FLUOR DE LYS[®] SIRT5 fluorometric drug discovery assay kit

The SIRS of the Price Drug Discovers Kit is a complete assay system designed to measure the lysyl desuccinylase activity of the recombinant human SIRTS included in the kit. A black 96-well microplate is packaged with the kit, but it should be noted that reagents of the FLUOR DE LYS® system have also been successfully employed in other formats, including cuvettes and 384-well plates. The SIRT5 Fluorometric Activity Assay is based on the unique FLUOR DE LYS®-Succinyl Substrate/Developer combination. The assay procedure has two steps. The FLUOR DE LYS®-Succinyl Substrate, which comprises a single lysine residue, NEsuccinylated on its side-chain, is first incubated with human recombinant SIRT5 together with the cosubstrate NAD+. Desuccinvlation of FLUOR DE LYS®-Succinyl sensitizes it so that, in the second step, treatment with the FLUOR DE LYS[®] Developer produces a fluorophore. Use of a succinylated, rather than acetylated substrate with SIRT5 results in readily observed saturation kinetics and a greater than 1000-fold increase in assay sensitivity.

Citations: 6

View Online »

Ordering Information

Order Online »

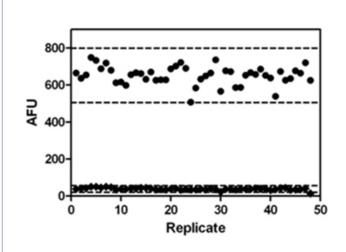
BML-AK513-0001

100 tests

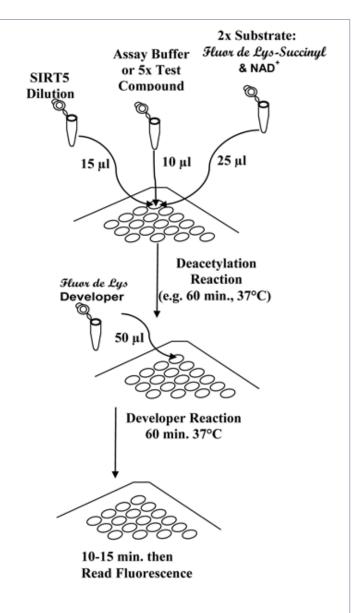
Manuals, SDS & CofA

View Online »

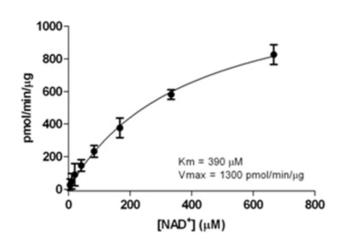
- Easy-to-use kits (two-step) for screening SIRT5 inhibitors/activators; includes enough active enzyme for entire plate
- Includes optimal substrate selected from a panel of succinylated sites
- 96-well plate included, but can be adapted to higher well format
- · Control inhibitors included
- Suitable for high-throughput screening (Z'-factors >0.73)
- Optimal, specific SIRT5 substrate means low enzyme concentration, making 'hit' validation easy



SIRT5 Z-factor analysis. SIRT5 (12 $\bar{\text{U}}/10~\text{ng}$) (\bullet) or buffer (



Performing the FLUOR DE LYS®-Succinyl SIRT5
Activity Assay. The procedure is done in two stages
First, the components of the desuccinylation reaction
(SIRT5, buffer or test compound, substrates) are mixed.
Following an incubation in which substrate
desuccinylation takes place, Developer is added and
mixed. This stops the deacetylation and produces the
fluorescent signal. The fluorescent signal develops and
can be read in less than 15 min.



Dependence of SIRT5 Kinetics on the Concentration of NAD+. Initial desuccinylation rates of SIRT5 (12 $\bar{\text{U}}/10$ ng) were determined with 20 min. incubations (37 °C) in the presence of 0.2 mM FLUOR DE LYS®-Succinyl and the indicated concentrations of NAD+. Reactions were stopped with FLUOR DE LYS® Developer/2 mM nicotinamide and fluorescence measured (Ex. 360 nm, Em. 460 nm). Each point represents the mean of three determinations and the error bars are standard deviations. The line is a non-linear least-squares fit to the Michaelis-Menten equation (Graphpad Prism). The Km for NAD+ was 390 μ M and the Vmax was 1300 pmol/min./ μ g.

Handling & Storage

Use/Stability

Store all components except the microplates and instruction booklet at -70°C. The SIRT5 enzyme, BML-SE555, must be handled with particular care in order to retain maximum enzymatic activity. Defrost it quickly in a RT water bath or by rubbing between fingers, then immediately store on an ice bath. The remaining, unused enzyme should be refrozen quickly, by placing at -70°C. If possible, snap freeze in liquid nitrogen or a dry ice/ethanol bath. To minimize the number of freeze/thaw cycles, aliquot into separate tubes and store at -70°C. The 20x Developer (BML-KI105) can be prone to precipitation if thawed too slowly. It is best to thaw this reagent in a room temperature water bath and, once thawed, transfer immediately onto ice. As with the SIRT5, it is best to refreeze unused portion in liquid nitrogen or a dry ice/ethanol bath.

Handling Avoid freeze/thaw cycles.

Long Term Storage -80°C

Shipping Dry Ice

Regulatory Status RUO - Research Use Only

Product Details

Alternative Name Sirtuin 5 fluorescent assay kit

Application Activity assay, Cell-based assays, Fluorescent detection,

HTS

Contents

BML-SE555-9090 SIRT5 (Sirtuin 5) (human, rec.)

Form: Dissolved in 25 mM TRIS, pH 7.5, 100 mM NaCl, 5

mM

DTT, 1 mg/mL BSA and 10% glycerol.

STORAGE: -70°C; AVOID FREEZE/THAW CYCLES! QUANTITY: 1200 U; See vial label for specific activity and protein concentration. One U = 1 pmol/min at 37 °C, 250

μΜ FLUOR DE LYS[®] Succinyl, Desuccinylase, 2000 μΜ

NAD+.

BML-KI590-0050 FLUOR DE LYS[®] Succinyl, Desuccinylase Substrate

FORM: 5 mM solution in DMSO (dimethylsulfoxide)

STORAGE: -70°C QUANTITY: 50 µl

BML-KI105-0300 FLUOR DE LYS[®] Developer Concentrate (20x)

FORM: 20x Stock Solution; Dilute in Assay Buffer before

use.

STORAGE: -70°C QUANTITY: 300 µl

BML-KI282-0500 NAD+ (Sirtuin Substrate)

2.7 mM KCl, 1 mM MgCl₂.

STORAGE: -70°C QUANTITY: 500 µl

BML-KI283-0500 Nicotinamide (Sirtuin Inhibitor)

FORM: 50 mM Nicotinamide in 50 mM TRIS-HCl, pH 8.0,

137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂.

STORAGE: -70°C QUANTITY: 500 µl

BML-KI285-0010 Suramin sodium (Sirtuin Inhibitor)

FORM: Solid MW: 1429.2

STORAGE: -70°C QUANTITY: 10 mg

SOLUBILITY: Water or Assay Buffer to 25 mM (10 mg in

0.27 ml)

BML-KI592-0030 FLUOR DE LYS[®] Desuccinylated Standard

FORM: 10 mM in DMSO (dimethylsulfoxide)

STORAGE: -70°C QUANTITY: 30 µl

BML-KI286-0020 Sirtuin Assay Buffer

(50 mM TRIS-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1

mM

Technical Info / Product Notes

Most sirtuin enzymes, also known as class III histone deactylases (class III HDACs), catalyze a reaction which couples deacetylation of protein N^e-acetyllysine residues to the formation of O-acetyl-ADP-ribose and nicotinamide, from the oxidized form of nicotinamide adenine dinucleotide (NAD⁺). Some sirtuins, notably human SIRT4 and SIRT6, are reported to catalyze an alternative reaction, the transfer of an ADP-ribosyl group from NAD+ to proteins, although the physiological relevance of these reactions is in question. Sirtuin homologs are found in all forms of life, including the archaea, the bacteria and both unicellular and multicellular eukaryotes. The founding exemplar of the group, Sir2 from baker's yeast (Saccharomyces cerevisiae), was named for its role in gene-silencing (Silent information regulator 2). Transcriptional silencing by Sir2 is linked to its deacetylation of lysines in the N-terminal tails of the histones in chromatin, hence the classification as a class III HDAC. Lysine deacetylation by sirtuins, however, extends beyond histones. Targets of sirtuin regulatory deacetylation include mammalian transcription factors such as p53, the cytoskeletal protein, tubulin, the bacterial enzyme, acetyl-CoA synthetase and its mammalian homologs.

SIRT5, along with two other mammalian sirtuins, SIRT3 and SIRT4, is localized to the mitochondria. The human SIRT5 gene is located in a chromosomal region in which abnormalities are associated with malignancies, suggesting a possible SIRT5 role in cancer. Thus far, the best studied of SIRT5's possible physiological roles is the deacetylation, and enhancement of the activity of the mitochondrial matrix enzyme carbamoyl phosphate synthase 1 (CPS1), the rate-limiting enzyme for urea synthesis in the urea cycle. Increased urea synthesis is required for removal of nitrogenous waste (ammonia) during periods of increased amino acid catabolism, including calorie restriction, fasting and the consumption of a high protein diet. Nakagawa et al. report that under these conditions, SIRT5 deacetylation of CPS1 is increased, along with CPS1 activity. At least in the instance of starvation, the increased SIRT5 activity may be attributed to increased levels of the sirtuin co-substrate NAD+ in the mitochondria, which in turn is due to induction of the NAD⁺ synthetic pathway enzyme nicotinamide phosphoribosyltransferase, (Nampt). It should be noted, however, that two proteomic studies of the mouse mitochondrial "acetylome" are in possible conflict with the CPS1 results of Nakagawa et al. One group observed that calorie restriction increased acetylation at 7 of 24 sites in CPS1, but did not lead to deacetylation at any sites. A comparison of the acetylated proteins of fed and fasted mice found that fasting induced the addition of 4 acetylated UniProt ID Q9NXA8

Last modified: May 29, 2024

