

ab283377– SIRT1 Inhibitor/Activator Screening Kit (Fluorometric)

For the screening of SIRT1 inhibitors and activators.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab283377>

Storage and Stability

On receipt entire assay kit should be stored at -80°C, protected from light. Upon opening, use kit within 1 year. Avoid repeated freeze/thaw for all non-buffer components.

Materials Supplied

Item	Quantity	Storage Condition
SIRT1 Assay Buffer	25 mL	-80°C
1 M DTT	0.4 mL	-80°C
Substrate (in DMSO)	0.2 mL	-80°C
NAD	1 vial	-80°C
SIRT1 Enzyme	0.2 mL	-80°C
Inhibitor (Nicotinamide, 4 mM)	0.9 mL	-80°C
Developer	1 mL	-80°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Fluorescence microplate reader.

Reagent Preparation

Before using the kit, spin the tubes prior to opening.

SIRT1 Assay Buffer: Store at 4°C or -20°C. Warm to 37°C and add DTT to final concentration of 2 mM just before use. Make fresh as needed.

1 M DTT: Store at -20°C. Thaw and keep on ice while in use. Use within two months.

Substrate: Aliquot and Store at -20°C. Avoid repeated freeze/thaw. Use fresh tip each time. Use within two months.

NAD: Reconstitute with 220 µl deionized water. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Before use, dilute NAD by adding 2 µl of NAD stock solution to 58 µl of SIRT1 Assay Buffer without DTT. Make as much as needed. Use within two months.

SIRT1 Enzyme: Thaw and mix gently by pipetting. Aliquot and store at -80°C. Use within two months.

Inhibitor (Nicotinamide): Store at -20°C. Keep on ice while in use.

Developer: Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use.

Assay Protocol

Enzyme Solution Preparation:

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 25 µl SIRT1 Enzyme Solution:

Item	Volume
SIRT1 Assay Buffer (with DTT)	23 µL
SIRT1 Enzyme	2 µL

2. Mix & add 25 µl of the SIRT1 Enzyme Solution into desired wells.

Screen compounds, Inhibitor Control, Enzyme Control & Blank Control Preparations:

1. Dissolve candidate inhibitors/activators at 1000X highest final test concentration into an appropriate solvent.
2. Dilute to 4X the desired test concentration with SIRT1 Assay Buffer (with DTT).
3. Add 25 µl Inhibitor (Nicotinamide), Assay Buffer or diluted test inhibitor/activator into SIRT1 Enzyme solution wells as Inhibitor Control, Enzyme Control [EC] (no inhibitor) or sample screen [S].
4. Add 50 µl SIRT1 Assay Buffer into one well as Blank Control (no enzyme).
5. Mix well, and incubate the plate for 5 min. at 37°C

Δ Note: High solvent concentration might affect the enzymatic activity. Prepare parallel well(s) as solvent control to test the effect of the solvent on enzyme activity.

Substrate preparation:

1. For each well, prepare 40 µl of Substrate solution.

Item	Volume
SIRT1 Assay Buffer (with DTT)	36 µL
Substrate	2 µL*
Diluted NAD	2 µL

2. Add 40 µl of the Substrate solution into each well. Mix & incubate at 37°C for 30-60 min.

Δ Note: To screen for Activators, dilute the Substrate 10x in DMSO and use 2 µl to prepare the Substrate Solution.

Develop:

1. Add 10 µl Developer into each well. Mix well and incubate for 10 min. at 37°C, protected from light.

Measurement:

Read fluorescence (Ex/Em = 400/505 nm).

Calculations:

1. Subtract the Blank Control reading from all readings to obtain Δ RFU for each reading.
2. Set the Δ RFU of Enzyme Control [EC] as 100%, and calculate % Inhibition or % Relative Activity of the test inhibitors or % Activation of activators as follows:

$$\% \text{ Relative Activity} = \frac{\Delta \text{RFU of } S}{\Delta \text{RFU of } EC} \times 100$$

$$\% \text{ Inhibition} = \frac{\Delta \text{RFU of } EC - \Delta \text{RFU of } S}{\Delta \text{RFU of } EC} \times 100$$

$$\% \text{ Activation} = \frac{\Delta \text{RFU of } S - \Delta \text{RFU of } EC}{\Delta \text{RFU of } EC} \times 100$$

$$\text{Fold Activation} = \frac{\Delta \text{RFU of } S}{\Delta \text{RFU of } EC} \times 100$$

Technical Support

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