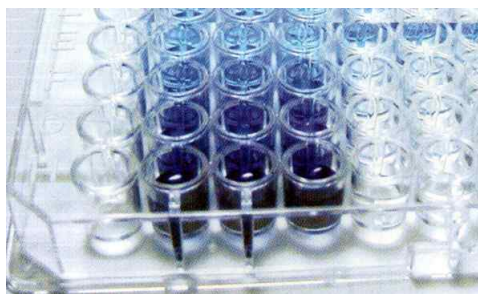


Sirtuin 2 (human) (IntraCellular) ELISA Kit

Cat. No. AG-45A-0030EK-KI01



Instruction Manual

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FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

AdipoGenTM 

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Introduction

Sirtuin 2 is a NAD-dependent histone deacetylase whose size is a calculated molecular mass of 39.5 kD. It is largely localized at the cytoplasm where it is physically associated with microtubules (1). SIRT2 was found to deacetylate lys40 of alpha-tubulin both in vitro and in vivo. SIRT2 colocalized and interacted in vivo with HDAC6, another tubulin deacetylase. Enzymatic analysis of recombinant sirtuin 2 in comparison to a yeast homolog of Sir2 protein showed a striking preference of SIRT2 for acetylated tubulin peptide as a substrate relative to acetylated histone H3 peptide. These observations established sirtuin 2 as a bona fide tubulin deacetylase (2). It has been also shown that sirtuin 2 is a regulator of mitotic progression that acts downstream from CDC14B in a pathway regulating mitotic exit or subsequent cytokinesis (3). Outeiro et al. identified a potent inhibitor of sirtuin 2 and found that inhibition of sirtuin 2 rescued alpha-synuclein toxicity and modified inclusion morphology in a cellular model of Parkinson disease. Genetic inhibition of sirtuin 2 via small interfering RNA similarly rescued alpha-synuclein toxicity, suggesting a link between neurodegeneration and aging via sirtuin 2 (4).

Assay Principles

The human intracellular Sirtuin 2 (iSirtuin 2) ELISA is to be used for quantitative determination of iSirtuin 2 in human cell lysates.

This kit is an enzyme-linked immunosorbent assay (ELISA). A monoclonal antibody specific for human iSirtuin 2 has been pre-coated onto 96 well microplate. Standards and samples are pipetted into the wells and any iSirtuin 2 present is bound by immobilized antibody. Bound iSirtuin 2 is captured by anti-human iSirtuin 2 polyclonal antibody. HRP conjugated anti-rabbit IgG is added. After washing, a substrate solution is added. The colors develop in proportion to the bounded iSirtuin 2 quantity. The color development is stopped and the intensity of color is measured.

Kit Components

- 1) Antibody coated 96-well plate, 12X 8-well strips
- 2) 5X Wash concentrate, 100 ml
- 3) 5X Diluent, 50 ml
- 4) 10X Lysis buffer, 12 ml
- 5) Secondary antibody, 12 ml
- 6) 100X Detector, 150 µl
- 7) Standard, recombinant human Sirtuin 2 expressed by *E. coli* cells, 1 vial, lyophilized
- 8) QC sample = a positive control of recombinant human Sirtuin 2 protein, 1 vial, lyophilized (For actual concentration of QC sample, see the 'Certificate of analysis' enclosed.)
- 9) Substrate, 12 ml
- 10) Stop solution, 12 ml

Reagents Description

Antibody coated 96-well plate, 12x8-well strips, with absorbed monoclonal antibody against human Sirtuin 2

5X Wash concentrate, buffered detergent solution, supplied as a 5X concentrate

5X Diluent, for sample and reagent dilution

10X Lysis buffer

1X Secondary antibody, polyclonal antibody against recombinant human Sirtuin 2

100X detector, HRP conjugated anti-rabbit IgG

Standard, 16.0 ng, recombinant human Sirtuin 2

QC sample, recombinant human Sirtuin 2

Substrate solution, chromogenic reagents

Stop solution, 1M H₃PO₄

Storage of Reagents

Reagents must be stored at 2-8°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 25°C. Diluted wash solution may be stored at room temperature for up to one month.

Materials Required but not Supplied

Precision single and multi-channel pipettes.

Disposable pipette tips.

Microtubes or equivalent for preparing dilutions.

Disposable plastic containers for preparing working reagents.

Reagent reservoirs.

Microwell or microstrip plate reader 450 nm

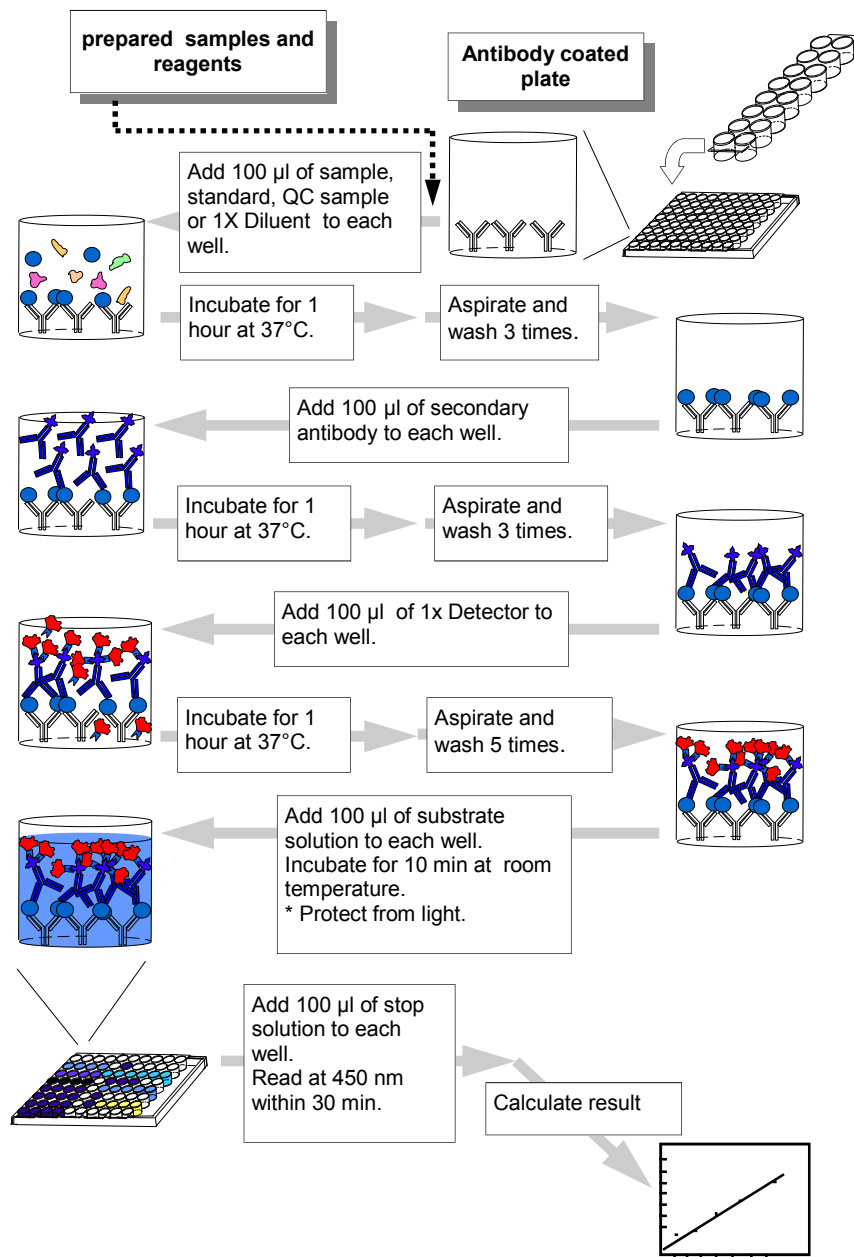
Deionized water

Phenyl methylsulfonyl fluoride (PMSF)

Sample Collection and Storage

Lysate Grow cell until 90% confluency. Scrap cells off the plate and transfer to an appropriate tube. Keep on ice and microcentrifuge at 1,200 rpm for 5 minutes at 4 °C. Remove supernatant, rinse cells once with ice-cold PBS. Remove PBS and add 200 µl ice-cold 1x lysis buffer supplemented with 1 mM phenyl methylsulfonyl fluoride (PMSF) to ten million cells of interest and incubate on ice for 30 minutes. Microcentrifuge at 12,000 rpm for 5 minutes at 4 °C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Use freshly prepared cell lysate samples.

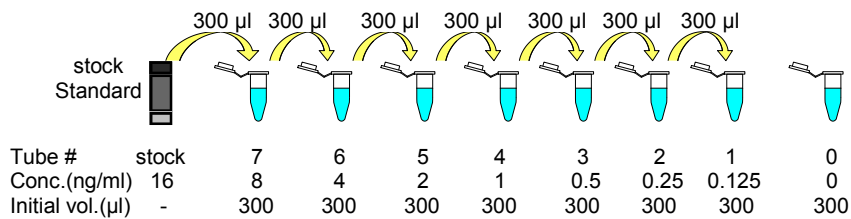
Flow Chart of Assay Procedure



Assay Procedure

1. Preparation of Reagents

- 1) Allow all samples and kit components to equilibrate to room temperature (20-25°C).
- 2) Plan the plate configuration and create a plate map. Calculate the amount of working reagents to use. It is recommended that standards and samples be run in duplicate.
- 3) Prepare **1X lysis buffer**. Dilute 10X Diluent 1:10 with deionized water (1 part 10X Diluent with 9 parts deionized water). Add 1 mM phenyl methylsulfonyl fluoride (PMSF) immediately before use.
- 4) Prepare **1X Wash Solution**. Dilute 5X Wash Concentrate 1:5 with deionized water (1 part 5X Wash Concentrate with 4 parts deionized water). The diluted 1X Wash Solution is stable for one month at room temperature.
- 5) Prepare **1X Diluent**. Dilute 5X Diluent 1:5 with deionized water (1 part 5X Diluent with 4 parts deionized water).
- 6) Prepare **1X Detector**. Dilute 100X Detector 1:100 with 1X Diluent (1 part 100X Detector with 99 parts 1X Diluent). Use the 1X Detector within one hour of preparation.
- 7) Warm **Substrate Solution** to room temperature before use.
- 8) Prepare working aliquots of the Standard as follows :
When opening the lyophilized Standard, remove cap gently as the lyophilizate may have become dislodged during shipping.



Add 1 ml of deionized water to the Standard vial to make a stock concentration of **16 ng/ml**. Mix well.

A recommended dilution scheme is as follows :

- a. Label 8 microcentrifuge tubes #0-7. Add 300 μ l of the 1X Diluent to the microcentrifuge tubes #0-7, respectively.
- b. Add 300 μ l of the stock Standard solution to tube #7 and vortex. This is Standard tube #7 with a concentration of 8 ng/ml.
- c. Standards #6 to #1 are then prepared by performing a 1:2 dilution of the preceding standard. Do not add any standard to the tube #0.

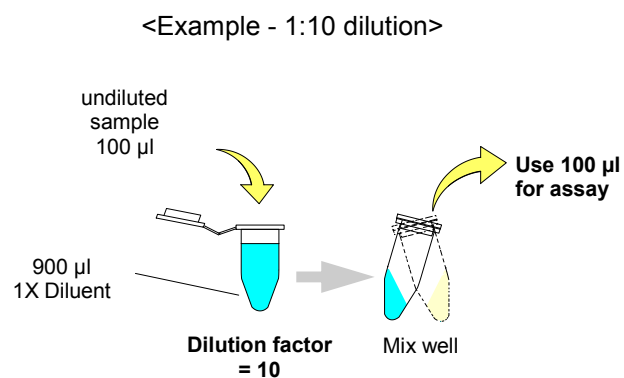
9) Reconstitute QC sample in 1 ml of deionized water. Mix well.

2. Sample Preparation

1) Dilute samples between 1:10 and 1:1000 and mix well.

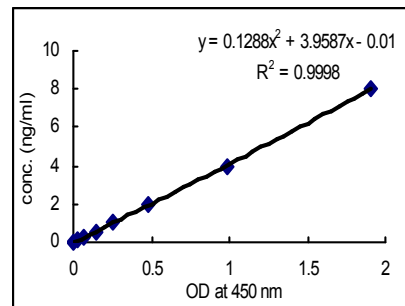
* If samples fall the outside range of assay, a lower or higher dilution may be required.

2) Use 100 μ l of the final diluted sample for ELISA.



3. Experiment procedure

- 1) Remove the appropriate number of microwell strips from the sealed foil pouch.
- 2) Pipette 100 µl of standards #0 to #7, the reconstituted QC sample and diluted sample into the antibody-coated plate according to the plate configuration. Use a new pipette tip for each standard or sample.
- 3) Incubate at 37°C for 1 hour.
- 4) Remove the solution and wash 3 times with 300 µl of 1X Wash Solution to each well.
- 5) Add 100 µl Secondary Antibody to each well.
- 6) Incubate at 37°C for 1 hour.
- 7) Remove the solution and wash 3 times with 300 µl of 1X Wash Solution to each well.
- 8) Add 100µl 1X Detector to each well.
- 9) Incubate at 37°C for 1 hour.
- 10) Remove the solution and wash 5 times with 300 µl of 1X Wash Solution to each well.
- 11) Add 100 µl of the Substrate Solution to each well.
- 12) Incubate at room temperature for 10 min.
* Protect from light.
- 13) Using the multi-channel pipette, add 100 µl Stop Solution to each well.
- 14) Read at 450 nm.
- 15) Subtract the absorbance of the blank from the readings for each standard and sample.
- 16) Construct a standard curve by plotting the known concentrations (Y) of standard versus the absorbance (X) of standard. A measurable range is typically shown between 0.125 ng/ml and 8 ng/ml.
- 17) Calculate iSirtuin 2 concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation.
- 18) The iSirtuin 2 concentrations calculated must be multiplied by dilution factor [see **2. Sample Preparation**] to obtain the concentrations of the undiluted samples.



Performance Characteristics

1) **Sensitivity** : 80 pg/ml

2) **Precision**

a. Intra-Assay (precision within an assay)

1 sample are tested 10 times to assess intra-assay precision.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
293E cells	8.949	0.711	7.948

b. Inter-Assay (precision between assays)

1 sample are tested 6 times to assess inter-assay precision.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
293E cells	9.697	0.787	8.118

3) **Recovery**

The recovery of Sirtuin 2 spiked to three different levels in one sample throughout the range of assay was evaluated.

Sample No.	Average recovery (%)	Range (%)
1	99.418	95-105

4) Specificity

a. Cross Reactivity

Analyte	Max. Conc. (ng/ml)	Cross Reactivity (%)
Human Sirtuin 2	4	100
Human Sirtuin 1	40	N. R.
Human Sirtuin 5	40	N. R.
Human Sirtuin 5 (intact form)	40	N. R.
Human Sirtuin 6	40	N. R.
Human Adiponectin	40	N. R.
Human Resistin	40	N. R.
Human RBP4	40	N. R.
Human Vaspin	40	N. R.
Human Progranulin	40	N. R.
Human GPX3	40	N. R.
Human FTO	40	N. R.
Human Visfatin	40	N. R.
Human Leptin	40	N. R.
Mouse FTO	40	N. R.
Mouse Visfatin	40	N. R.

N. R. : No Cross-reactivity

5) Linearity - Effect of Lysate Dilution

To assess the linearity of the assay, one lysate sample are first diluted as indicated below prior to sample preparation as described in the protocol.

Sample No.	Sample Dilution	Expected (ng/ml)	Observed (ng/ml)	% Of Expected
293E cells	1:10	9.67	9.67	100
	1:20	4.84	4.68	96.78
	1:40	2.42	2.38	98.24

$$\% \text{ of expected} = \text{observed} / \text{expected} \times 100\%$$

References

1. North, B. J., Marshall, B. L., Borra, M. T. et al. 2003 The human Sir2 ortholog, SIRT2, is an NAD(+)-dependent tubulin deacetylase. *Mol. Cell* 11: 437-444.
2. Dryden, S. C., Nahhas, F. A., Nowak, J. E. et al. 2003 Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle. *Mol. Cell. Biol.* 23: 3173-3185.
3. Outeiro, T. F., Kontopoulous, E., Altmann, S. M. et al. 2007 Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of Parkinson's disease. *Science* 317: 516-519.

Troubleshooting Guide

Problem	Possible Cause	Solution
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be appropriate for the system.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of detector too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.
	Technique problem	Proper mixing of reagents and wash steps are critical.

AdipoGen Inc.

Room #401, Venture Building B,
Songdo TechnoPark, 7-50
Songdo-dong, Yeonsu-gu,
Incheon, Korea

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TECHNICAL INFORMATION

Web www.adipogen.com

E-mail adipogen-kr@adipogen.com

Phone +82-32-858-1470

Fax +82-32-831-1470