



ab123457

SIRT1 Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human SIRT1 protein.

This product is for research use only and is not intended for diagnostic use.

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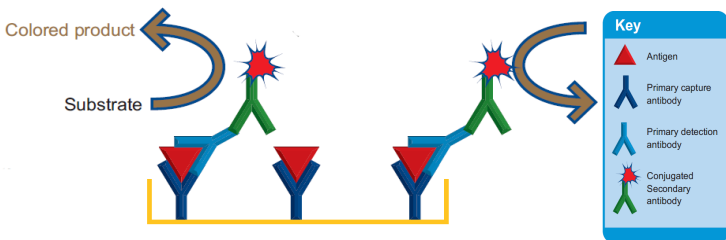
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1. Introduction

Principle: ab123457 SIRT1 Human ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Human SIRT1 in cell and tissue lysates. The assay employs an antibody specific for Human SIRT1 protein coated onto well plate strips. Standards and samples are pipetted into the wells and analyte present in the sample is bound to the wells by the immobilized antibody. The wells are washed and an anti-SIRT1 primary detector antibody is added. After washing away unbound primary detector antibody, HRP-conjugated secondary detector antibody specific for the primary detector antibody is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of analyte bound. The developing blue color is measured at 600 nm. Optionally the reaction can be stopped by adding hydrochloric acid which changes the color from blue to yellow and the intensity can be measured at 450 nm.



Background: SIRT1 - silent mating type information regulation 2 homolog (homolog of yeast Sir2) – encodes a member of the sirtuins family of deacetylases. The Sirtuin 1 protein (gene SIRT1) is responsible for epigenetic gene silencing after recruitment to the nucleus. Sirtuin1 deacetylates proteins, including histones, in a wide (and growing) variety of processes in apoptosis and senescence, muscle differentiation and may serve as a cytosolic NAD⁺/NADH sensor. This enzyme may also regulate the circadian clock of the cell in response to metabolic conditions. Sirtuin 1 is inhibited by nicotinamide and may be activated by resveratrol, a component of red wine. Resveratrol may participate in activation of sirtuin proteins, and may therefore participate in an extended lifespan as it has been observed in yeast.

Specific examples of activity: SIRT1 deacetylates 'Lys-382' of p53/TP53 and impairs its ability to induce proapoptotic program and modulate cell senescence. SIRT1 deacetylates TAF1B and thereby represses rDNA transcription by the RNA polymerase I. SIRT1 deacetylates 'Lys-266' of SUV39H1, leading to its activation. SIRT1 deacetylates 'Lys-26' of HIST1H1E. SIRT1 is involved in HES1- and HEY2-mediated transcriptional repression. SIRT1 inhibits skeletal muscle differentiation by deacetylating PCAF and MYOD1. SIRT1 may serve as a sensor of the cytosolic ratio of NAD(+)/NADH, which is essential in skeletal muscle cell differentiation. SIRT1 deacetylates

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'Lys-16' of histone H4 (in vitro). SIRT1 is a 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. SIRT1 deacetylates H2A. In case of HIV-1 infection, SIRT1 interacts with and deacetylates the viral Tat protein. Deacetylates APEX1 at 'Lys-6' and 'Lys-7'. SIRT1 stimulates cellular AP endonuclease activity by promoting the association of APEX1 to XRCC1.

2. Assay Summary

Bring all reagents to room temperature.

Prepare all the reagents, samples, and standards as instructed.



Add 50 μ L standard or sample to each well used.

Incubate 2 hours at room temperature.



Aspirate and wash each well two times. Add 50 μ L of prepared 1X Detector Antibody to each well.

Incubate 1 hour at room temperature.



Aspirate and wash each well two times. Add 50 μ L of prepared 1X HRP Label to each well.

Incubate 1 hour at room temperature.



Aspirate and wash each well three times.

Add 100 μ L TMB Development Solution to each well.

Immediately begin recording the color development with elapsed time for 15 minutes at 600 nm. Alternatively add a Stop solution at a user-defined time and read at 450 nm.

3. Kit Contents

Item	Quantity
20X Buffer	20 mL
Extraction Buffer	15 mL
10X Blocking Buffer	6 mL
TMB Development Solution	12 mL
10X SIRT1 Detector Antibody	1 mL
10X HRP Label	1 mL
Human SIRT1 standard (1 µg)	1 vial
SIRT1 Microplate (12 x 8nn antibody coated well strips)	1

4. Storage and Handling

Store all components at 4°C. This kit is stable for at least 6 months from receipt. After reconstitution the standard should be stored at -80°C. Unused microplate strips should be returned to the pouch containing the desiccant and resealed.

5. Additional Materials Required

- Microplate reader capable of measuring absorbance at 600 nm (or 450 nm after addition of Stop solution - not supplied).
- Method for determining protein concentration (BCA assay recommended).
- Deionized water
- Multi and single channel pipettes
- PBS (1.4 mM KH_2PO_4 , 8 mM Na_2HPO_4 , 140 mM NaCl, 2.7 mM KCl, pH 7.3)
- Tubes for standard dilution
- Stop solution (optional) – 1N hydrochloric acid
- Optional plate shaker for all incubation steps

6. Preparation of Reagents

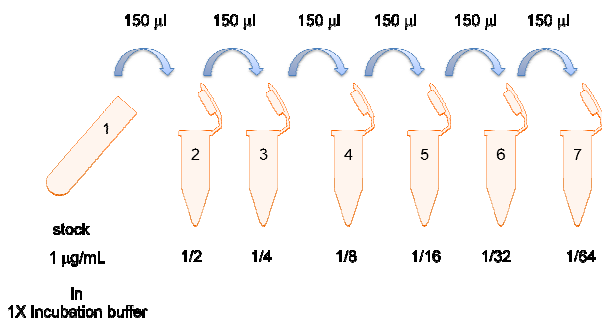
- 6.1 Equilibrate all reagents and samples to room temperature (18-25°C) before use.
- 6.2 Prepare 1X Wash Buffer by adding 20 mL 20X Buffer to 380 mL nanopure water. Mix gently and thoroughly.

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- 6.3 Prepare 1X Incubation Buffer by adding 6 mL 10X Blocking Buffer to 54 mL 1X Wash Buffer. Unused 1X Incubation buffer may be stored at -20°C for 6 months after performing the ELISA.
- 6.4 Prepare the 1X Detector Antibody by diluting the 10X SIRT1 Detector Antibody 10-fold with 1X Incubation Buffer immediately prior to use. Prepare 0.5 mL for each 8 well strip used.
- 6.5 Prepare the 1X HRP label by diluting the stock 10X HRP Label 10-fold with 1X Incubation Buffer immediately before use. Prepare 0.5 mL for each 8 well strip used.
- 6.6 Reconstitute the standard by adding 1 mL 1X Incubation buffer to the stock Human SIRT1 standard (1 µg) tube by pipette. Allow to sit for 10 minutes and repeat pipetting or vortex to ensure thorough reconstitution. This 1 µg/mL stock of standard material is then used to generate a standard curve in labeled tubes as described below. Any remaining stock material can be stored at -80°C.
- 6.7 To prepare serially diluted standards, label six tubes #2-7. Label the reconstituted stock Human SIRT1 standard from step 6.6 tube #1. Add 150 µL of 1X Incubation buffer to each of tubes #2 through #7. Transfer 150 µL from tube #1 to tube

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#2. Mix thoroughly. With a fresh pipette tip transfer 150 μ L from #2 to #3. Mix thoroughly. Repeat for Tubes #4 through #7. Use 1X Incubation buffer as the zero standard tube labeled #8. Use fresh standards for each assay.



7. Sample Preparation

Note: Extraction buffer can be supplemented with phosphatase inhibitors, PMSF and protease inhibitor cocktail prior to use. Supplements should be used according to manufacturer's instructions.

7.1 Cell lysates

7.1.1 Collect non adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 10 min at 4°C.

- 7.1.2 Rinse cells twice with PBS.
- 7.1.3 Solubilize cell pellet at 2×10^7 /mL in Extraction Buffer.
- 7.1.4 Incubate on ice for 20 minutes. Centrifuge at 16,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

7.2 Tissue lysates

- 7.2.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).
- 7.2.2 Suspend the homogenate to 25 mg/mL in PBS.
- 7.2.3 Solubilize the homogenate by adding 4 volumes of Extraction Buffer to a sample protein concentration of 5 mg/mL.
- 7.2.4 Incubate on ice for 20 minutes. Centrifuge at 16,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay

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samples immediately or aliquot and store at -80°C .

The sample protein concentration in the extract may be quantified using a protein assay.

The sample should be diluted to within the working range of the assay in 1X Incubation Buffer. As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Data Analysis.

8. Assay Procedure

Equilibrate all reagents and samples to room temperature before use. It is recommended all samples and standards be assayed in duplicate.

- 8.1 Prepare all reagents, working standards, and samples as directed in the previous sections.
- 8.2 Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and seal.
- 8.3 Add 50 μL of each serially diluted Human SIRT1 standard (tubes 2 – 8) test sample per well. It is recommended to include a dilution series of a control (normal) sample as a

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reference. Also include a 1X Incubation buffer as a zero standard.

- 8.4 Cover/seal the plate and incubate for 2 hours at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.
- 8.5 Aspirate each well and wash, repeat this once more for a total of **two** washes. Wash by aspirating or decanting from wells then dispensing 300 μ L 1X Wash buffer into each well as described above. Complete removal of liquid at each step is essential to good performance. After the last wash, remove the remaining buffer by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.
- 8.6 Immediately before use prepare sufficient (0.5 mL/strip used) 1X Detector Antibody (step 6.4) in 1X Incubation buffer. Add 50 μ L 1X Detector antibody to each well used. Cover/seal the plate and incubate for 1 hour at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.
- 8.7 Repeat the aspirate/wash procedure above.

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- 8.8 Immediately before use prepare sufficient (0.5 mL/strip used) 1X HRP label in 1X Incubation buffer (step 6.5). Add 50 μ L 1X HRP label to each well used. Cover/seal the plate and incubate for 1 hour at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.
- 8.9 Repeat the aspirate/wash procedure above, however, performing a total of **three** washes.
- 8.10 Add 100 μ L TMB Development Solution to each empty well and immediately record the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nM
Time:	up to 15 min.
Interval:	20 sec. - 1 min.
Shaking:	Shake between readings

(*Alternative*– In place of a kinetic reading, at a **user defined**, time record the endpoint OD data at (i) 600 nm or (ii) stop the reaction by adding 100 μ L stop solution (1N HCl) to each well and record the OD at 450 nm.

8.11 Analyze the data as described below.

9. Data Analysis

Average the duplicate standard readings and plot against their concentrations after subtracting the zero standard reading. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Read SIRT1 protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in 1X Incubation buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

TYPICAL STANDARD CURVE - For demonstration only.

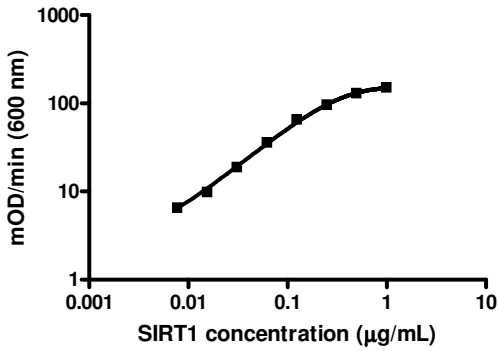


Figure 1. Example standard curve. A dilution series of recombinant SIRT1 in the working range of the assay.

TYPICAL EXPERIMENTAL RESULTS - For demonstration only.

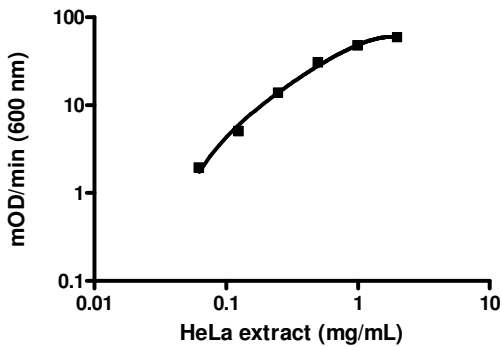


Figure 2. Example HeLa dilution series and working range.

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Typical working ranges	
Sample Type	Range
Tissue extract (e.g. Heart)	None detected
Whole cultured cell extract	0.060 - 1 mg/mL
Human SIRT1 standard	0.008 - 0.5 µg/mL

SENSITIVITY

Determined minimum detectable dose = 8 ng/mL SIRT1 standard.

LINEARITY OF DILUTION

Linearity of dilution determined by comparing dilution series of HeLa cells.

Sample Type	% Expected Value
1:1	100
1:2	112
1:4	96
1:8	97
1:16	99

REPRODUCIBILITY

Parameter	CV%
Average Intra (n= 8)	7
Inter (n=3 days)	7.5

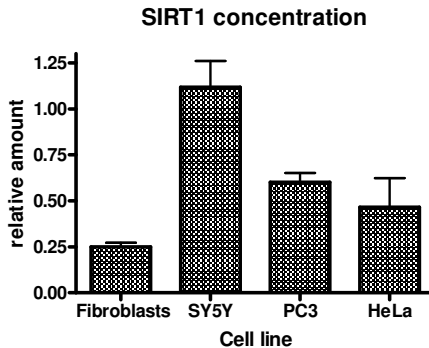
RECOVERY

Sample Type	Average Recovery (%)	Range (%)
10% serum 30-500 ng/mL SIRT1	106	98-112

10. Specificity

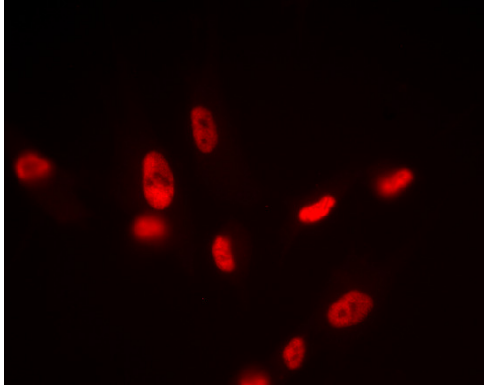
Species– Human. Rat, mouse are not suitable with this kit, other species are untested.

The expression level of SIRT1 varies by cell type. Shown below, four cell types are analyzed by SIRT1 expression level relative to the Human HepG2 cell line. This relative relationship is confirmed by Western blotting using antibody Abcam ab13533.



The SIRT1 detector antibody in this ELISA is available as antibody ab110304.

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Immunocytochemistry image of ab110304 stained Human HDFn cells. The cells were paraformaldehyde fixed (4%, 20 min) and Triton X-100 permeabilized (0.1%, 15min). The cells were incubated with the antibody (19A7AB4, 0.5 $\mu\text{g}/\text{ml}$) for 2h at room temperature or over night at 4°C. 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. Target protein locates to the nucleus.

11. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C , all other assay components 4°C . Keep substrate solution protected from light

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Abcam in the USA

Abcam Inc
1 Kendall Square, Ste B2304
Cambridge,
MA 02139-1517
USA

Toll free: 888-77-ABCAM (22226)

Fax: 866-739-9884

Abcam in Europe

Abcam plc
330 Cambridge Science Park
Cambridge
CB4 0FL
UK

Tel: +44 (0)1223 696000

Fax: +44 (0)1223 771600

Abcam in Japan

Abcam KK
2-2-1 Nihonbashi
Horidome-cho,
Chuo-ku, Tokyo
103-0012
Japan

Tel: +81-(0)3-6231-094

Fax: +81-(0)3-6231-0941

Abcam in Hong Kong

Abcam (Hong Kong) Ltd
Unit 225A & 225B, 2/F
Core Building 2
1 Science Park West Avenue
Hong Kong Science Park
Hong Kong

Tel: (852) 2603-682

Fax: (852) 3016-1888

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