abcam

Product datasheet

Anti-SIRT1 antibody [EPR18239] ab189494

ועלשעבע RabMAb

★★★★ 4 Abreviews 70 References 画像数 13

製品の概要

製品名 Anti-SIRT1 antibody [EPR18239]

製品の詳細 Rabbit monoclonal [EPR18239] to SIRT1

由来種 Rabbit

アプリケーション 適用あり: IP, ICC/IF, WB, IHC-P, Flow Cyt (Intra)

種交差性 交差種: Mouse, Rat, Human

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: Mouse testis tissue lysate; HEK293, MDA-MB-231, F9, HeLa and A549 whole cell lysates;

> Rat E18 brain tissue lysate. IHC-P: Human testis and skeletal muscle tissue; Mouse testis tissue; Rat skeletal muscle tissue. ICC/IF: HeLa and F9 cells. Flow Cyt (intra): HeLa and F9 cells. IP: F9

whole cell lysate.

特記事項 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**.

製品の特性

製品の状態 Liquid

保存方法 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.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

ポリ/モノ モノクローナル クローン名 EPR18239

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab189494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
IP		1/30.
ICC/IF		1/100.
WB	★★★★☆ (3)	1/1000. Detects a band of approximately 110, 120 kDa (predicted molecular weight: 80 kDa).
IHC-P	★★★★ (1)	1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/60.

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 antagonistmediated 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 SIRT1deacetylated 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 transcriptionindependent 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. Deacetylates 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

lsoform 2: lsoform 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. lsoform 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

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.

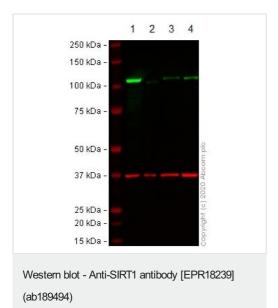
Proteolytically cleaved by cathepsin B upon TNF-alpha treatment to yield catalytic inactive but stable SirtT1 75 kDa fragment (75SirT1).

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



All lanes : Anti-SIRT1 antibody [EPR18239] (ab189494) at 1/1000 dilution

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: 80 kDa **Observed band size:** 110 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab189494 observed at 110 kDa. Red - loading control, <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab189494 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 ab189494 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 hour at room temperature before imaging.

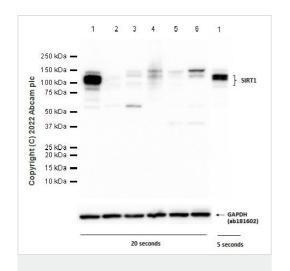


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SIRT1 antibody
[EPR18239] (ab189494)

Immunohistochemical analysis of paraffin-embedded human skeletal muscle tissue labeling SIRT1 with ab189494 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Cytoplasmic staining in human skeletal muscle (PMID: 23332867) is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) ready to use.

Perform heat-mediated antigen retrieval using Citrate, pH 6.0.



Western blot - Anti-SIRT1 antibody [EPR18239] (ab189494)

All lanes : Anti-SIRT1 antibody [EPR18239] (ab189494) at 1/1000 dilution

Lane 1: Mouse testis tissue lysate

Lane 2: Mouse colon tissue lysate

Lane 3: Mouse kidney tissue lysate

Lane 4: Mouse lymph node tissue lysate

Lanes 5-6: Mouse liver tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 80 kDa

Observed band size: 75.110.120 kDa

Blocking buffer and concentration: 5% NFDM/TBST **Diluting buffer and concentration:** 5% NFDM/TBST

Exposure time: 20,5 seconds

This antibody detects strong band in testis but weak band in other tissues like colon, kidney, lymph node and liver. Please upload more lysate or use lower dilution when testing these tissues.

We are unsure as to the identity of the band around 130kDa.

ab189494 MERGED

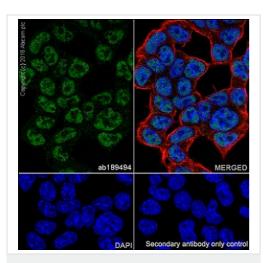
DAPI Secondary antibody only control

Immunocytochemistry/ Immunofluorescence - Anti-SIRT1 antibody [EPR18239] (ab189494)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% methanol-permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling SIRT1 with ab189494 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and weakly cytoplasmic staining in HeLa cell line.

The nuclear counterstain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.

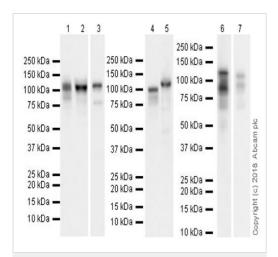


Immunocytochemistry/ Immunofluorescence - Anti-SIRT1 antibody [EPR18239] (ab189494)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% methanol-permeabilized F9 (mouse embryonic testicular cancer cell line) cells labeling SIRT1 with ab189494 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and weakly cytoplasmic staining in F9 cell line.

The nuclear counterstain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) (**ab195889**) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.



Western blot - Anti-SIRT1 antibody [EPR18239] (ab189494)

Lanes 1-6: Anti-SIRT1 antibody [EPR18239] (ab189494) at 1/1000 dilution

Lane 7: Anti-SIRT1 antibody [EPR18239] (ab189494) at 1/5000 dilution

Lane 1: Mouse testis tissue lysate at 20 µg

Lane 2 : F9 (mouse embryonic testicular cancer cell line) whole cell lysate at 20 µg

Lane 3: HeLa (human epithelial cell line from cervix

adenocarcinoma) whole cell lysate at 20 μg

Lane 4: Rat E18 brain tissue lysate at 20 µg

Lane 5 : A549 (human lung carcinoma cell line) whole cell lysate at $20~\mu g$

Lane 6: Human testis tissue lysate at 10 µg

Lane 7: Rat testis tissue lysate at 10 µg

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Developed using the ECL technique.

Predicted band size: 80 kDa

Observed band size: 110,120 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure times:

Lanes 1 & 2: 8 seconds

Lane 3: 32 seconds

Lanes 4 & 5: 67 seconds

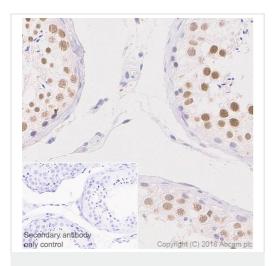
Lane 6: 59 seconds

Lane 7: 10 seconds

The expression profile is consistent with what has been described in the literature (PMID: 21474819).

The 75 kDa band is a cleaved fragment of SIRT1 (PMID: 25770475, PMID: 21305533), while the approximately 85 kDa

band likely represents a splice variant (PMID: 20975832).

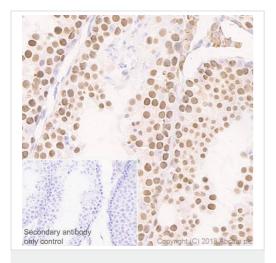


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SIRT1 antibody
[EPR18239] (ab189494)

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling SIRT1 with ab189494 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Mainly nuclear staining in human testis (PMID: 17197703) is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) ready to use.

Perform heat-mediated antigen retrieval using Citrate, pH 6.0.

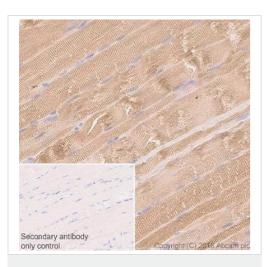


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SIRT1 antibody
[EPR18239] (ab189494)

Immunohistochemical analysis of paraffin-embedded mouse testis tissue labeling SIRT1 with ab189494 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Mainly nuclear staining in mouse testis (PMID: 17197703) is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) ready to use.

Perform heat-mediated antigen retrieval using Citrate, pH 6.0.



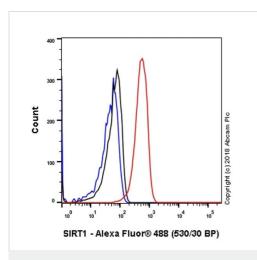
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SIRT1 antibody
[EPR18239] (ab189494)

Immunohistochemical analysis of paraffin-embedded rat skeletal muscle tissue labeling SIRT1 with ab189494 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Cytoplasmic staining in rat skeletal muscle (PMID: 23332867) is observed. Counter stained with Hematoxylin.

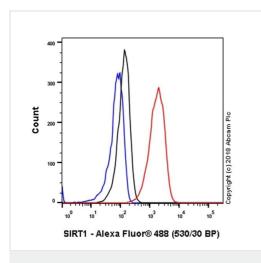
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) ready to use.

Perform heat-mediated antigen retrieval using Citrate, pH 6.0.



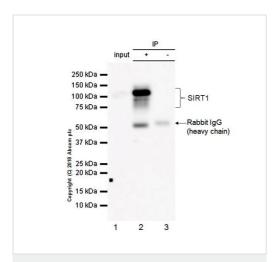
Flow Cytometry (Intracellular) - Anti-SIRT1 antibody [EPR18239] (ab189494)

Intracellular flow cytometric analysis of4% paraformaldehyde-fixed, 90% methanol-permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling SIRT1 with ab189494 at 1/60 dilution (red) compared with a rabbit lgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit lgG H&L (Alexa Fluorr® 488) (ab150077), at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-SIRT1 antibody [EPR18239] (ab189494)

Intracellular flow cytometric analysis of 4% paraformal dehyde-fixed, 90% methanol-permeabilized F9 (mouse embryonic testicular cancer cell line) cell line labeling SIRT1 with ab 189494 at 1/60 dilution (red) compared with a rabbit lgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit lgG H&L (Alexa Fluorr \$\mathbb{8}488)\$ (ab150077), at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-SIRT1 antibody [EPR18239] (ab189494)

SIRT1 was immunoprecipitated from 0.35 mg of F9 (mouse embryonic testicular cancer cell line) whole cell lysate with ab189494 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab189494 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

Lane 1: F9 whole lysate 10 µg (Input).

Lane 2: ab189494 IP in F9 whole cell lysate.

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab189494 in F9 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 50 seconds.



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