Product Manual

SIRT2 Fluorometric Drug Discovery Kit

A FLUOR DE LYS® Fluorescent Assay System

Catalog # BML-AK556

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

Yeast Sir2 (Silent information regulator 2)[1] is the founding exemplar of the 'sirtuins', an apparently ancient group of enzymes which occurs in eukaryotes, the archaea and eubacteria[2]. Originally described as a factor required for maintenance of silencing at telomeres and matin-Gtype loci, Sir2 was subsequently shown to be an enhancer of mother-cell replicative lifespan[3]. The sirtuins represent a distinct class of trichostatin A-insensitive protein-lysyl-deacetylases (class III HDACs) and have been shown to catalyze a reaction that couples lysine deacetylation to the formation of nicotinamide and *O*-acetyl-ADP-ribose from NAD^+ and the abstracted acetyl group[4-6]. There are seven human sirtuins, which have been designated SIRT1- SIRT7[7]. Like Sir2, human SIRT2 is a class I sirtuin, although not as closely related to Sir2 as human SIRT1[7]. Thus far, SIRT2 is the only human sirtuin to have had its three-dimensional structure determined[8] and its catalytic core has the same basic two-domain architecture and central groove as other sirtuins[9-13]. SIRT2 is a cytoplasmic protein[14-16], although a lesser amount of nuclear localization[15, 17] and an interaction with the homeobox transcription factor HOXA10[17] have also been reported. A complex comprising HDAC6 and SIRT2 colocalizes with the microtubule network and both enzymes can deacetylate α-tubulin lysine-40 in purified tubulin heterodimers or microtubules[15]. SIRT2 protein levels rise during mitosis and it becomes phosphorylated[16]. Overexpression of SIRT2 delays exit of cells from mitosis, suggesting that it may play a role in cell cycle regulation[16].

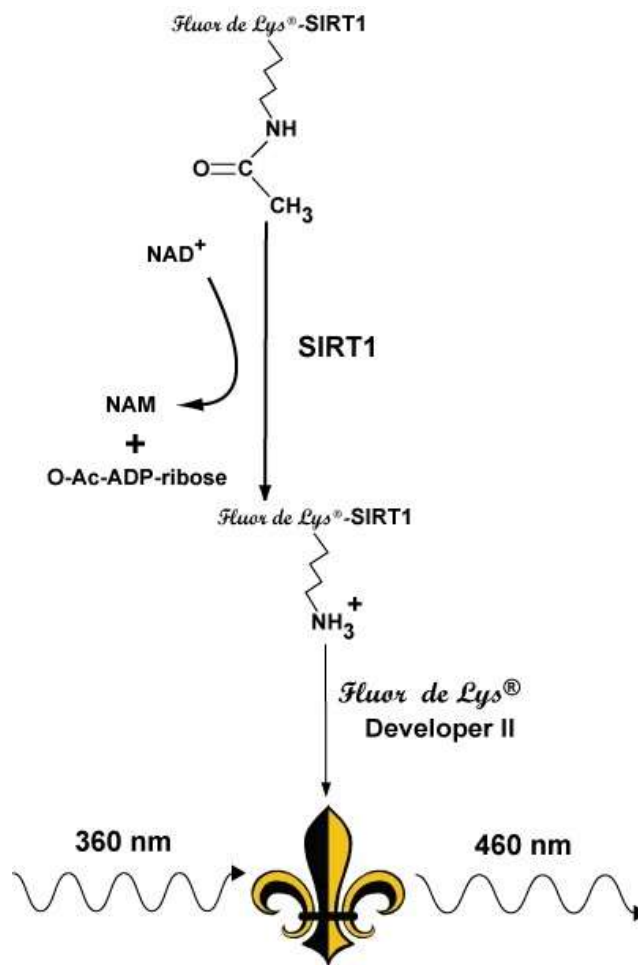

Figure 1. Reaction Scheme of the SIRT2 Fluorescent Activity Assay. NAD^+- dependent deacetylation of the substrate by recombinant human SIRT2 sensitizes it to Developer II, which then generates a fluorophore (symbol). The fluorophore is excited with 360 nm light and the emitted light (460 nm) is detected on a fluorometric plate reader. NAD+ is consumed in the reaction to produce nicotinamide (NAM) and O-acetyl-ADP-ribose.

The SIRT2 Fluorescent Activity Assay/Drug Discovery Kit is a complete assay system designed to measure the lysyl deacetylase activity of the recombinant human SIRT2 included in the kit. For convenience, two types of 96-well microplates come packaged with the kit, but it should be noted that the reagents have also been successfully employed in other formats, including cuvettes and 384-well plates.The SIRT2 Fluorescent Activity Assay is based on the unique FLUOR DE LYS® SIRT2 Substrate/Developer II combination. The FLUOR DE LYS® SIRT2 Substrate is a peptide comprising amino acids 317-320 of human p53 (Gln-Pro-Lys-Lys(Ac)). The assay's fluorescence signal is generated in proportion to the amount of

deacetylation substrate produced by SIRT2 action. FLUOR DE LYS® SIRT2 was the substrate deacetylated most efficiently by SIRT2 from among a panel of substrates patterned on p53, histone H3 and histone H4 acetylation sites (see Fig. 2, FLUOR DE LYS® SIRT2 is labeled 'p53-320').

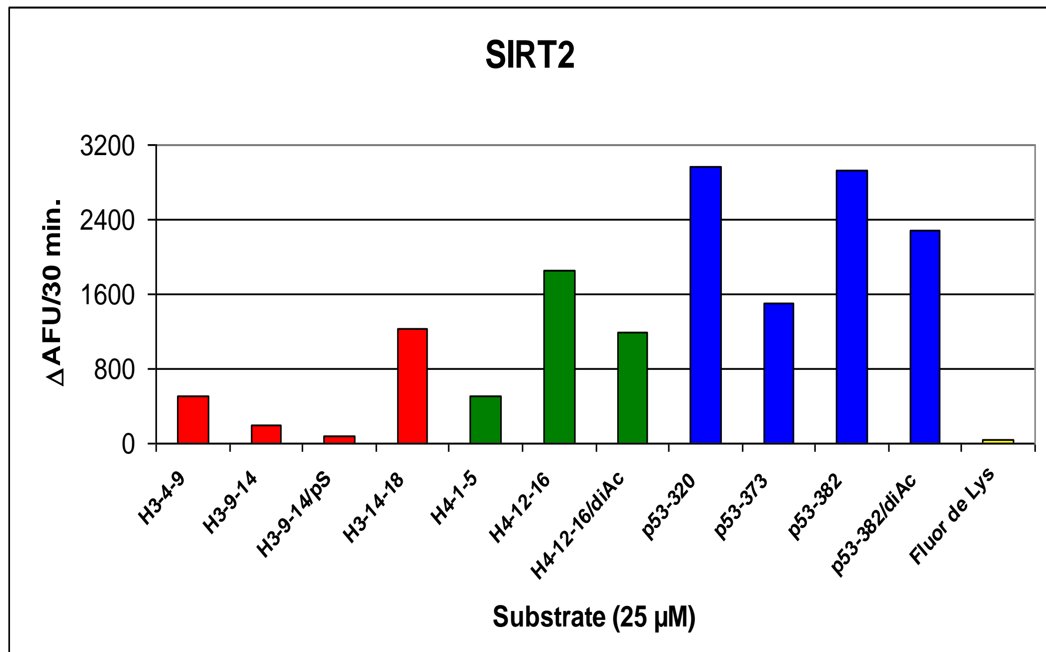

Figure 2. SIRT2 Peptide Substrate Preferences. Initial rates of deacetylation were determined for a series of fluorogenic acetylated peptide substrates based on short stretches of human histone H3, H4 and p53 sequence. Recombinant human SIRT2 (SE-251), was incubated for 30 min at 37°C with 25 µM of the indicated fluorogenic acetylated peptide substrate and 500 µM NAD^+. Reactions were stopped by the addition of Developer II/2 mM nicotinamide and the deacetylation-dependent fluorescent signal was allowed to develop for 45 min. Fluorescence was then measured in the wells of a clear microplate with a CytoFluor™ II fluorescence plate reader (PerSeptive Biosystems, Ex. 360 nm, Em. 460 nm, gain=85).

The assay procedure has two steps (Fig. 1). First, the FLUOR DE LYS® SIRT2 Substrate, which comprises the p53 sequence Gln-Pro-Lys-Lys(ε-acetyl), is incubated with human recombinant SIRT2 together with the cosubstrate NAD^+. Deacetylation of FLUOR DE LYS® SIRT2 sensitizes it so that, in the second step, treatment with the FLUOR DE LYS® Developer II produces a fluorophore.

The protocols and application examples described below emphasize conditions suitable for the screening of potential inhibitors or activators of SIRT2. Nicotinamide (BML-KI283) and suramin sodium (BML-KI285), are included as positive controls for SIRT2 inhibition (see Fig. 4). Although inhibitor screens are typically done at relatively low substrate concentrations, the kit does include enough substrate to perform kinetic studies over a full range of relevant concentrations (see Figures 5 & 6).

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MATERIALS PROVIDED

BML-SE251-0500 SIRT2 (Sirtuin 2, hSir2^{SIRT2})(human, recombinant)

FORM: Recombinant enzyme dissolved in 25 mM Tris, pH 7.5, 100 mM NaCl, 5 mM dithiothreitol and 10% glycerol. See vial label for activity and protein concentrations.

STORAGE: -80°C; AVOID FREEZE/THAW CYCLES!

QUANTITY: 500 U; One U=1 pmol/min at 37°C, 500 µM, FLUOR DE LYS® SIRT2 Substrate (BML-KI179), 500 µM NAD^+

BML-KI179-0005 FLUOR DE LYS® SIRT2, Deacetylase Substrate

FORM: 5 mM solution in 25 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$

STORAGE: -80°C

QUANTITY: 100 µL

BML-KI176-1250 FLUOR DE LYS® Developer II Concentrate (5x)

FORM: 5x Stock Solution; Dilute in Assay Buffer before use.

STORAGE: -80°C

QUANTITY: 5 x 250 µL

BML-KI282-0500 NAD^+ (Sirtuin Substrate)

FORM: 50 mM β-Nicotinamide adenine dinucleotide (oxidized form) in 25 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$.

STORAGE: -80°C

QUANTITY: 500 µL

BML-KI283-0500 Nicotinamide (Sirtuin Inhibitor)

FORM: 50 mM Nicotinamide in 25 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$.

STORAGE: -80°C

QUANTITY: 500 µL

BML-KI285-0010 Suramin sodium (Sirtuin Inhibitor)

FORM: Solid

MW: 1429.2

STORAGE: -80°C

QUANTITY: 10 mg

SOLUBILITY: Water or Assay Buffer to 25 mM (10 mg in 0.27 mL)

BML-KI142-0030 FLUOR DE LYS® Deacetylated Standard

FORM: 10 mM in DMSO (dimethylsulfoxide)

STORAGE: -80°C

QUANTITY: 30 µL

BML-KI286-0020 Sirtuin Assay Buffer

(50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1 mg/mL BSA)

STORAGE: -80°C

QUANTITY: 20 mL

80-2407 ½ Volume NBS Microplates

1 clear and 1 white, 96-well, non-binding

STORAGE: Room temperature.

OTHER MATERIALS NEEDED

- Microplate reading fluorimeter capable of excitation at a wavelength in the range 350-380 nm and detection of emitted light in the range 450-480 nm.
- Pipetman or multi-channel pipetman capable of pipetting 2-100 µL accurately
- Ice bucket to keep reagents cold until use.
- Microplate warmer or other temperature control device

PROTOCOL

Notes on Storage: *Store all components except the microtiter plate at -80°C for the highest stability. Components with storage temperatures other than -80°C can be stored at the temperature listed OR at -80°C. The SIRT2 enzyme,* ***BML-SE251****, 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 -80°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 -80°C. The 5x Developer II (****BML-KI176****) 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.*

Some Things To Consider When Planning Assays:

1. The assay is performed in two stages. The first stage, during which the SIRT2 acts on the Substrate, is done in a total volume of 50 µL. The second stage, which is initiated by the addition of 50 µL of Developer II, including a SIRT2 inhibitor, stops SIRT2 activity and produces the fluorescent signal. See “Preparing Reagents For Assay” and Table 1 (p. 13).

   Two types of ½-volume, 96-well microplates are provided with the kit. The signal obtained with the opaque, white plate can be ~5-fold greater than that obtained with the clear plate. As long as the fluorimeter to be used is configured so that excitation and emission detection occur from above the well, the white plate should significantly increase assay sensitivity.

   Should it be necessary, for convenience in adding or mixing reagents, there is some leeway for change in the reaction volumes. The wells of the microplates provided can readily accommodate 150 µL. If planning a change to the volume of the Developer, it should be noted that it is important to keep two factors constant: 1) concentration of SIRT2 inhibitor (1 mM nicotinamide) in the final mix; 2) 10 µL/well amount of Developer II Concentrate (BML-KI176). See “Preparing Reagents For Assay”, Step #5, (p. 13).

2. Experimental samples should be compared to a “Time Zero” (sample for which 1x Developer II/2 mM

nicotinamide is added immediately before mixing of the SIRT1 with substrate) and/or a negative control (no enzyme).

3. For many applications, including inhibitor screening, a signal approximately proportional to the initial enzyme rate is desirable. Particularly if a sub-K_m substrate concentration is chosen (see point 4. below) the rate will immediately begin to decline as substrate is used up. In the case of SIRT2, inhibition by one of the reaction products, nicotinamide, will also contribute to this effect. A preliminary time course experiment will aid in the selection of an incubation time, which yields a signal that is both sufficiently large and proportional to enzyme rate.
4. The K_m of SIRT2 for the FLUOR DE LYS® SIRT2 Substrate has been measured at 186 μM at 3 mM NAD^+ (Fig. 5). The K_m for NAD^+, determined at 1 mM FLUOR DE LYS® SIRT2 Substrate, was 547 μM (Fig. 6). Use of substrate concentrations at or below K_m will help avoid substrate competition effects, which could mask the effectiveness of competitive inhibitors or activators which act to lower substrate K_m's.
5. The effects of some enzyme modulators, such as covalent inhibitors, may be time-dependent. In other cases, time dependence may be indicative of artifacts such as the formation of aggregates. Two schemes for order of reagent mixing are outlined in the notes under Table 1. One includes a preincubation of enzyme and test compound. The other presents substrates and test compound to the enzyme simultaneously.
6. It is conceivable that some compounds being screened for modulation of SIRT2 activity may interfere with the action of the FLUOR DE LYS® Developer II. It is therefore important to confirm that apparent "hits" are in fact acting only via SIRT1 effects. One approach to this involves retesting the candidate compound in a reaction with the FLUOR DE LYS® Deacetylated Standard (BML- KI142) plus the FLUOR DE LYS® Developer II. A detailed retesting procedure is described below, in the section "Uses Of The FLUOR DE LYS® Deacetylated Standard"

(p. 15). In some cases, it may be possible to avoid this retesting by means of measurements taken during the fluorescence development phase of the initial SIRT2 assay.

Preparing Reagents For Assay:

1. Defrost all kit components and keep these, and all dilutions described below, on ice until use. Note that it is best to rapidly thaw both the SIRT2 enzyme (BML-SE251) and the 5x Developer II (BML-KI176). (See 'Notes on Storage', above.) All undiluted kit components are stable for several hours on ice.

2. Assuming 5 U of SIRT2 (BML-SE251) per assay, dilute a sufficient amount to 1 U/µL in Assay Buffer (BML-KI286) to provide for the assays to be performed (slightly more than # of wells x 5 µL). Subsequent dilutions of five-fold to 0.2 U/µL or three fold to 0.33 U/µL will be made, depending on whether test compounds will be added with substrate or preincubated with the enzyme (see Performing the Assay and Table 1, p. 13).

3. Prepare dilution(s) of suramin, nicotinamide and/or Test Compounds in Assay Buffer (BML-KI286). Since 10 µL will be used per well (Table 1), and since the final volume of the SIRT2 reaction is 50 µL, these inhibitor dilutions will be 5x their final concentration. Suramin sodium is soluble in both water and Assay Buffer (10 mg in 0.27 ml = 25 mM). High concentrations of both ethanol and DMSO affect SIRT2 activity and appropriate solvent controls should always be included.

4. Prepare a dilution of the substrates, FLUOR DE LYS® SIRT2 (BML-KI282, 50 mM), in Assay Buffer (BML-KI286), that will be 3.33x the desired final concentrations. For inhibitor screening, substrate concentrations at or below the K_m are recommended. This 3.33x stock will constitute 60% of a 2x substrate stock, prepared either with or without added test compounds (see Performing the Assay and Table 1, below).

5. Shortly before use (<30 min.), prepare sufficient 1x FLUOR DE LYS® Developer II plus nicotinamide (2 mM) for the assays to be performed (50 µL per well). One

mL will contain 760 μL Assay Buffer, 200 μL 5x Developer II and 40 μL 50 mM nicotinamide. Addition of nicotinamide to the Developer II insures that SIRT2 activity stops when the Developer II is added. Keep diluted Developer II on ice until use.

Performing the Assay:

1. Table 1 gives examples of solutions and volumes for use in various types of SIRT2 assays. These are mixtures for the first, deacetylation phase, of the assay. The SIRT2 reaction is initiated by mixing 25 μL of a 2x substrate solution with 25 μL containing the enzyme. The notes below Table 1 (‡) describe schemes for mixing the stock solutions prepared above (Preparing Reagents for Assay) so that the test compounds are added as part of the 2x substrate solution (1) or are preincubated with the enzyme (2).

TABLE 1. COMPOSITION OF EXAMPLE ASSAY MIXTURES (PER WELL VOLUMES)

Sample	**Assay Buffer**	**SIRT2 (1 U/μL))**	**Test Cmpd. or Solvent Control (5x)**	**Substrates** FLUOR DE LYS®SIRT2 **plus NAD⁺**
Blank (No Enzyme)	25 μL	0	10 μL	15 μL
Time Zero⌘	10 μL + 10 μL‡	5 μL	10 μL	15 μL⌘
Control	10 μL + 10 μL‡	5 μL	10 μL	15 μL
Suramin	10 μL + 10 μL‡	5 μL	10 μL	15 μL
Test Sample	10 μL + 10 μL‡	5 μL	10 μL	15 μL

‡ The Assay Buffer amount is written as a split “10 μL + 10 μL” in reference to two possibilities for the order in which test compounds are mixed with the SIRT2 enzyme:

1) If substrate and test compound are to be mixed with the enzyme simultaneously, then the entire 20 μL would be mixed with 5 μL of enzyme or a master mix consisting of 0.2

U/µL SIRT2 in Assay Buffer could be dispensed at 25 µL per well. In this case, substrates plus test compound (25 µL) could be added from a mother plate in which the wells contain a mixture of 40% 5x Test Compound and 60% 3.33x Substrates.

If the test compound is to be preincubated with enzyme prior to substrate addition, 15 µL of an enzyme master mix consisting of 0.33 U/µL SIRT2 in Assay Buffer could be dispensed per well and then mixed with 10 µL of 5x Test Compound. The reaction would then be initiated by addition of 25 µL of 2x Substrates in Assay Buffer (40% Assay Buffer, 60% 3.33x Substrates).

⌘ **NOTE:** In a 'Time Zero' sample, the substrate addition is made after the addition of 1x Developer II/2 mM nicotinamide.

2) Add 25 µL of 0.2 U/µl SIRT2 or 15 µL of 0.33 U/µL SIRT2 plus 10 µL 5x Test Compound or 25 µL Assay Buffer to appropriate wells of the assay plate.
3) Warm the assay plate and 2x substrate solutions to 37°C.
4) Initiate SIRT2 reactions by adding 25 µL 2x substrate solutions to the assay wells and thoroughly mixing. DO NOT ADD SUBSTRATE TO "TIME ZERO" WELLS.
5) Allow SIRT2 reactions to proceed for desired length of time and then stop by addition of 1x Developer II/2 mM nicotinamide (50 µL). Add 25 µL of 2x Substrate solution to "Time Zero" samples. Incubate plate at room temperature for at least 45 min. Signal development can be accelerated by higher temperature (30-37°C).
6) Read samples in a microplate-reading fluorimeter capable of excitation at a wavelength in the range 350-380 nm and detection of emitted light in the range 450-480 nm. Completion of signal development can be assessed by taking fluorescence readings at 5 min. intervals. The Developer reaction is complete when the fluorescence readings reach a maximum and plateau. Signals are stable for at least 60 min. beyond this time.

Preparation of a Standard Curve:

1. The exact concentration range of the FLUOR DE LYS® Deacetylated Standard (BML-KI142) that will be useful for preparing a standard curve will vary depending on the

fluorimeter model, the gain setting and the exact excitation and emission wavelengths used. We recommend diluting some of the standard to a relatively low concentration with Assay Buffer (1 to 5 µM). The fluorescence signal should then be determined, as described below, after mixing 50 µL of the diluted standard with 50 µL of 0.2x Developer II. The estimate of AFU (arbitrary fluorescence units)/µM obtained with this measurement, together with the observed range of values obtained in the enzyme assays can then be used to plan an appropriate series of dilutions for a standard curve. Provided the same wavelength and gain settings are used each time, there should be no need to prepare a standard curve more than once.

2. After ascertaining an appropriate concentration range, prepare, in Assay Buffer, a series of FLUOR DE LYS® Deacetylated Standard dilutions that span this range. Pipet 50 µL of each of these dilutions, and 50 µL of Assay Buffer as a 'zero', to a set of wells on the microplate.
3. Prepare enough of a 0.2x dilution of FLUOR DE LYS® Developer II in Assay Buffer for addition of 50 µL to each of the standard wells.
4. Mix 50 µL of the 0.2x Developer II with the 50 µL in each standard well and incubate 5-10 min. at room temperature (25°C).
5. Read samples in a microplate-reading fluorimeter capable of excitation at a wavelength in the range 350-380 nm and detection of emitted light in the range 450-480 nm.
6. Plot fluorescence signal (y-axis) versus concentration of the FLUOR DE LYS® Deacetylated Standard (x-axis). Determine slope as AFU /µM. See example in Fig. 3.

Testing of Potential SIRT2 Inhibitors for Interference with the FLUOR DE LYS® Developer II or the Fluorescence Signal:

1. The FLUOR DE LYS® Developer is formulated so that, under normal circumstances, the reaction goes to completion in less than 30 min. at 25°C. That, together with the recommended 45 min. reaction time, should help

insure that in most cases, even when some retardation of the development reaction occurs, the signal will fully develop prior to the reading of the plate.

2. A convenient step to control for substances that interfere with the Developer reaction or the fluorescence signal itself may be built directly into an inhibitor screening protocol. After waiting for the signal from the SIRT2 reaction to fully develop and stabilize (usually less than 45 min., see 1. above), the fluorescence is recorded and a ‘spike’ of FLUOR DE LYS® Deacetylated Standard is added (e.g. 5 µL of 20 µM Deacetylated Standard (BML-KI142) in Assay Buffer; i.e. amount equivalent to 5 µM/100 pmol in the 50 µL SIRT2 reaction). Sufficient Developer reactivity should remain to produce a full signal from this ‘spike’. When the new, increased fluorescence level has fully developed (<15 min.), the fluorescence is read and the difference between this reading and the first one can provide an internal standard, in terms of AFU/µM, for appropriate quantitation of each well. This is particularly useful in cases where the development reaction itself is not compromised but the fluorescence signal is diminished. Highly colored test compounds, for example, may have such an effect. As discussed further below (see 3.), interference with the development reaction *per se* will be reflected in the kinetics of signal development, both that due to the initial SIRT2 reaction and that due to a subsequent Deacetylated Standard ‘spike’.

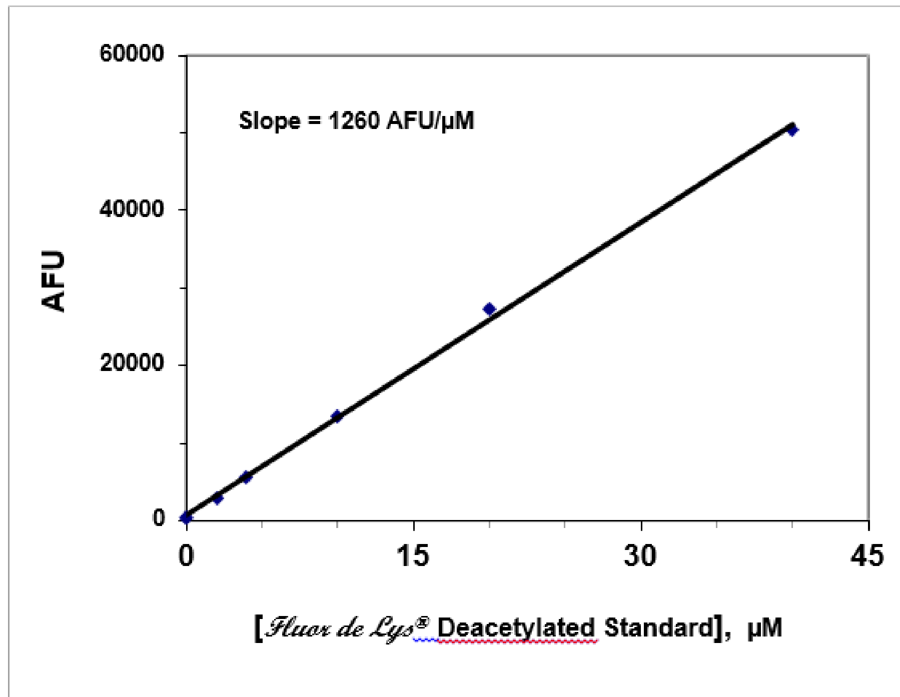

Figure 3. Fluorescence Standard Curve. Fifty µL aliquots of FLUOR DE LYS® Deacetylated Standard, in Assay Buffer at the indicated concentrations, were mixed with 50 µL 0.2x Developer II and incubated 15 min., 25°C. Fluorescence was then measured in the wells of the clear microplate with a CytoFluor™ II fluorescence plate reader (PerSeptive Biosystems, Ex. 360 nm, Em. 460 nm, gain=85)

3. It should be possible to identify many cases in which there is interference with the development reaction by taking a series of fluorescence readings immediately following addition of the FLUOR DE LYS® Developer (e.g. readings at 5 min. intervals for 45 min.). The fluorescence of control samples (no inhibitor) will change very little after the third or fourth reading. Samples containing compounds which inhibit SIRT2, but which do not interfere with Developer II, will display similarly rapid kinetics, although a lower final fluorescence. Nicotinamide (100 µM) provides a good model of this behavior. Any sample in which the approach to the final fluorescence is substantially slower than in the above examples should be suspected of interference with the development reaction. For samples in which little or no fluorescence has developed, it may be impossible to assess the development kinetics.
4. Absolute certainty regarding interference with the

Developer II reaction can only be obtained through an assay in which the compound in question is tested for its effect on the reaction of FLUOR DE LYS® Deacetylated Standard with the Developer. Using a standard curve such as that described in the previous section, determine the concentration of Deacetylated Standard that will yield a signal similar to that produced after development of a control (no inhibitor) SIRT2 reaction. Mix 40 µL of the diluted Standard with 10 µL inhibitor or 10 µL Assay Buffer (see Table 2). Initiate development by adding 50 µL of 0.2x Developer II to each well. Follow fluorescence development by reading at 1 or 2 min. intervals for 30 min. If a test inhibitor sample reaches its final fluorescence significantly more slowly than the control then there may be interference with the Developer II reaction. Compounds that decrease the final fluorescence signal without slowing the kinetics of its development may be quenching the fluorescence signal rather than interfering with the Developer II reaction (see point 2. above).

5. Once it is determined that a particular substance does interfere with the Developer reaction, it may be possible to adjust reaction conditions to eliminate this effect. In cases where the same final fluorescence is achieved, but more slowly than the control, simply extending the incubation time after addition of the Developer II would be sufficient. Other possible adjustments include increasing the volume of Developer II used per well (e.g. to 100 µL). Both approaches may be used separately or in combination.

TABLE 2. ASSAY MIXTURES FOR TEST COMPOUND RETESTING WITH FLUOR DE LYS® DEACETYLATED STANDARD

Sample	Test Compound or Solvent Control (5x)	Diluted[6] FLUOR DE LYS® deAc. Standard (1.25x)	DEVELOPER II (0.2x).
Control	10 µL	40 µL	50 µL
Test Compound	10 µL	40 µL	50 µL

[6]The appropriate dilution of the FLUOR DE LYS® Deacetylated Standard, in Assay Buffer may be determined from the standard curve and should be the concentration producing a fluorescent signal equal to that produced by control (no Test Compound) samples in the SIRT2 assay. The dilution in Assay Buffer is prepared at 1.25x this concentration to compensate for the 4/5 dilution due to addition of 10 µL of Assay Buffer or 5x Test Compound.

Preparation of a Standard Curve:

The *SIRT2 Fluorescent Activity Assay/Drug Discovery Kit* has been used for investigating SIRT2 kinetics as a function of the concentrations of FLUOR DE LYS® SIRT2 Substrate and NAD+ (Figures 5&6) as well as for the discovery and characterization of activators and inhibitors of the enzyme (Figure 4).

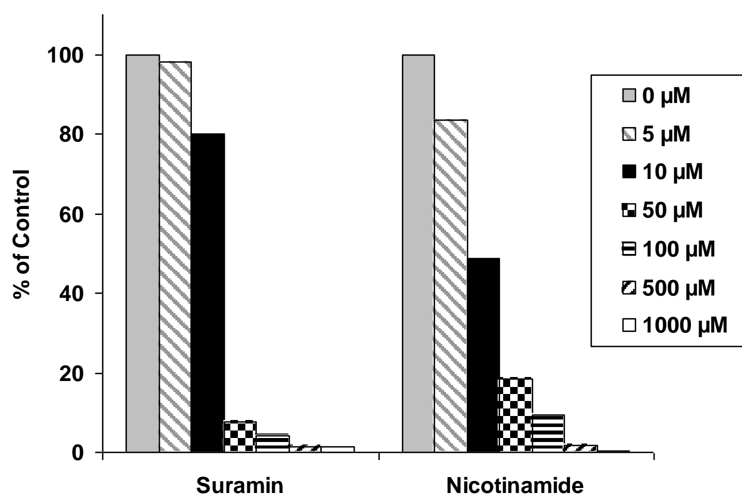

Figure 4. Inhibitors of SIRT2. Initial deacetylation rates of SIRT2 were determined at 25 µM FLUOR DE LYS® SIRT2, 25 µM NAD^+ (37°C) in the presence of the indicated concentrations of suramin or nicotinamide. Reactions were stopped with FLUOR DE LYS® Developer II/2 mM nicotinamide and fluorescence measured (CytoFluor™II, PerSeptive Biosystems, Ex. 360 nm, Em. 460 nm, gain=85).

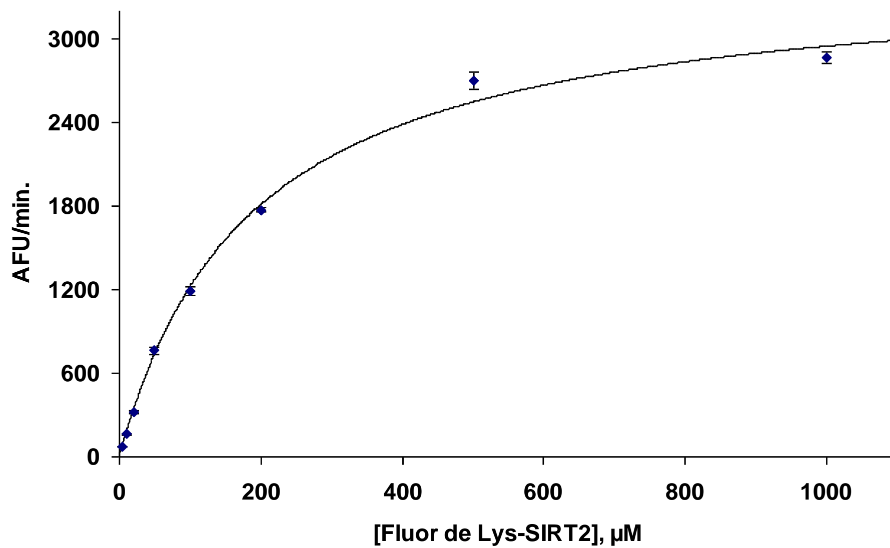

Figure 5. Dependence of SIRT2 Kinetics on the Concentration of FLUOR DE LYS® SIRT2. Initial deacetylation rates of SIRT2 were determined with 10 min. incubations (37°C) in the presence of 3 mM NAD^+. Reactions were stopped with FLUOR DE LYS® Developer II/2 mM nicotinamide and fluorescence measured (CytoFluor™II, PerSeptive Biosystems, Ex. 360 nm, Em. 460 nm, gain=85). Each point represents the mean of three determinations and the error bars are standard errors. The line is a non-linear least squares fit to the Michaelis-Menten equation. The K_m for FLUOR DE LYS® SIRT2 was 186 µM and the V_{max} was 3510 AFU/min.

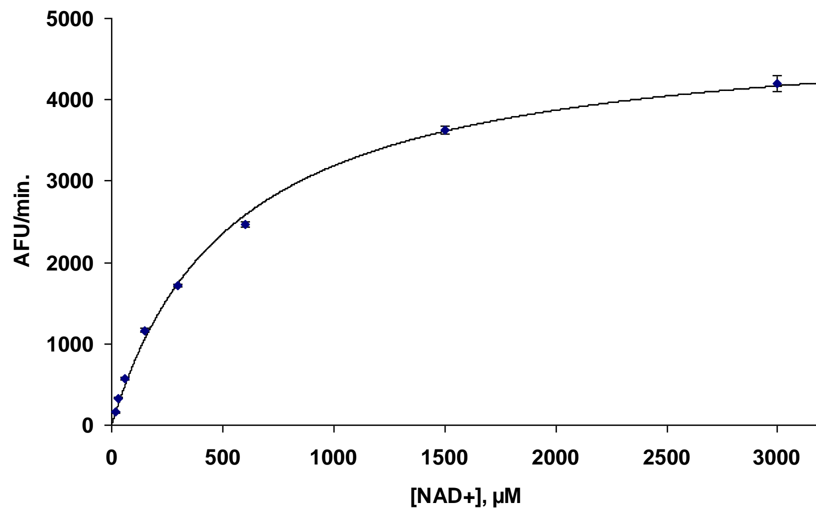

Figure 6. Dependence of SIRT2 Kinetics on the Concentration of NAD^+. Initial deacetylation rates of SIRT2 were determined with 20 min. incubations (37°C) in the presence of 1 mM FLUOR DE LYS® SIRT2. Reactions were stopped with FLUOR DE LYS® Developer II/2 mM nicotinamide and fluorescence measured (CytoFluor™II, PerSeptive Biosystems, Ex. 360 nm, Em. 460 nm, gain=85). Each point represents the mean of three determinations and the error bars are standard errors. The line is a non-linear least squares fit to the Michaelis-Menten equation. The K_m for NAD^+ was 547 µM and the V_{max} was 4931 AFU/min.

THE APPLICATION EXAMPLES, DESCRIBED HEREIN, ARE INTENDED ONLY AS GUIDELINES. THE OPTIMAL CONCENTRATIONS OF SUBSTRATES AND INHIBITORS, ASSAY VOLUMES, BUFFER COMPOSITION, AND OTHER EXPERIMENTAL CONDITIONS MUST BE DETERMINED BY THE INDIVIDUAL USER. NO WARRANTY OR GUARENTEE OF PARTICULAR RESULTS, THROUGH THE USEOF THESE PROCEDURES, IS MADE OR IMPLIED.

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