cell lysate
Lane 6 : RBL-1 whole cell lysate
Lane 7 : Human Testis tissue lysate
Lane 8 : Human Colon tissue lysate
Lane 9 : Mouse Testis tissue lysate
Lane 10 : Mouse Colon tissue lysate
Lane 11 : Rat Testis tissue lysate
Lane 12 : Rat Colon tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 81 kDa
Observed band size: 110 kDa

Exposure time: 4 minutes

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% Milk before being incubated with ab110304 overnight at 4°C. Antibody binding was detected using an anti-mouse antibody conjugated to HRP, and visualised using ECL development solution.

Western blot - Anti-SIRT1 antibody [19A7AB4] (ab110304)

This image is courtesy of an anonymous Abreview

All lanes : Anti-SIRT1 antibody [19A7AB4] (ab110304) at 1/1000 dilution

All lanes : rat Skeletal muscle

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Mouse at 1/6000 dilution

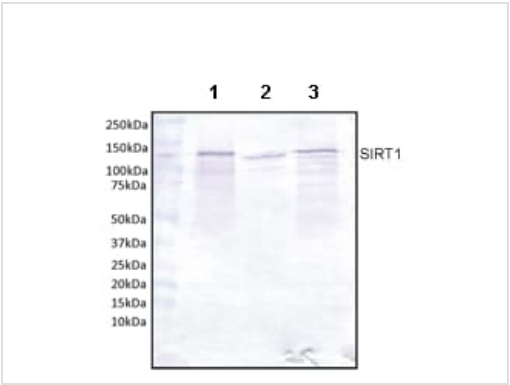
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 81 kDa
Observed band size: 120 kDa

Exposure time: 13 minutes

Blocked with 3% milk (TBS-tween) at 4C for 16 hours



Western blot - Anti-SIRT1 antibody [19A7AB4] (ab110304)

All lanes : Anti-SIRT1 antibody [19A7AB4] (ab110304) at 0.125 µg/ml

Lane 1 : HepG2 cell lysate(Human)
Lane 2 : H9C2 cell lysate (Rat)
Lane 3 : MEF cell lysate (Mouse)

Predicted band size: 81 kDa

WB Conditions
Primary Antibody: 0.25 µg/mL in 10X Blocking Buffer (**ab126587**). 3hrs at room temperature.

Secondary Antibody: 1:5,000 in 10X Blocking Buffer (**ab126587**). 3hrs at room temperature.

ab110304 detects a band of approximately 110 kDa (110-121 kDa) which is likely to be due to post translational glycosylation. SIRT1 is known to bind to several other proteins, and the 121kDa band could also be due to the presence of one of these complexes (ensure samples are adequately reduced and denatured).

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