# FLUOR DE LYS® Green SIRT5 fluorometric drug discovery assay kit

## A Sensitive Fluorometric Assay for Screening SIRT5 Inhibitors

The FLUOR DE LYS® Green 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 Green SIRT5 Fluorescent Activity Assay is based on the unique FLUOR DE LYS®-Succinyl GreenSubstrate/Developer combination. The assay procedure has two steps. The FLUOR DE LYS®-Succinyl GreenSubstrate, which comprises a lysine residue, $N^{\varepsilon}$-succinylated on its side-chains, is first incubated with human recombinant SIRT5 together with the cosubstrate $NAD^{+}$. Desuccinylation of FLUOR DE LYS®-Succinyl Green 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

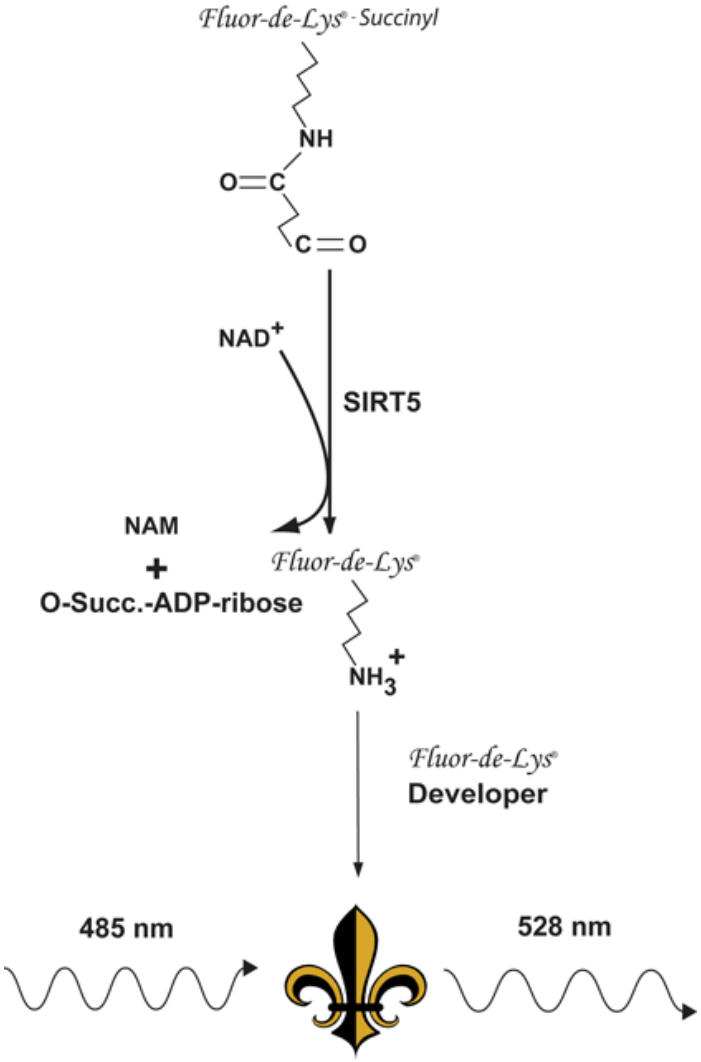
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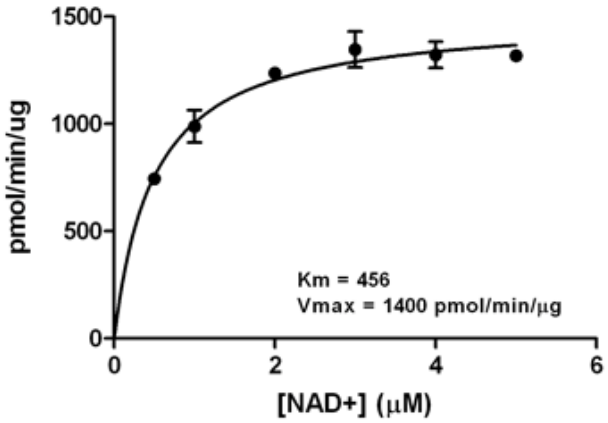
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|---|---|
| **BML-AK514-0001** | 100 tests |

## 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
- While the FLUOR DE LYS®-*Succinyl* substrate (Prod. No. BML-KI590, in FLUOR DE LYS® SIRT5 Fluorometric Drug Discovery Assay Kit, Prod. No. BML-AK413) contains an AMC ('blue') fluorophore that uses commonly-used wavelengths, FLUOR DE LYS®-*Succinyl Green* substrate has longer excitation/emission wavelengths, avoiding interference often seen with screening compounds at shorter wavelengths.




Reaction Scheme of the SIRT5 Fluorescent Activity Assay*. NAD+-dependent desuccinylation of the substrate by recombinant human SIRT5 sensitizes it to Developer, which then generates a fluorophore (symbol). The fluorophore is excited with 485 nm light and the emitted light (528 nm) is detected on a fluorometric plate reader. NAD+ is consumed in the reaction to produce nicotinamide (NAM) and O-succinyl-ADP-ribose.




Dependence of SIRT5 Kinetics on the Concentration of NAD+. Initial desuccinylation rates of SIRT5 (10 ng) were determined with 20 min. incubations (37°C) in the presence of 0.5 mM FLUOR DE LYS®-Succinyl Green and the indicated concentrations of NAD+. Reactions were stopped with FLUOR DE LYS® Developer/2 mM nicotinamide and fluorescence measured. 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. The Km for NAD+ was 456 µM and the Vmax was 1400 pmol/min/µg.

## Handling & Storage

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| **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

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|---|---|
| **Alternative Name** | Green sirtuin 5 fluorescent assay kit |
| **Application** | Activity assay, Cell-based assays, Fluorescent detection, HTS |

**Contents**

**BML-SE555-9090 SIRT5 (Sirtuin 5) (human, recombinant)**
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 Green*, Desuccinylase, 2000 µM $NAD^+$.

**BML-KI591-0050 FLUOR DE LYS®–*Succinyl Green***, 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 b-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

**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-KI605-0030FLUOR DE LYS®–*Green* Desuccinylated Standard**
FORM: 1 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

**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

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