

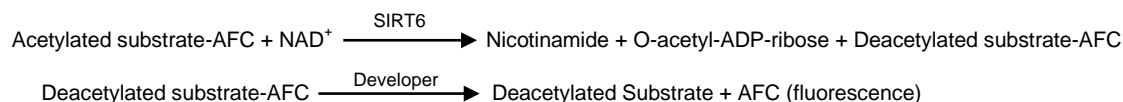
SIRT6 Inhibitor Screening Kit (Fluorometric)

7/14

(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 = 395/541 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
Substrate	0.2 ml	Red	K323-100-2
Cofactor	Lyophilized	Purple	K323-100-3
Developer	1 ml	Orange	K323-100-4
SIRT6 Enzyme	Lyophilized	Green	K323-100-5
Enzyme Reconstitution Buffer	500 µl	Yellow	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:** Bring to 37°C before use.
- **Substrate:** Once thawed, aliquot and store at -80°C.
- **Cofactor:** Reconstitute with 220 µl ddH₂O. 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:** Reconstitute in 410 µl cold Enzyme Reconstitution Buffer and mix gently by pipetting. After reconstituting, aliquot enzyme and store at -80°C.

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	21 µl
SIRT6 Enzyme	4 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.
3. **Substrate preparation:** For each well, prepare 40 µl of Substrate solution.

SIRT6 Assay Buffer	36 µl
Substrate	2 µl
Cofactor	2 µl

 Add 40 µl of the Substrate solution into each well. Mix & incubate at 37°C for 60 min.
4. **Develop:** Add 10 µl Developer into each well. Mix well and incubate for 10 min. at 37°C, protected from light.
5. **Measurement:** Read fluorescence (Ex/Em = 395/541 nm).

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

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

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

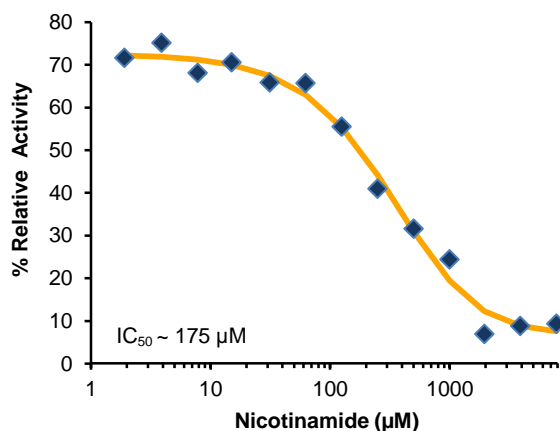


Figure: Inhibition of SIRT6 Enzyme activity by Nicotinamide using kit protocol

VII. Related Products:

SIRT6, human recombinant (7578)	Sirtinol (2062)
SIRTs Blocking Peptides	SIRTs Antibodies
HDAC Activity Colorimetric Assay Kit (K331)	HDACs Blocking Peptides
HDAC Inhibitor Drug Screening Kit (K340)	4-hydroxynonenal (2083)
HDAC Activity Fluorometric Assay Kit (K330)	HDACs Antibodies
HDAC3 Activity Assay Kit (K343)	HDAC3, human recombinant (7613)
HDAC3 Inhibitor Screening Kit (K363)	HDAC8 Activity Assay Kit (K348)
HDAC8 Inhibitor Screening Kit (K368)	HDAC8, human recombinant (K7618)
DiscoveryPak™ HDAC Inhibitor Set (K851, K876)	Cambinol (1653)
HDAC1 IP & Activity Assay Kit (K342)	Sirtuin 1 (human intracellular) ELISA Kit (K4923)
HDAC2 IP & Activity Assay Kit (K341)	Sirtuin 2 (human intracellular) ELISA Kit (K4924)
HDAC3 IP & Activity Assay Kit (K344)	Sirt2 Inhibitor Screening kit (K322)
InSitu HDAC Activity Fluorometric Assay Kit (K339)	Hyperforin, DCHA (2153)
Salermide (1873)	SIRT1 Inhibitor, EX-527 (1652)
SIRT2 Inhibitor, AGK2 (1651)	SIRT2 Inhibitor, AK-7 (1857)
SIRT2 Inhibitor, B2 (2085)	Sirtinol (2062)
Suramin Hexasodim Salt (1874)	

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