

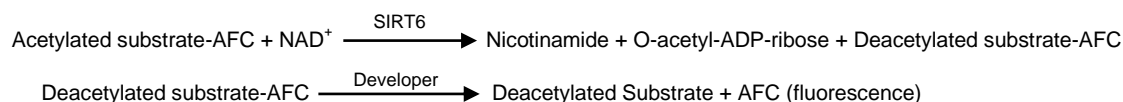
# SIRT6 Inhibitor Screening Kit (Fluorometric)

Rev 04/19

(Catalog # K323-100; 100 assays; Store kit at -20°C)

## I. Introduction:

SIRT6 or Sirtuin 6 proteins are a class of proteins that possess either histone deacetylase or mono-ribosyltransferase activity. SIRT6 is a nuclear sirtuin that has been associated with aging, cellular protection, sugar metabolism and certain types of cancer. Broad therapeutic applications are foreseen for SIRT6 inhibitors, including uses in diabetes, immune-mediated disorders, and cancer. Unlike other known protein deacetylases, which simply hydrolyze acetyl-lysine residues, the sirtuin-mediated deacetylation reaction hydrolyzes acetyl-lysine and NAD. This hydrolysis yields the deacetylated substrate, O-acetyl-ADP-ribose and nicotinamide, itself an inhibitor of sirtuin activity. Studies suggest that the human sirtuins may function as intracellular regulatory proteins with mono-ADP-ribosyltransferase activity. In BioVision's Sirtuin 6 inhibitor screening kit, Sirtuin 6 deacetylates the substrate, followed by cleavage of the deacetylated substrate to release the fluorescent group, which is detected fluorometrically at Ex/Em = 400/505 nm. In the presence of SIRT inhibitor, deacetylation is impeded, preventing cleavage of the substrate and release of the fluorescent group. This kit provides a rapid, simple, sensitive, and reliable test, which is suitable for high-throughput screening of SIRT6 inhibitors. Inhibitor control (Nicotinamide) is included to compare the efficacy of the test inhibitors.



## II. Application:

- Screening/characterizing/studying SIRT6 inhibitors

## III. Kit Contents:

| Components                     | K323-100 | Cap Code | Part Number |
|--------------------------------|----------|----------|-------------|
| SIRT6 Assay Buffer             | 25 ml    | WM       | K323-100-1  |
| 1 M DTT                        | 0.4 ml   | Green    | K323-100-2  |
| Substrate (DMSO)               | 0.2 ml   | Red      | K323-100-3  |
| Cofactor                       | 1 vial   | Purple   | K323-100-4  |
| Developer                      | 1 ml     | Orange   | K323-100-5  |
| SIRT6 Enzyme                   | 100 µl   | Amber    | K323-100-6  |
| Inhibitor (Nicotinamide, 4 mM) | 0.9 ml   | Blue     | K323-100-7  |

## IV. User Supplied Reagents and Equipments:

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

## V. Storage Condition and Reagent Preparation:

Store kit at -20°C, protected from light. Avoid repeated freeze/thaw for all non-buffer components. Briefly centrifuge small vials before opening. Read entire protocol before performing the assay. Use kit within two months.

- **SIRT6 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:** Once thawed, aliquot and store at -80°C.
- **Cofactor:** Reconstitute with 220 µl ddH<sub>2</sub>O. Aliquot and Store at -80°C. Avoid repeated freeze/thaw cycles. Use within two months.
- **Developer:** Store at -80°C once thawed. Keep on ice while in use.
- **SIRT6 Enzyme:** Thaw and mix gently by pipetting. Aliquot and store at -20°C. After opening, use within two months.

## VI. SIRT6 Inhibitor Screening Assay Protocol:

1. **Enzyme Solution Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare 25 µl SIRT6 Enzyme solution:

|                    |       |
|--------------------|-------|
| SIRT6 Assay Buffer | 24 µl |
| SIRT6 Enzyme       | 1 µl  |

Mix & add 25 µl of the SIRT6 Enzyme Solution into desired wells.

2. **Screen compounds, Inhibitor Control, Enzyme Control & Blank Control Preparations:** Dissolve candidate inhibitors at 1000X highest final test concentration into an appropriate solvent. Dilute to 4X the desired test concentration with SIRT6 Assay Buffer. Add 25 µl Inhibitor, Assay Buffer or diluted test inhibitor into SIRT6 Enzyme solution wells as Inhibitor Control (Nicotinamide), Enzyme Control [EC] (no inhibitor) or sample screen [S]. Mix well, and incubate for 5 min. at 37°C. Add 50 µl SIRT6 Assay Buffer into one well as Blank Control.

**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.

3. **Substrate preparation:** Dilute Cofactor by adding 2 µl of Cofactor solution to 58 µl of SIRT6 Assay Buffer (without DTT) just before use. Make as much as needed. For each well, prepare 40 µl of Substrate solution.

FOR RESEARCH USE ONLY!

SIRT6 Assay Buffer 36  $\mu$ l  
Substrate 2  $\mu$ l  
Diluted Cofactor 2  $\mu$ l

Add 40  $\mu$ l of the Substrate solution into each well. Mix & incubate at 37°C for 60 min.

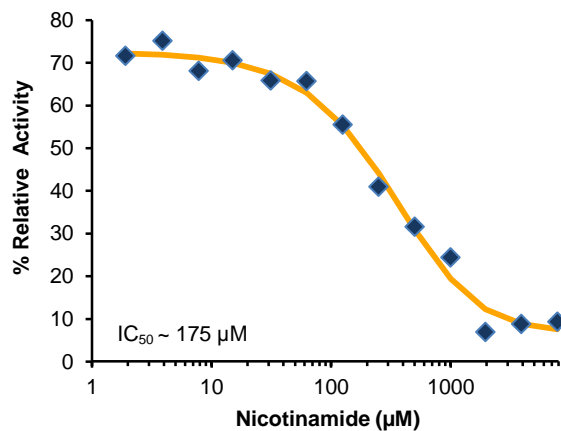
4. **Develop:** Add 10  $\mu$ l Developer into each well. Mix well and incubate for 10 min. at 37°C, protected from light.

5. **Measurement:** Read fluorescence (Ex/Em = 400/505 nm).

6. **Calculation:** Subtract the Blank Control reading from all readings to obtain  $\Delta$ RFU for each reading. Set the  $\Delta$ RFU of Enzyme Control [EC] as 100%, and calculate % Inhibition or % Relative Activity of the test inhibitors as:

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

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



**Figure:** Inhibition of SIRT6 Enzyme activity by Nicotinamide using kit protocol

#### VII. Related Products:

SIRT6, human recombinant (7578)

SIRT6s Blocking Peptides

HDAC Activity Colorimetric Assay Kit (K331)

HDAC Inhibitor Drug Screening Kit (K340)

HDAC Activity Fluorometric Assay Kit (K330)

HDAC3 Activity Assay Kit (K343)

HDAC3 Inhibitor Screening Kit (K363)

HDAC8 Inhibitor Screening Kit (K368)

DiscoveryPak™ HDAC Inhibitor Set (K851, K876)

HDAC1 IP & Activity Assay Kit (K342)

HDAC2 IP & Activity Assay Kit (K341)

HDAC3 IP & Activity Assay Kit (K344)

InSitu HDAC Activity Fluorometric Assay Kit (K339)

Salermide (1873)

SIRT2 Inhibitor, AGK2 (1651)

SIRT2 Inhibitor, B2 (2085)

Suramin Hexasodium Salt (1874)

Sirtinol (2062)

SIRT6s Antibodies

HDACs Blocking Peptides

4-hydroxynonenal (2083)

HDACs Antibodies

HDAC3, human recombinant (7613)

HDAC8 Activity Assay Kit (K348)

HDAC8, human recombinant (K7618)

Cambinol (1653)

Sirtuin 1 (human intracellular) ELISA Kit (K4923)

Sirtuin 2 (human intracellular) ELISA Kit (K4924)

Sirt2 Inhibitor Screening kit (K322)

Hyperforin, DCHA (2153)

SIRT1 Inhibitor, EX-527 (1652)

SIRT2 Inhibitor, AK-7 (1857)

Sirtinol (2062)

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