FLUOR DE LYS® SIRT5 fluorometric drug discovery assay kit

A Sensitive Fluorometric Assay for Screening SIRT5 Inhibitors

The SIRT5 Fluorometric Drug Discovery Kit is a complete assay system designed to measure the lysyl desuccinylase activity of the recombinant human SIRT5 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, *Nε*-succinylated on its side-chain, is first incubated with human recombinant SIRT5 together with the cosubstrate NAD+. Desuccinylation 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.

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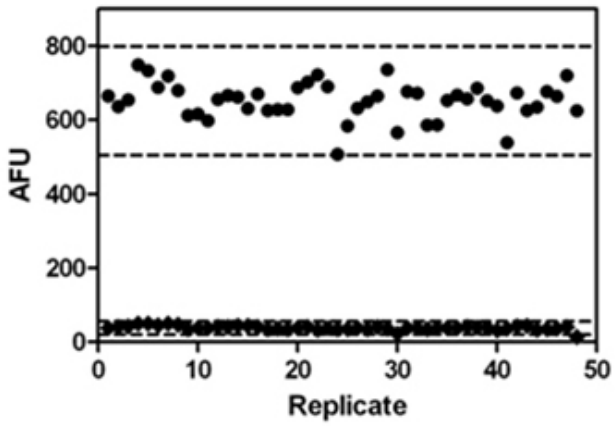
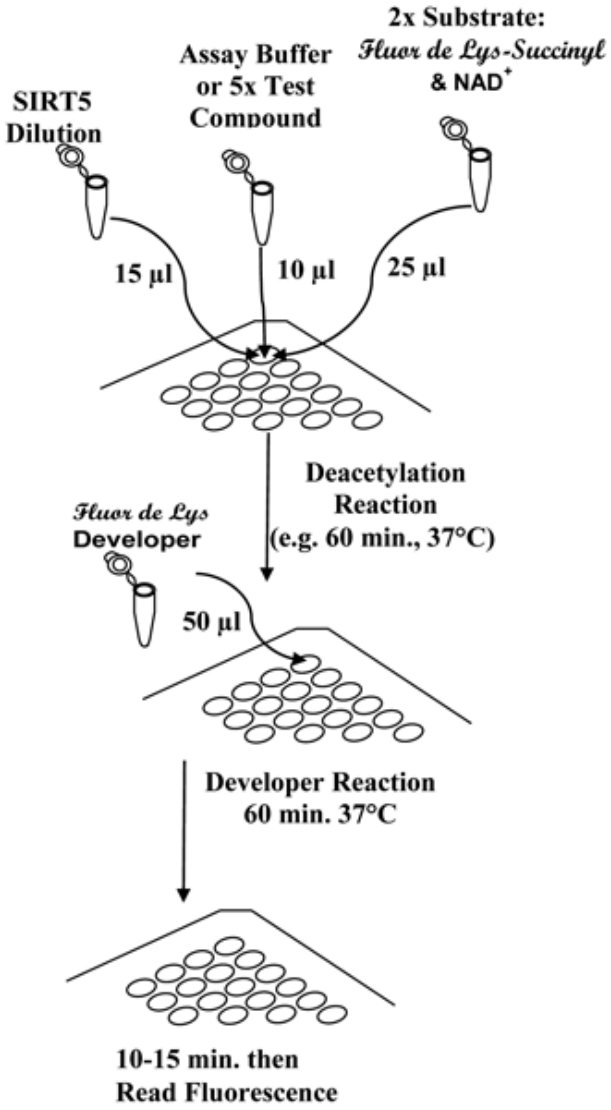
BML-AK513-0001	100 tests

Manuals, SDS & CofA View Online »

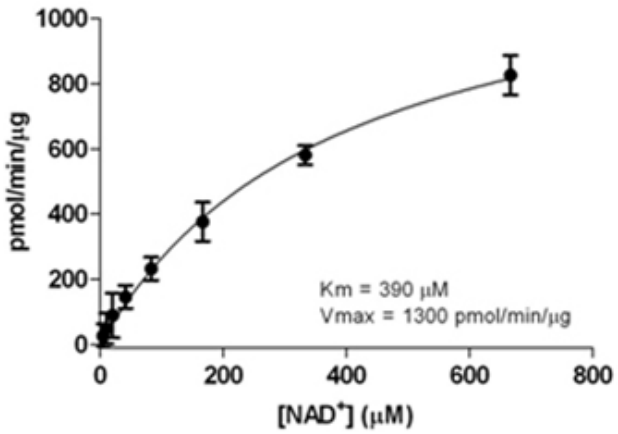
- Easy-to-use kits (two-step) for screening SIRT5 inhibitors/activators; includes enough active enzyme for entire plate
- Includes optimal substrate selected from a panel of succinylated sites
- 96-well plate included, but can be adapted to higher well format
- Control inhibitors included
- Suitable 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 Ū/10 ng) (●) 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 Ū/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 µM and the Vmax was 1300 pmol/min./µg.

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 µM FLUOR DE LYS®*Succinyl*, Desuccinylase, 2000 µM 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)
FORM: 50 mM β-Nicotinamide adenine dinucleotide (oxidized form) in 50 mM TRIS-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$.
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 $MgCl_2$.
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 $MgCl_2$, 1 mg/ml BSA)
STORAGE: -70°C
QUANTITY: 20 ml

80-2409 1/2 Volume Black NBS Microplate
STORAGE: Room temperature.

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 sites to CPS1, while only one of five sites present in the fed condition disappeared upon fasting.

An alternative view of SIRT5's physiological function is that it may primarily involve catalysis of reactions other than deacetylation. SIRT5's deacetylase activity is detectable but weak with an acetylated histone H4 peptide and with chemically acetylated histones or bovine serum albumin. The catalytic efficiency of SIRT5 with an acetylated histone H3 peptide (k_{cat}/K_m = 3.5 s^{-1} M^{-1}) is orders of magnitude lower than several human and yeast sirtuins (SIRT1, SIRT2, Sir2, Hst2) and more than 20-fold lower than the next weakest deacetylase tested, human SIRT3. Although there is a seeming conflict between the idea of SIRT5 as a non-deacetylase and its effects on CPS1, it should be noted that the rate of SIRT5 deacetylation of CPS1 has not been quantified; the deacetylation was only shown in a qualitative way by western blotting with anti-acetyllysine. Further, although SIRT5 performs an NAD^+-dependent activation of CPS1 and an NAD^+-dependent deacetylation of CPS1, no mechanistic link between the deacetylation and the activation has been established. The *in vitro* SIRT5/CPS1 activation experiments were performed with crude mitochondrial matrix lysates, from SIRT5 knockout mice, serving as the CPS1 source. Conceivably, the CPS1 harbored another modification, in addition to acetylation, that SIRT5 reversed in an NAD^+-dependent reaction. Consistent with this possibility is recently presented evidence that mitochondrial proteins are lysine succinylated and that SIRT5 can desuccinylate

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