CREATIVE BIOMART®
Assay Kit

SIRT1 Inhibitor/Activator Screening Kit

Product Information

Cat.No. Kit-2156

Product Overview

In the SIRT1 Inhibitor/Activator screening Kit, Sirtuin 1 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 SIRT1 inhibitor, deacetylation is impeded, preventing cleavage of the substrate and release of the fluorescent group. The SIRT1 activator enhances SIRT1 activity resulting in a higher fluorescent signal in comparison to the control. This kit provides a rapid, simple, sensitive, and reliable test, which is suitable for high-throughput screening of SIRT1 inhibitors/activators. Inhibitor control (Nicotinamide) is included to compare the efficacy of the test inhibitors.

Size

100 assays

Description

SIRT1 or Sirtuin 1 is a member of the silent information regulator 2 family. SIRT1, an NAD-dependent histone deacetylase can deacetylate histones and a number of nonhistone substrates, including p53. It is predominantly localized in the nucleus of normal cells. In cancer cells however, it is predominantly localized in the cytoplasm. SIRT1 has been shown to regulate a wide range of cellular functions that affect metabolic homeostasis and aging. SIRT1 exerts anti-apoptotic, anti-oxidative, and anti-inflammatory effects against cellular injury, and protects the cells through the regulation of mitochondrial biogenesis, autophagy, and metabolism in response to the cellular energy and redox status. SIRT1 also promotes vasodilation and protects vascular tissues. Activation and inhibition of SIRT1 is being targeted for various diseases. 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.

Applications

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SIRT1 Inhibitor/Activator Screening Kit

Screening/characterizing/studying SIRT1 inhibitors and activators

Storage

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.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 -20°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

Kit Components

SIRT1 Assay Buffer: 25 ml1 M DTT: 0.4 mlSubstrate (in DMSO): 0.2 mlNAD: 1 vialSIRT1 Enzyme: 0.2 mlInhibitor (Nicotinamide, 4 mM): 0.9 mlDeveloper: 1 ml

Detection method Fluorescence (Ex/Em = 400/505 nm)

Features & Benefits

• Simple and reliable test to screen SIRT1 inhibitors/activators• High-throughput suitable• Includes Inhibitor Control (Nicotinamide)

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