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Product Information

Sirtuin 1 Human Intracellular ELISA Kit

Catalog Number **EPI015**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Sirtuin 1 is the human ortholog for the yeast Sir2 (silent information regulator 2) protein, regulating epigenetic gene silencing as a possible anti-aging effect. Sirtuin 1 is an NAD(+)-dependent histone deacetylase, which deacetylates Lys^{9} and Lys^{14} of histone H3 and Lys^{16} of histone H4, involved in various cellular functions such as transcription, energy sensing, and differentiation. Sirtuin 1 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.

The Sirtuin 1 Human Intracellular ELISA Kit 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 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 the excess polyclonal antibody, HRP conjugated anti-rabbit IgG (Detector) 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.

Components

The kit is sufficient for 100 assays in 96 well plates.

Pre-coated Multiwell Plate, 12 × 8 well strips Catalog Number EPI015A	1 each
Wash Buffer 10× Catalog Number EPI015B	50 mL
Diluent 5× Catalog Number EPI015C	50 mL
Lysis Buffer 10× Catalog Number EPI015D	12 mL
Detection Antibody Catalog Number EPI015E	12 mL
Detector 100× (HRP-conjugated anti-IgG) Catalog Number EPI015F	150 µL
Human Sirtuin 1 Standard (4 ng) Catalog Number EPI015G	1 vl
Human Sirtuin 1 QC Sample Catalog Number EPI015H	1 vl
TMB Substrate Solution Catalog Number EPI015I	12 mL
Stop Solution Catalog Number EPI015J	12 mL
Plate Sealers Catalog Number EPI015K	3 each

Reagents Required but Not Provided.

- Phosphate Buffered Saline (Catalog Number P5368 or equivalent)
- Phenylmethanesulfonyl fluoride (Catalog Number P7626 or equivalent)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Reagents from the kit with a volume less than 100 µL should be centrifuged.

To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Bring reagents to room temperature before use. Crystals could appear in the 10× solutions due to high salt concentration in the solutions. Crystals are readily dissolved at room temperature or at 37 °C before dilution of the buffer solutions.

Use ultrapure water for the preparation of reagents.

1× Wash Buffer – Dilute 10× Wash Buffer 1:9 with water to obtain 1× Wash Buffer.

1× Diluent – Dilute 5× Diluent 1:4 with water to obtain 1× Diluent.

1× Lysis Buffer – Dilute 10× Lysis Buffer 1:9 with water to obtain 1× Lysis Buffer.

Detection Antibody and TMB Substrate Solutions are ready to use. Warm to room temperature before use.

1× Detector – Dilute 100× Detector 1:99 with 1× Diluent to obtain 1× Detector.
Note: The diluted Detector must be used within 1 hour of preparation.

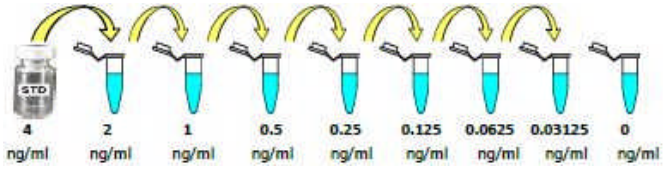
QC Sample Preparation – Reconstitute Human Sirtuin 1 QC Sample with 1 mL of water. Mix the QC Sample to ensure complete reconstitution. Allow to sit for a minimum of 15 minutes. The QC Sample is ready to use. Do not dilute it.

Preparation of Standards
1. Reconstitute Human Sirtuin 1 Standard with 1 mL of water to produce Stock Standard Solution (4 ng/mL). Mix the Stock Standard Solution to ensure complete reconstitution.
2. Allow to sit for a minimum of 15 minutes.
3. Store the Stock Standard Solution in 300 µL aliquots at –20 °C.
4. Prepare 2-fold serial dilutions of Stock Standard Solution with 1× Diluent (see Table 1).

Table 1.
Sirtuin 1 Standards

Sirtuin 1 Standard	1× Diluent	Final Sirtuin 1 Concentration
300 µL of (4 ng/mL)Stock Standard Solution	300 µL	2 ng/mL
300 µL of 2 ng/mL	300 µL	1 ng/mL
300 µL of 1 ng/mL	300 µL	0.5 ng/mL
300 µL of 0.5 ng/mL	300 µL	0.25 ng/mL
300 µL of 0.25 ng/mL	300 µL	0.125 ng/mL
300 µL 0.125 ng/mL	300 µL	0.0625 ng/mL
300 µL 0.0625 ng/mL	300 µL	0.03125 ng/mL
No Standard	300 µL	0 ng/mL

Figure 1.
Serial Dilution of Stock Standard Solution (4 ng/mL)



Storage/Stability
The kit is shipped on wet ice. All components should be stored at 2–8 °C. Keep Substrate Solution protected from light.

Procedure
Read the entire protocol before performing the assay. All standards, QC sample, and samples should be run in duplicate.

Sample Preparation
1. Grow cells to 90% confluency.
2. Scrap cells off the plate and transfer to an appropriate tube.
3. Keep on ice and microcentrifuge at 1,200 rpm for 5 minutes at 2–8 °C.
4. Remove supernatant and rinse cells once with ice-cold PBS.
5. Remove PBS and add 200 µL of ice-cold 1× Lysis Buffer supplemented with 1 mM phenyl methylsulfonyl fluoride (PMSF) to 10^7 cells.
6. Incubate on ice for 30 minutes.
7. Microcentrifuge at 12,000 rpm for 5 minutes at 2–8 °C and transfer the supernatant to a new tube. The supernatant is the cell lysate.
8. Cell lysates have to be diluted in 1× Diluent. As a starting point 10 to 1,000-fold dilutions are recommended.
   Note: Use freshly prepared cell lysate samples. Samples containing visible precipitates must be clarified before use.

Assay Reaction

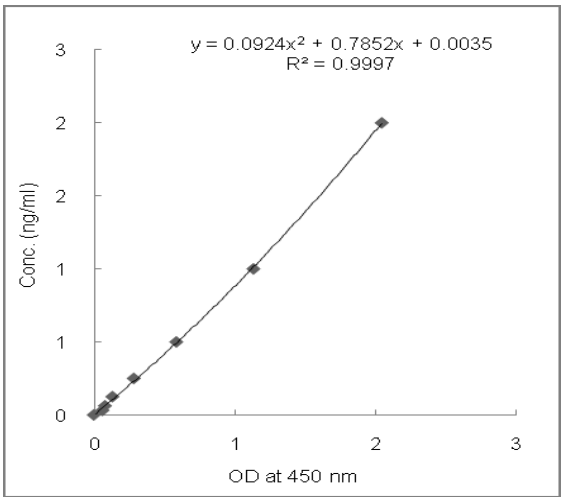
1. Determine the number of 8 well strips needed for assay and insert them into the frame for current use. The extra strips should be returned to the foil pouch and can be stored at 2–8 °C up to 1 month.
2. Add 100 µL of the Stock Standard Solution, serial dilutions of the Stock Standard Solution, Samples, and QC Sample into the appropriate wells in duplicate.
   Note: Once reagents have been added to the 8 well strips, DO NOT let the strips dry at any time during the assay.
3. Cover plate with plate sealer and incubate for 1 hour at 37 °C.
4. Aspirate and wash 3 times with 300 µL of 1× Wash Buffer.
5. Add 100 µL of Detection Antibody to each well and tap gently on the side of the plate to mix.
6. Cover plate with plate sealer and incubate for 1 hour at 37 °C.
7. Aspirate and wash 3 times with 300 µL of 1× Wash Buffer.
8. Add 100 µL of the 1× Detector to each well.
9. Cover plate with plate sealer and incubate for 1 hour at 37 °C.
10. Remove plate form 37 °C, aspirate, and wash 5 times with 300 µL of 1× Wash Buffer.
11. After last wash, tap inverted plate on a stack of paper towels. Complete removal of liquid is essential for good performances.
12. Add 100 µL of the TMB Substrate Solution to each well.
13. Allow the color to develop at room temperature in the dark for 10 min.
14. Stop the reaction by adding 100 µL of Stop Solution to each well.
15. Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
16. Measure the OD at 450 nm in an ELISA plate reader within 30 minutes.

Results

Calculations

1. Average the duplicate readings for each Standard solution, QC Sample, and Test Sample, and subtract the average blank value (obtained with the 0 ng/mL point).
2. Generate a Standard Curve by plotting the average absorbance on the horizontal x-axis versus the corresponding concentration (ng/mL) on the vertical y-axis.
3. Calculate the Test Sample Sirtuin 1 concentrations by interpolation of the Standard Curve regression curve in the form of a quadratic equation (see Figure 2).

Figure 2.
Sirtuin 1 Standard Curve



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

Product Profile

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].

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.

Troubleshooting Guide

Problems	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 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.

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