# Sirtuin 1 (human intracellular) ELISA Kit

(Catalog #K4923-100; 100 assays; Store kit at 4°C)

## I. Description:

Sirtuin 1 is the human ortholog for the yeast Sir2 (silent information regulator 2) protein regulating epigenetic gene silencing as a possible antiaging effect. Sirtuin 1 is an NAD(+)-dependent histone deacetylase, which deacetylate lysines 9 and 14 of histone H3 and lysine-16 of histone H4, involved in various cellular functions such as transcription, energy sensing, and differentiation. Sirtuin1 plays an important role in regulating adipogenesis via repression of PPAR and the gluconeogenic/glycolytic pathways in liver in response to fasting signals through the transcriptional coactivator PGC1A deacetylated at specific lysine residues in an NAD(+)-dependent manner. This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for quantitative determination of human Sirtuin 1 in cells. A monoclonal antibody specific for Sirtuin 1 has been precoated onto the 96-well microtiter plate. Standards and samples are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, Sirtuin 1 is recognized by the addition of a purified polyclonal antibody specific for Sirtuin 1 (Detection Antibody). After removal of excess polyclonal antibody, HRP conjugated anti-rabbit IgG (HRP) is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of Sirtuin 1 in the samples. This ELISA is specific for the measurement of natural and recombinant human Sirtuin 1. It does not cross-react with human Sirtuin 2, human Sirtuin 5, human Sirtuin 6, human adiponectin, human resistin, human RBP4, human vaspin, human progranulin, human GPX3, human FTO, human Nampt, human leptin, mouse FTO, mouse Nampt. The assay range is 0.031 – 2 ng Sirtuin 1/ml and a detection limit of 30 pg/ml (based on adding two standard deviations to the mean value of the (50) zero standards).

## II. Kit Contents:

| Component | 100 Assays | Part Number |
|---|---|---|
| Pre-coated Microtiter Plate | 6 x 16 well strips | K4923-100-1 |
| Wash Buffer (10X) | 2 x 30 ml | K4923-100-2 |
| ELISA Buffer (10X) | 2 x 30 ml | K4923-100-3 |
| Lysis Buffer (10X) | 12 ml | K4923-100-4 |
| Detection Antibody | 60 µl | K4923-100-5 |
| HRP 100X (HRP conjugated anti-IgG) | 150 µl | K4923-100-6 |
| Human Sirtuin 1 Standard (lyophilized) | 4 ng | K4923-100-7 |
| TMB Substrate Solution | 12 ml | K4923-100-8 |
| Stop Solution | 12 ml | K4923-100-9 |
| Plate Sealers | 2 | K4923-100-10 |
| Gel Minibags | 2 | K4923-100-11 |

## III. Storage Conditions:

Reagents must be stored at 2 - 8°C when not in use. Bring reagents to room temperature before use. Do not expose reagents to temperatures greater than 25°C.

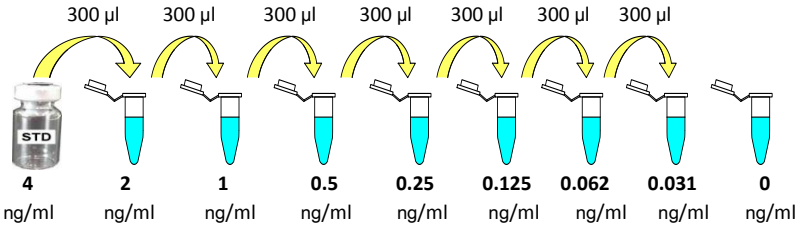
## IV. Assay Procedure (Read the ENTIRE Protocol Before Proceeding)

### 1. Test Samples/Standards/QC Sample: (We recommend these be run in duplicate)

a) **Cell Lysates**: 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 Lysis Buffer 1X 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. Cell Lysates have to be diluted in ELISA Buffer 1X. Samples containing visible precipitates must be clarified before use. **Note**: As a starting point, 1/10 to 1/1,000 dilutions of cell lysates are recommended! If sample values fall outside the detection range of the assay, a lower or higher dilution may be required.

b) **Standards**: Reconstituted with 1 ml of deionized water. This reconstitution produces a stock solution of 4 ng/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. NOTE: The reconstituted standard is aliquoted and stored at -20°C. Prepare Standard Curve using 2-fold serial dilutions with 1X Diluent:

| To obtain | Add | Into |
|---|---|---|
| 2 ng/ml | 300 µl of SIRTUIN 1 (4 ng/ml) | 300 µl of 1X ELISA Buffer |
| 1 ng/ml | 300 µl of SIRTUIN 1 (2 ng/ml) | 300 µl of 1X ELISA Buffer |
| 0.5 ng/ml | 300 µl of SIRTUIN 1 (1 ng/ml) | 300 µl of 1X ELISA Buffer |
| 0.25 ng/ml | 300 µl of SIRTUIN 1 (0.5 ng/ml) | 300 µl of 1X ELISA Buffer |
| 0.125 ng/ml | 300 µl of SIRTUIN 1 (0.25 ng/ml) | 300 µl of 1X ELISA Buffer |
| 0.06 ng/ml | 300 µl of SIRTUIN 1 (0.125 ng/ml) | 300 µl of 1X ELISA Buffer |
| 0.031 ng/ml | 300 µl of SIRTUIN 1 (0.062 ng/ml) | 300 µl of 1X ELISA Buffer |
| 0 ng/ml | 300 µl of 1X Diluent | Empty tube |




### 2. Reagent Preparation: (Prepare just the appropriate amounts for the assay)

a) **1X Wash Buffer:** Dilute 10X Wash Buffer 1: 9 with $dH_2O$ to obtain 1X Wash Buffer.
b) **1X ELISA Buffer:** Dilute 10X Wash Buffer 1: 9 with $dH_2O$ to obtain 1X ELISA Buffer.
c) **1X Lysis Buffer:** Dilute with deionized water 1:10 to obtain Lysis Buffer 1X. Add 1 mM PMSF immediately before use.
d) **Detection Antibody (DET):** Dilute to 1:200 in 1X ELISA Buffer. The diluted Detection Antibody is not stable and cannot be stored.
e) **HRP 100X (HRP Conjugated anti-rabbit IgG):** Dilute 1:100 in 1X ELISA Buffer. Note: The diluted HRP must be used within 1 hr of preparation

### 3. Assay Protocol:

1. Determine the number of 16-well strips needed for assay and insert them into the frame for current use. The extra strips should be resealed in the foil pouch and can be stored at 4°C for up to 1 month.
2. Add 100 µl of the Standards, Samples and QC Sample into the appropriate wells in duplicate.
3. Cover plate with plate sealer and incubate for 1 hr at 37°C.
4. Aspirate and wash x 3 with 300 µl of 1X Wash Buffer. After the last wash, complete removal of liquid is essential for good performance.
5. Add 100 µl Detection Antibody (DET) to each well and tap gently on the side of the plate to mix.
6. Cover plate with plate sealer and incubate for 1 hr at 37°C.
7. Aspirate and wash x 3 with 300 µl of 1X Wash Buffer.
8. Add 100 µl of the 1X HRP to each well.
9. Cover plate with plate sealer and incubate for 1 hr at 37°C.
10. Remove plate from 37°C, aspirate and wash x 5 with 300 µl of 1X Wash Buffer. After last wash, tap inverted plate on a stack of paper towels. Complete removal of liquid is essential for good performance.
11. Add 100 µl of the TMB Substrate Solution to each well.
12. Allow the color to develop at room temperature in the dark for 10 min.
13. Stop the reaction by adding 100 µl of Stop Solution to each well.
14. Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added. **Caution:** Stop Solution is a Corrosive Solution
15. Measure the OD at 450 nm in an ELISA plate reader within 30 min.

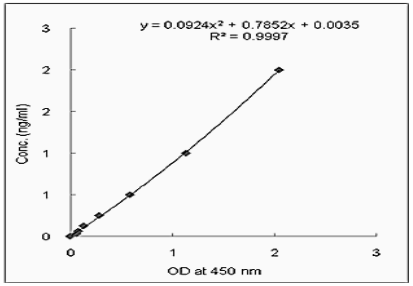
**3. Calculations:**

a) Average the duplicate readings for each Standard, control and test samples and subtract the average blank value (obtained with the 0 ng/ml point).

b) Generate a Standard Curve by plotting the average absorbance on the horizontal (X) axis vs. the corresponding concentration (ng /ml) on the vertical (Y) axis. (See Typical Data below)

c) Calculate the Test Sample Sirtuin 1 concentrations by interpolation of the Standard Curve regression curve as shown below in the form of a quadratic equation.

d) If the Test Samples were diluted, multiply the interpolated values by the dilution factor to calculate the corrected human Sirtuin 1 concentrations.




**VI. Performance Characteristics**:

1. Intra-assay Precision: (3) samples of known concentrations of human Sirtuin 1 were assayed in replicates (4) times to test precision within an assay.

| Samples | Mean (ng/ml) | SD | CV (%) | n |
|---|---|---|---|---|
| 293E cells | 30.765 | 0.742 | 2.413 | 4 |
| HT-29 cells | 7.980 | 0.645 | 8.082 | 4 |
| Molt4 cells | 10.502 | 0.748 | 7.121 | 4 |

2. Inter-assay Precision: (3) samples of known concentrations of human Sirtuin 1 were assayed in (6) separate assays to test precision between assays.

| Samples | Mean (ng/ml) | SD | CV (%) | n |
|---|---|---|---|---|
| 293E cells | 30.778 | 2.326 | 7.558 | 6 |
| HT-29 cells | 7.675 | 0.687 | 8.951 | 6 |
| Molt4 cells | 10.655 | 0.430 | 4.037 | 6 |

3. Recovery: Different human cell lysates were spiked with known concentrations of human Sirtuin 1 and the recoveries averaged 98 % (range from 90 % to 110 % )

| Samples | Average (%) | Range (%) |
|---|---|---|
| 293E cells | 92.703 | 90-100 |
| HT-29 cells | 98.497 | 95-105 |
| Molt4 cells | 104.333 | 100-110 |

**VII. Technical Hints and Limitations:**

- It is recommended that all standards, controls and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100 µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the 16-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep Substrate Solution protected from light.
- The Stop Solution consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

**VIII. Troubleshooting:**

| PROBLEM | POSSIBLE CAUSES | SOLUTIONS |
|---|---|---|
| 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 followed as indicated in the manual. |
| | 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 HRP 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. |

***FOR RESEARCH USE ONLY! Not to be used on humans.***