



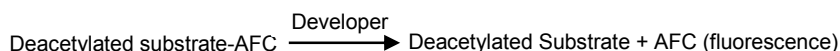
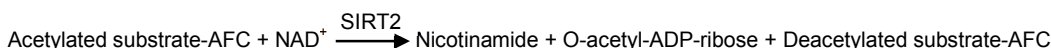
SIRT2 Inhibitor Screening Assay Kit (Fluorometric)

rev.2/13

(Catalog # K322-100; 100 assays; Store kit at -20°C)

I. Introduction:

Sirtuin or Sir2 proteins are a class of proteins that possess either histone deacetylase or mono-ribosyltransferase activity. Sirtuins have been implicated in influencing aging and regulating transcription, apoptosis and stress resistance, as well as energy efficiency and alertness during low-calorie situations. Unlike other known protein deacetylases, which simply hydrolyze acetyl-lysine residues, the sirtuin-mediated deacetylation reaction couples lysine deacetylation to NAD hydrolysis. This hydrolysis yields O-acetyl-ADP-ribose, the deacetylated substrate 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 inhibitor screening Kit, Sirtuin deacetylates the substrate and then the developer cleaves the deacetylated substrate to release the fluorescent group, which can be detected fluorometrically at Ex/Em = 395/541 nm. In the presence of SIRT inhibitor, the deacetylation will be impeded which prevents the cleavage of the substrate to release the fluorescent group. The kit provides a rapid, simple, sensitive, and reliable test, which is also suitable for high-throughput screening of SIRT2 inhibitors. Inhibitor control (Nicotinamide) is included to compare the efficacy of the test inhibitors.



II. Application:

- Screening and characterization of Sirtuin inhibitors.

III. Kit Contents:

Components	K322-100	Cap Code	Part Number
Assay Buffer	25 ml	WM	K322-100-1
Substrate	0.2 ml	Red	K322-100-2
Cofactor	1 vial	Purple	K322-100-3
Developer	1 ml	Orange	K322-100-4
SIRT2 Enzyme	0.5 ml	Green	K322-100-5
Inhibitor Control (Nicotinamide)	1 ml	Blue	K322-100-6

IV. User Supplied Reagents and Equipments:

- 96-well plate with flat clear bottom, black wall plates are preferred for fluorescence reading
- Fluorescence microplate reader

V. Storage and Handling:

Store kit at -20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials before opening. Read the entire protocol before performing the assay.

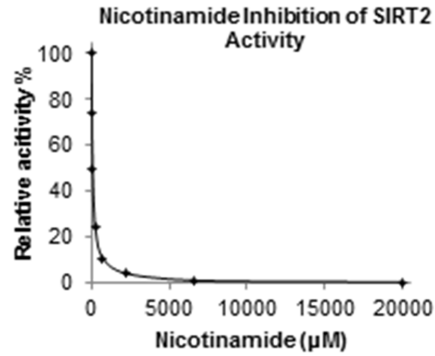
VI. Reagent Preparation:

Cofactor: Reconstitute with 220 µl ddH₂O. Aliquot and Store at -20°C. Avoid repeated freeze/thaw cycles. Use within one month.

VII. SIRT2 Inhibitor Screening Assay Protocol:

- Enzyme:** For each well, add 5 µl SIRT2 Enzyme.
- Screen compounds, inhibitor control and blank control preparations:**
Dissolve candidate inhibitors into proper solvent. Dilute to 2X the desired test concentration with Assay Buffer. Add 45 µl diluted test inhibitors, Inhibitor Control or Assay Buffer into SIRT2 enzyme wells as sample screen [S], Inhibitor Control (Nicotinamide), or Enzyme Control [EC] (no inhibitor). Mix well, and incubate for 5 minutes at 37°C.
- Substrate preparation:** For each well, prepare 40 µl of substrate solution.
36 µl Assay Buffer
2 µl Substrate
2 µl Cofactor solution
Mix, add 40 µl of the substrate solution into each well. Mix, incubate at 37°C for 60 minutes.
- Measurement:** Read fluorescence (R₀) at Ex/Em = 395/541 nm.
- Develop:** Add 10 µl Developer in each well. Mix well and incubate for 10 minutes at 37°C, protected from light. Read again fluorescence (R₁) at Ex/Em = 395/541 nm.
- Calculation:** The RFU of fluorescence generated by hydrolyzation of substrate is $\Delta\text{RFU} = R_1 - R_0$. Set the ΔRFU of enzyme control [EC] as 100%, and calculate the relative % inhibition of the test inhibitors as:

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



VIII. Related Products:

SIRT2, human recombinant	Sirtinol
SIRTs Blocking Peptides	SIRTs Antibodies
HDAC Colorimetric Activity Assay Kit	HDACs Blocking Peptides
HDAC Inhibitor Drug Screening Kit	Trichostatin A
HDAC Fluorometric Activity Assay Kit	HDACs Antibodies
HDAC3 Activity Assay Kit	HDAC3, human recombinant
HDAC3 Inhibitor Screening Kit	HDAC8 Activity Assay Kit
HDAC8 Inhibitor Screening Kit	HDAC8, human recombinant
DiscoveryPak™ HDAC Inhibitor Set	Tubastatin A
Sirtuin 2 (human intracellular) Elisa Kit	

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