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Product datasheet

SIRT1 Activity Assay Kit (Fluorometric) ab156065

2 Abreviews 84 References 6 Images

Overview

Product name	SIRT1 Activity Assay Kit (Fluorometric)
Detection method	Fluorescent
Sample type	Cell culture extracts, Tissue Extracts
Assay type	Enzyme activity
Assay time	1h 00m
Species reactivity	**Reacts with:** Mouse, Rat, Human
Product overview	SIRT1 Activity Assay Kit (Fluorometric) (ab156065) detects SIRT1 activity in lysates. Primarily, the SIRT1 Activity Assay Kit is designed for the rapid and sensitive evaluation of SIRT1 inhibitors or activators using crude SIRT1 fraction or purified SIRT1. Additionally, any cultured primary cell, cell line, or tissue homogenate can be assayed for SIRT1 activity with the SIRT1 Activity Assay Kit if the appropriate antibody directed against SIRT1 (Anti-SIRT1 antibody (**ab7343**)) is used for immunoprecipitation. Abcam's SIRT1 Activity Assay Kit (Fluorometric) has been shown to detect the activity of Sirtuins, at least SIRT1 in Human or animal cell lysates or in column fractions. The assay shows good linearity of sample response. The assay may be used to follow the purification of Sirtuins or may be used to detect the presence of Sirtuins in cell lysates. Applications for this kit include: 1. Monitoring the purification of SIRT1. 2. Screening inhibitors or activators of SIRT1. 3. Detecting the effects of pharmacological agents on SIRT1.
Notes	Histone Deacetylases (HDACs) are a class of enzymes responsible for the deacetylation of lysine residues on the N-terminal part of the core histones (H2A, H2B, H3 and H4), allowing the histones to wrap the DNA more tightly. HDAC proteins occur in four groups (class I, class IIA, class IIB, class III, class IV) based on function and DNA sequence similarity. Classes I, IIA and IIB are considered "classical" HDACs whose activities are inhibited by trichostatin A (TSA), whereas class III is a family of NAD+-dependent proteins (sirtuins) not

affected by TSA. Class IV is considered an atypical class on its own, based solely on DNA sequence similarity to the others.

Platform Microplate reader

Properties

Storage instructions Please refer to protocols.

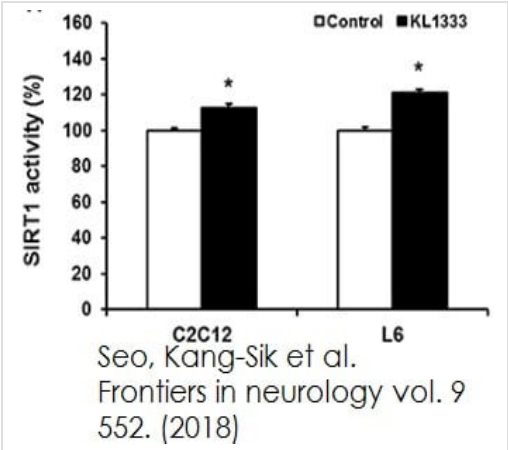
Components	100 tests
Developer	1 x 500µl
Fluoro-Deacetylated Peptide (0.2 mM)	1 x 100µl
Fluoro-Substrate Peptide (0.2 mM)	1 x 500µl
NAD (2 mM)	1 x 500µl
Recombinant SIRT1	1 x 500µl
SIRT Assay Buffer	2 x 1ml
Stop Solution	2 x 1ml

Function 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.
Tissue specificity	Widely expressed.
Sequence similarities	Belongs to the sirtuin family. Class I subfamily. Contains 1 deacetylase sirtuin-type domain.
Post-translational modifications	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.
Cellular localization	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).

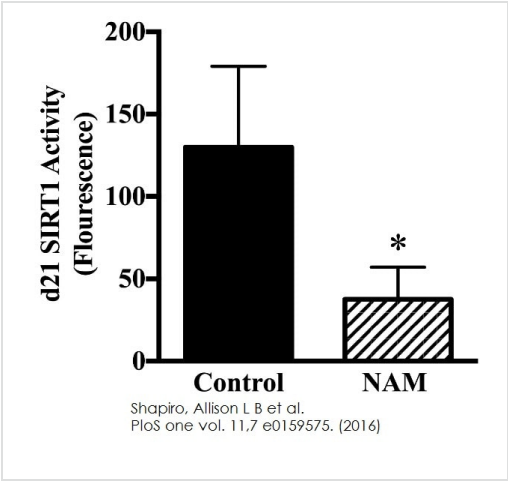
Images



C2C12 and L6 myoblasts were treated with 1 and 2 µM KL1333 for 1 h, respectively. SIRT1 activities in both cell lines were analyzed using a fluorescence-based assay.

Functional Studies - SIRT1 Activity Assay Kit (Fluorometric) (ab156065)

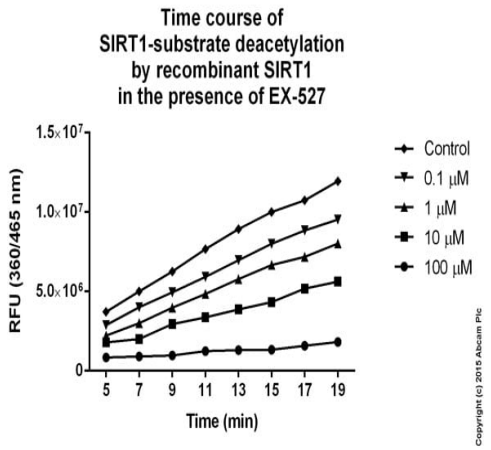
Seo, Kang-Sik et al., Frontiers in neurology?vol. 9 552., Fig 2, doi:10.3389/fneur.2018.00552



A representative sample of 9 sets of cells from the 46 experimental sets was used for SIRT1 enzyme activity at day 21 in vehicle-control and NAM conditions only

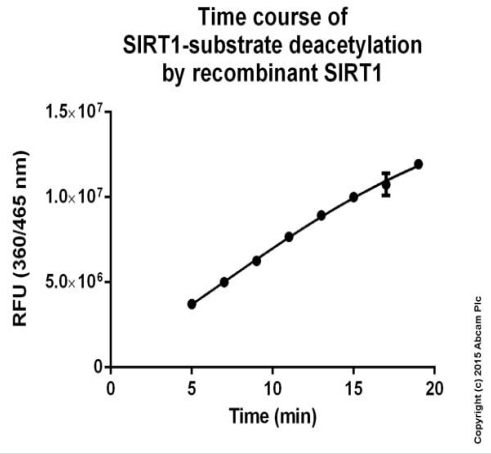
Functional Studies - SIRT1 Activity Assay Kit (Fluorometric) (ab156065)

Shapiro, Allison L B et al., PloS one?vol. 11,7 e0159575., Fig 2, doi:10.1371/journal.pone.0159575



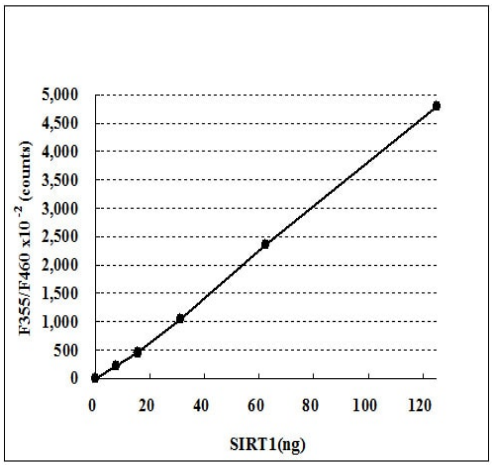
SIRT1 Activity Assay Kit (ab156065)

Time course of SIRT1-substrate deacetylation by recombinant SIRT1 in the presence of EX-527 (**ab141506**)



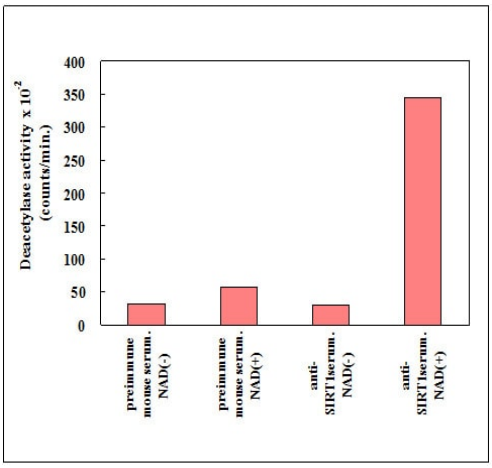
SIRT1 Activity Assay Kit (ab156065)

Time course of SIRT1-substrate deacetylation by recombinant SIRT1



ab156065 - SIRT1 Activity Assay Kit (Fluorometric)

Dose dependency curve of recombinant SIRT1 activity



ab156065 - SIRT1 Activity Assay Kit (Fluorometric)

Measurement of 293T cell endogenous SIRT1 activity in a sample previously immunoprecipitated with an anti-SIRT1 antibody.

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