Signosis
Innovative Plate Assay Solutions

SIRT Activity Assay Kit

Catalog Number EA-7061 **(For Research Use Only)**

Introduction

Sirtuin (SIRT) is an enzyme that plays a crucial role in various cellular processes including metabolism and stress response. SIRT functions as a NAD+ dependent deacetylase, which removes acetyl groups from proteins and alters their activity.

Principle of the assay

The SIRT activity assay kit can measure the activity of SIRT using a FRET-based peptide substrate. The peptide has a fluorophore on the N-terminus, quencher on the C-terminus, and an acetylated lysine side chain. Before the peptide is cleaved, the quencher prevents fluorescence from being emitted. When a reaction mixture containing the SIRT enzyme and a Lys-protease is added to the peptide substrate, SIRT first deacetylates the acetyl group on the lysine. Then, once the lysine is exposed, the Lys-protease will cut the peptide on the carboxyl side of the lysine, splitting the peptide in half and separating the fluorophore from the quencher. Once the fluorophore is separated from the quencher, it will emit fluorescence that can be measured at Ex/Em: 350/460 nm.

Materials Provided

- SIRT Substrate (-80°C)
- Protease Stock (-80°C)

Cell Sample Preparation

1. Wash the cells once with PBS before lysing the cells.
2. For a 96-well culture plate, add 40 µL of lysis buffer to each well and incubate at room temperature for 10 minutes.
3. Pipette the lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
4. If necessary, homogenize the cell lysates with a sonicator.
5. The cell lysates may be assayed directly or stored at -80°C.
6. Use PBS to dilute the cell sample to the appropriate concentration, if necessary.

Tissue Sample Preparation

1. Weigh tissue sample and add 1 mL of lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C.
6. Use PBS to dilute the tissue sample to the appropriate concentration, if necessary.

Assay Procedure

1. Reaction mix preparation: calculate the amount of each reagent needed to make the reaction mix according to the table below.

Component	Reaction Mix (per well/sample)
SIRT substrate	1 µL
1x Protease stock	1 µL
PBS	48 µL
Total	50 µL

2. Add 50 µL of reaction mix to each well of the plate.
3. Add 50 µL of sample to each well with reaction mix and mix thoroughly.
4. Measure the fluorescence of the plate at Ex/Em: 350/460 nm. Obtain kinetic data for SIRT activity by making multiple readings of the plate. Start at one-minute intervals and increase the interval time as the signal begins to stabilize.