SIRT1 ELISA Kit Mouse (OKEH03539)

Instructions for Use

For the quantitative measurement of SIRT1 in Mouse biological samples.

This product is intended for research use only.

v1.0

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1. Background

Principle

Aviva Systems Biology's SIRT1 ELISA Kit (Mouse) (OKEH03539) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for SIRT1 has been pre-coated onto a 96-well plate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for SIRT1 is added, incubated and followed by washing. Avidin-Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by HRP generating a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm is quantitatively proportional to the amount of sample SIRT1 captured in the well.

Target Background

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, metabolism, 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, which increases its DNA binding ability and enhances 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-537' and 'Lys-540' 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 through histone deacetylation in a BCL6-dependent manner and leading to neuronal differentiation. Regulates the circadian expression of several core clock genes, including ARNTL/BMAL1, RORC, PER2 and CRY1 and plays a critical role in maintaining a controlled rhythmicity in histone acetylation, thereby contributing to circadian chromatin remodeling. Deacetylates ARNTL/BMAL1 and histones at the circadian gene promoters in order to facilitate repression by inhibitory components of the circadian oscillator. Deacetylates PER2, facilitating its ubiquitination and degradation by the proteosome. Protects cardiomyocytes against palmitate-induced apoptosis (PubMed:11250901, PubMed:11672522, PubMed:12651913, PubMed:12887892, PubMed:12960381, PubMed:15175761, PubMed:15220471, PubMed:15632193, PubMed:15744310, PubMed:15788402, PubMed:16098828, PubMed:16366736, PubMed:16790548, PubMed:16892051, PubMed:17098745, PubMed:17347648, PubMed:17620057, PubMed:17901049, PubMed:17936707, PubMed:18004385, PubMed:18296641, PubMed:18371449, PubMed:18477450, PubMed:18662546, PubMed:18662547, PubMed:18687677, PubMed:19299583, PubMed:19356714, PubMed:20817729, PubMed:21176092, PubMed:21187328, PubMed:21189328, PubMed:21622680, PubMed:23160044). Deacetylates XBP1 isoform 2; deacetylation decreases protein stability of XBP1 isoform 2 and inhibits its transcriptional activity. Involved in the CCAR2-mediated regulation of PCK1 and NR1D1. Deacetylates CTNB1 at 'Lys-49' (By similarity). In POMC (pro-opiomelanocortin) neurons, required for leptin-induced

activation of PI3K signaling (PubMed:20620997).By similarity
 <p>Manually curated information which has been propagated from a related experimentally characterized protein.</p>

  <p>Moreâ€¦</p> Manual assertion inferred from sequence similarity toiUniProtKB:Q96EB6 (SIR1_HUMAN)35 Publications
 <p>Manually curated information for which there is published experimental evidence.</p>

  <p>Moreâ€¦</p> Manual assertion based on experiment iniRef.3"Negative control of p53 by Sir2alpha promotes cell survival under stress." Luo J., Nikolaev A.Y., Imai S., Chen D., Su F., Shiloh A., Guarente L., Gu W. Cell 107:137-148(2001) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION, INTERACTION WITH TP53, ENZYME REGULATION, MUTAGENESIS OF HIS-355.Ref.4"Acetylation of TAF(I)68, a subunit of TIF-IB/SL1, activates RNA polymerase I transcription." Muth V., Nadaud S., Grummt I., Voit R. EMBO J. 20:1353-1362(2001) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION IN DEACETYLATION OF TAF1B.Ref.5"The absence of SIR2alpha protein has no effect on global gene silencing in mouse embryonic stem cells." McBurney M.W., Yang X., Jardine K., Bieman M., Th'ng J., Lemieux M. Mol. Cancer Res. 1:402-409(2003) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION.Ref.7"Sir2 regulates skeletal muscle differentiation as a potential sensor of the redox state." Fulco M., Schiltz R.L., Iezzi S., King M.T., Zhao P., Kashiwaya Y., Hoffman E., Veech R.L., Sartorelli V. Mol. Cell 12:51-62(2003) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION, INTERACTION WITH MYOD1 AND PCAF, MUTAGENESIS OF HIS-355.Ref.8"Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice." Cheng H.-L., Mostoslavsky R., Saito S., Manis J.P., Gu Y., Patel P., Bronson R., Appella E., Alt F.W., Chua K.F. Proc. Natl. Acad. Sci. U.S.A. 100:10794-10799(2003) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION.Ref.9"Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma." Picard F., Kurtev M., Chung N., Topark-Ngarm A., Senawong T., Machado De Oliveira R., Leid M., McBurney M.W., Guarente L. Nature 429:771-776(2004) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION IN ADIPODIGENESIS, FUNCTION IN FAT MOBILIZATION, INTERACTION WITH PPARG AND NCOR1.Ref.10"Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases." Hallows W.C., Lee S., Denu J.M. Proc. Natl. Acad. Sci. U.S.A. 103:10230-10235(2006) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION IN DEACETYLATION OF ACSS2, FUNCTION IN REGULATION OF ACCS2.Ref.11"SIRT1 deacetylates and positively regulates the nuclear receptor LXR." Li X., Zhang S., Blander G., Tse J.G., Krieger M., Guarente L. Mol. Cell 28:91-106(2007) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION IN DEACETYLATION OF NR1H3 AND NR1H2, FUNCTION IN REGULATION OF NR1H3.Ref.12"SIRT1 regulates apoptosis and Nanog expression in mouse embryonic stem cells by controlling p53 subcellular localization." Han M.K., Song E.K., Guo Y., Ou X., Mantel C., Broxmeyer H.E. Cell Stem Cell 2:241-251(2008) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION IN APOPTOSIS.Ref.13"Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity." Daitoku H., Hatta M., Matsuzaki H., Aratani S., Ohshima T., Miyagishi M., Nakajima T., Fukamizu A. Proc. Natl. Acad. Sci. U.S.A. 101:10042-10047(2004) [PubMed] [Europe PMC] [Abstract]Cited for: INTERACTION WITH FOXO1, FUNCTION IN DEACETYLATION OF FOXO1, MUTAGENESIS OF HIS-355.Ref.15"Increased dosage of mammalian Sir2 in pancreatic beta cells enhances glucose-stimulated insulin secretion in mice." Moynihan K.A., Grimm A.A., Plueger M.M., Bernal-Mizrachi E., Ford E., Cras-Meneur C., Permutt M.A., Imai S. Cell Metab. 2:105-117(2005) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION IN REGULATION OF INSULIN SECRETION.Ref.16"SIRT1 deacetylation and repression of p300 involves lysine residues 1020/1024 within the cell cycle regulatory domain 1." Bouras T., Fu M., Sauve A.A., Wang F., Quong A.A., Perkins N.D., Hay R.T., Gu W., Pestell R.G. J. Biol. Chem. 280:10264-10276(2005) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION.Ref.17"Nuclear trapping of the forkhead transcription factor FoxO1 via Sirt-dependent deacetylation promotes expression of glucogenetic genes." Frescas D., Valenti L., Accili D. J. Biol. Chem. 280:20589-20595(2005) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION IN REGULATION OF FOXO1.Ref.18"Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1." Rodgers J.T., Lerin C., Haas W., Gygi S.P., Spiegelman B.M., Puigserver P. Nature 434:113-118(2005) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION IN DEACETYLATION OF PPARGC1A, FUNCTION IN REGULATION OF GLUCOSE HOMEOSTASIS, INDUCTION.Ref.20"Interactions between E2F1 and SirT1 regulate apoptotic response to DNA damage." Wang C., Chen L., Hou X., Li Z., Kabra N., Ma Y., Nemoto S., Finkel T., Gu W., Cress W.D., Chen J. Nat. Cell Biol. 8:1025-1031(2006) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION, INTERACTION WITH E2F1, MUTAGENESIS OF HIS-355.Ref.21"Sirt1 regulates insulin secretion by repressing UCP2 in

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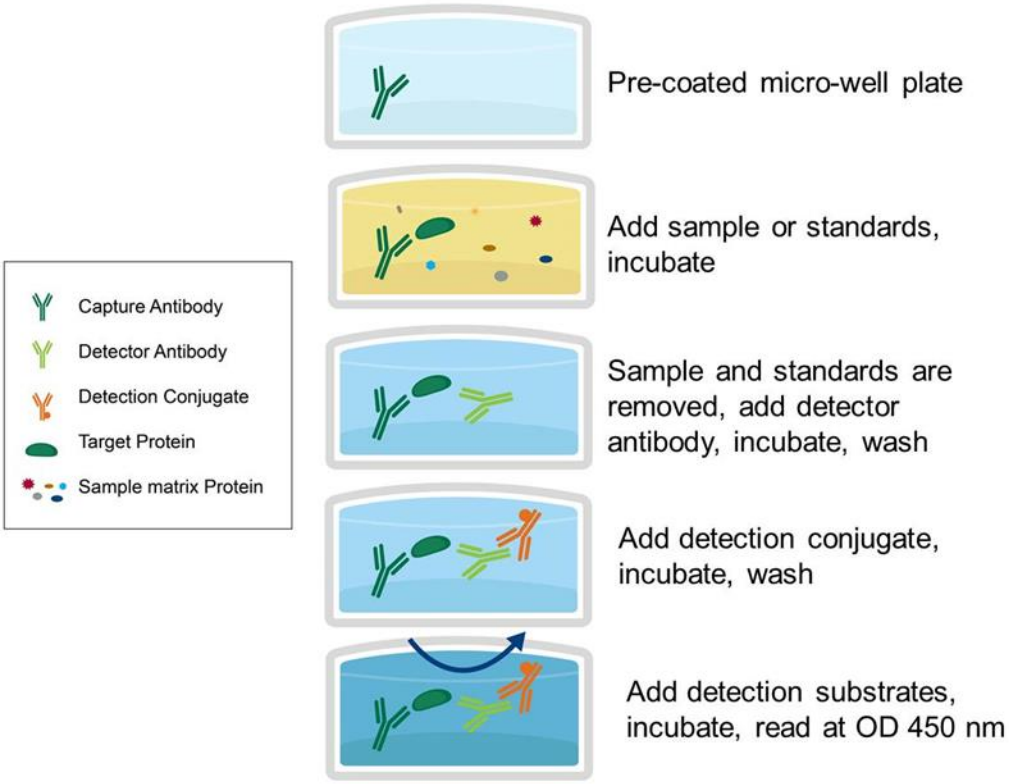
A.A., Brace C.S., Wang T., Stormo G.D., Imai S. Aging Cell 10:305-317(2011) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION, INTERACTION WITH HNF1A.Ref.49"Disruption of a Sirt1-dependent autophagy checkpoint in the prostate results in prostatic intraepithelial neoplasia lesion formation." Powell M.J., Casimiro M.C., Cordon-Cardo C., He X., Yeow W.S., Wang C., McCue P.A., McBurney M.W., Pestell R.G. Cancer Res. 71:964-975(2011) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION IN AUTOPHAGY, DISRUPTION PHENOTYPE.Ref.50"MicroRNA-195 promotes palmitate-induced apoptosis in cardiomyocytes by down-regulating Sirt1." Zhu H., Yang Y., Wang Y., Li J., Schiller P.W., Peng T. Cardiovasc. Res. 92:75-84(2011) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION IN PALMITATE-INDUCED APOPTOSIS, INDUCTION, DOWN-REGULATION BY PALMITATE.Ref.52"BCL6 controls neurogenesis through Sirt1-dependent epigenetic repression of selective Notch targets." Tiberi L., van den Ameele J., Dimidschstein J., Piccirilli J., Gall D., Herpoel A., Bilheu A., Bonnefont J., Iacovino M., Kyba M., Bouschet T., Vanderhaeghen P. Nat. Neurosci. 15:1627-1635(2012) [PubMed] [Europe PMC] [Abstract]Cited for: FUNCTION IN NEUROGENESIS, INTERACTION WITH BCL6. 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.By similarity
 <p>Manually curated information which has been propagated from a related experimentally characterized protein.</p>

 <p>Moreâ€¦</p> Manual assertion inferred from sequence similarity toiUniProtKB:Q96EB6 (SIR1_HUMAN) 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.By similarity
 <p>Manually curated information which has been propagated from a related experimentally characterized protein.</p>

 <p>Moreâ€¦</p> Manual assertion inferred from sequence similarity toiUniProtKB:Q96EB6 (SIR1_HUMAN)

General Specifications	
Specificity	Mouse SIRT1 UniProt: Q923E4 GeneID: 93759 Target Alias: AA673258, MGC150273, mSIR2a, NAD-dependent deacetylase sirtuin-1, Sir2, Sir2a, Sir2alpha, SIR2alpha, Sir2l1, SIR2L1, SIR2-like protein 1
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

• Open kit immediately upon receipt. Store components at -20°C (NOTE: exceptions below) for 6 months or until expiration date. Avoid any freeze/thaw cycles.

4. Kit Components

• The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
SIRT1 Microplate	96 Wells (12 x 8 Well strips)	-20°C for 6 months
SIRT1 Lyophilized Standard	2 vials	
100X Biotinylated SIRT1 Detector Antibody	1 x 120 µL	
100X Avidin-HRP Conjugate	1 x 120 µL	
Sample Diluent	1 x 20 mL	
Detector Antibody Diluent	1 x 12 mL	
Conjugate Diluent	1 x 12 mL	
25X Wash Buffer	1 x 30 mL	Store at 4°C for 6 months
Stop Solution	1 x 10 mL	
TMB Substrate	1 x 10 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.

• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

• Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.

• Automated plate washer (optional).

• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.

• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.

• Absorbent paper or paper toweling.

• Distilled or deionized ultrapure water.

• 37°C Incubator (optional)

7. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing bilirubin, precipitates or fibrin strands or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

• **TMB Substrate** is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use immediately.

8.1 Standard

8.1.1 Prepare a fresh standard curve for each assay performed. Reconstituted standards cannot be stored for later use. For further directions, please refer to the Certificate of Analysis.

8.2 1X Biotinylated SIRT1 Detector Antibody

8.2.1 Prepare the **1X Biotinylated SIRT1 Detector Antibody** immediately prior to use by diluting the **100X Biotinylated SIRT1 Detector Antibody** 1:100 with **Detector Antibody Diluent**.

8.2.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Biotinylated SIRT1 Detector Antibody** to 990 µL **Detector Antibody Diluent**.

8.2.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.3 1X Avidin-HRP Conjugate

8.3.1 Prepare the **1X Avidin-HRP Conjugate** immediately prior to use by diluting the **100X Avidin-HRP Conjugate** 1:100 with **Conjugate Diluent**.

8.3.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Avidin-HRP Conjugate** to 990 µL **Conjugate Diluent**.

8.3.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.4 1X Wash Buffer

8.4.1 If crystals have formed in the **25X Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

8.4.2 Add the entire 30 mL contents of the **25X Wash Buffer** bottle to 720 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

8.4.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.4.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.5 Microplate Preparation

• Micro-plates are provided ready to use and do not require rinsing or blocking.

• Unused well strips should be returned to the original packaging, sealed and stored at 4°C.

• Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation Guidelines

9.1 Sample Preparation and Storage

- Samples must be tested to determine if the kit is valid.
- Store samples to be assayed at 4°C for 24 hours prior being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

General Sample Preparation Guidelines:

- **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Plasma** - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 4°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Tissue Homogenates** – Rinse 100 mg of tissue with 1X PBS, then homogenize in 1 mL of 1X PBS and store overnight at -20°C. Perform two freeze-thaw cycles to break the cell membranes, then centrifuge the homogenate for 5 minutes at 5,000 x g, 2-8°C. Remove the supernatant and assay immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.
- **Cell Lysates -** Adherent cells should be detached with trypsin and then collected by centrifugation (suspension cells can be collected by centrifugation directly). Wash cells three times in cold PBS. Resuspend cells in PBS (1×) and ultrasonicate the cells 4 times (or freeze cells at ≤ -20°C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle 3 times.) Centrifuge at 1,500 x g for 10 minutes at 2 - 8°C to remove cellular debris.
- **Cell culture supernatants and other biological fluids** – Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Recombinant Proteins: Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g. antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products

9.2 Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

- Prior to performing the full experiment, test a serially diluted representative sample.

  - Alternately, a small pool of several samples can also be used with this same method if sample volume is limited.

  -or-

  - Refer to published literature for expected concentrations and derive the optimal dilution level based on the expected dynamic range of the kit

- Dilute samples using **Sample Diluent**.
- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Optimal results for intra- and inter-assay reproducibility will be obtained when performing incubation steps at 37°C as indicated below.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

10.2 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the **SIRT1 Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.3 Cover the plate with the well plate sealer and incubate at 37°C for 2 hours.

10.4 Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.5 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6 Add 100 µL of prepared **1X Biotinylated SIRT1 Detector Antibody** to each well.

10.7 Cover with the well-plate sealer and incubate at 37°C for 60 minutes.

10.8 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.9 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10 Wash plate 3 times with **1X Wash Buffer** as follows:

10.10.1 Add 300 µL of **1X Wash Buffer** to each assay well.

10.10.2 Incubate for 1 minute.

10.10.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10.5 Repeat steps 10.10.1 through 10.10.4 **two** more times.

10.11 Add 100 µL of prepared **1X Avidin-HRP Conjugate** into each well, cover with plate sealer and incubate at 37°C for 60 minutes.

10.12 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.13 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.14 Wash plate **5 times** with **1X Wash Buffer** as in Step 10.10.

10.15 Add 90 µL of **TMB Substrate** to each well, cover with plate sealer and incubate at 37°C **in the dark** for 15-30 minutes. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.
(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

10.16 Add 50 µL of **Stop Solution** to each well. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the **TMB Substrate**.

10.17 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.16. If wavelength correction is available, set to 540 nm or 570 nm.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:

$$(\text{Relative } OD_{450}) = (\text{Well } OD_{450}) - (\text{Mean Blank Well } OD_{450})$$

The standard curve is generated by plotting the mean replicate **Relative OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software. A standard curve should be generated each time the test is performed.

Note: if wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

USA

Aviva Systems Biology, Corp.
7700 Ronson Road, Suite 100
San Diego, CA 92111

Phone: 858-552-6979
Toll Free: 888-880-0001
Fax: 858-552-6975

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