FLUOR DE LYS®
SIRT3 fluorometric drug discovery assay kit

A FLUOR DE LYS® fluorescent assay system. The SIRT3 Fluorescent Activity Assay/Drug Discovery Kit is a complete assay system designed to measure the lysyl deacetylase activity of the recombinant human SIRT3 included in the kit. The kit is ideal for chemical library screening for candidate inhibitors or activators or kinetic assay of the enzyme under varying conditions. The FLUOR DE LYS® SIRT3 assay is based on the FLUOR DE LYS® Substrate and FLUOR DE LYS® Developer combination. The assay procedure has two steps. First, the FLUOR DE LYS® SIRT2 Substrate, which comprises a unique peptide based on amino acids 317-320 of p53 (Gln-Pro-Lys-Lys(Ac)), is incubated with SIRT3. Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the FLUOR DE LYS® Developer II produces a fluorophore.

Like Sir2 and human SIRTs 1 and 2, SIRT3 is a class I sirtuin. The sirtuins are currently the subject of intense research interest with respect to their roles in gene silencing, aging, and oxidative stress responses. Unlike SIRT1, which is located in nuclei, or SIRT2, which is primarily cytoplasmic, with a lesser amount of nuclear localization, SIRT3 is synthesized as an inactive precursor protein whose mature, active form is located in the mitochondrial matrix. Although its substrates are as yet unidentified, SIRT3's mitochondrial localization suggests some interesting possibilities for SIRT3 function. NAD /NADH are essential to mitochondrial electron transport and ATP production. Thus SIRT3 could, *via* its requirement for NAD , act as a sensor and transducer of metabolic signals. Alternatively, since mitochondrial NAD concentrations may be unresponsive to metabolic changes, but depleted by opening of the mitochondrial permeablity pore, SIRT3 could be acting as a modulator of apoptotic signals that affect mitochondrial permeability.

Citations: 24 View Online »

- Useful for inhibitor screening or characterizing enzyme kinetics
- Includes optimal substrate selected from a panel of acetylated sites in p53 and histones
- Supplied with enough recombinant enzyme for 96 assays (1 x 96-well plate)

Ordering Information

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BML-AK557-0001	96 wells

Manuals, SDS & CofA

View Online »

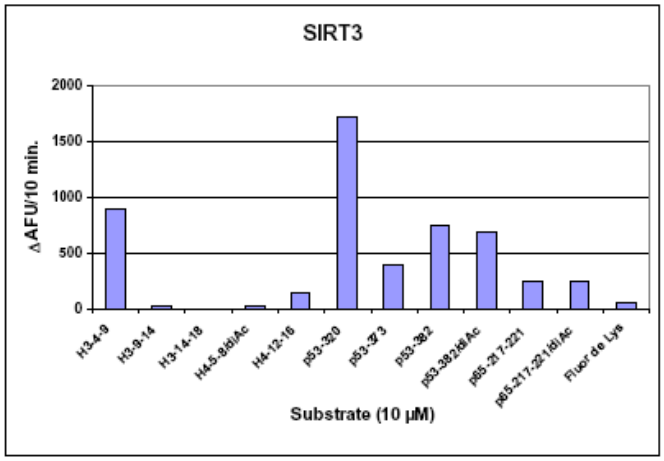

Figure :. SIRT3 Peptide Substrate Preferences. Initial rates of deacetylation were determined for a series of fluorogenic acetylated peptide substrates. based on native acetylation sites in human histones H3 and H4, and the transcription factors p53 and p65 sequence. Recombinant human SIRT3 (Prod. No. BML-SE270), was incubated for 10 min at 37°C with 10 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. at 37°C. Fluorescence was then measured in the wells of a white microplate (Prod. No. BML-KI110) with a CytoFluor™II fluorescence plate reader at 5 min intervals until development reached a plateau (PerSeptive Biosystems, Ex. 360 nm, Em. 460 nm, gain=85). The substrate labeled ‘p53-320’ is FLUOR DE LYS®-SIRT2 (Prod. No. BML-KI179).

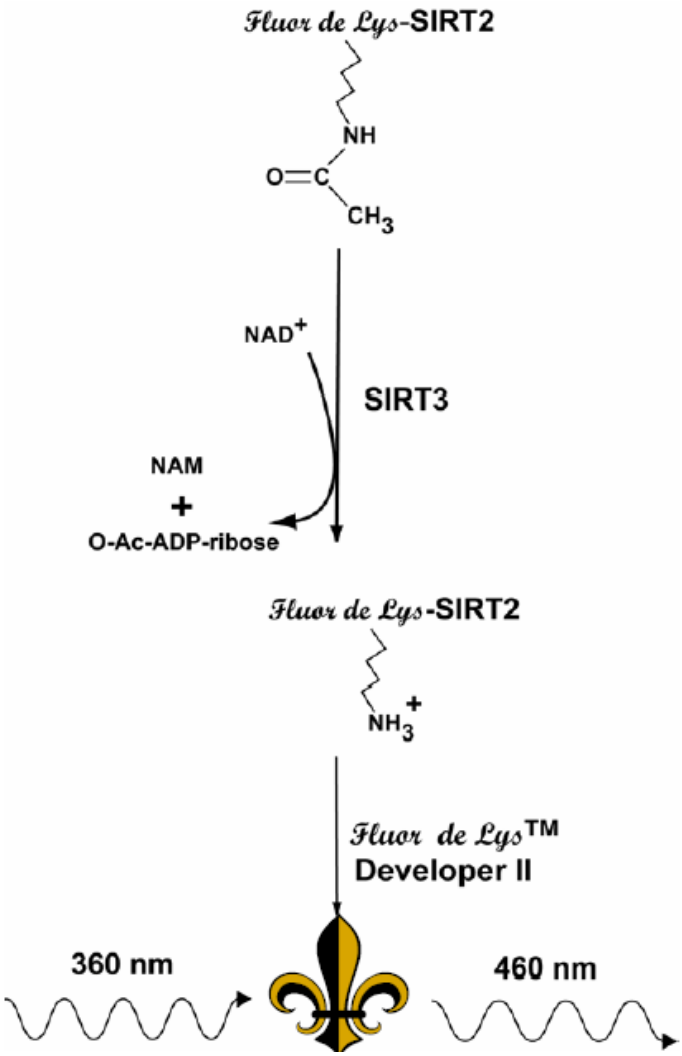

Figure 1: Reaction Scheme of the SIRT3 Fluorescent Activity Assay. NAD+-dependent deacetylation of the substrate by recombinant human SIRT3 sensitizes it to Developer II, which then generates a fluorophore (symbol). The fluorophore is excited with 360 nm light and the emitted light (460 nm) is detected on a fluorometric plate reader. NAD+ is consumed in the reaction to produce nicotinamide (NAM) and O-acetyl-ADP-ribose.

Handling & Storage

Use/Stability	Store all components except the microplates and instruction booklet at -70°C for the highest stability. The SIRT3 enzyme, Prod. No. BML-SE270, 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 extract 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 5x Developer II (^Prod. No. 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.
Long Term Storage	-80°C
Shipping	Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	Sirtuin 3 fluorescent assay kit
Application	Activity assay, Cell-based assays, Fluorescent detection, HTS

Contents	**SIRT3** (Sirtuin 3) (human, recombinant) (Prod. No. BML-SE270) (500 U, one U=1 pmol/min at 37°C, 500µM, FLUOR DE LYS®-SIRT2 substrate (Prod. No. BML-KI179), 500µM NAD ; Recombinant enzyme dissolved in 25mM TRIS, pH 7.5, 100mM sodium chloride, 5mM dithiothreitol and 10% glycerol. See vial label for activity and protein concentrations) Storage: -70°C, avoid freeze/thaw cycles! **FLUOR DE LYS®-SIRT2,** Deacetylase substrate (Prod. No. BML-KI179) (100µl; 5mM solution in 25mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride). Note: this SIRT2 substrate is also hydolyzed by SIRT3. **FLUOR DE LYS® Developer II Concentrate** (5x) (Prod. No. BML-KI176) (5 x 250 µl; 5x Stock Solution; Dilute in Assay Buffer before use) Storage: -70°C **NAD** (Sirtuin Substrate) (Prod. No. BML-KI282) (500 µl; 50 mM β-Nicotinamide adenine dinucleotide (oxidized form) in 50mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride) Storage: -70°C **Nicotinamide** (Sirtuin Inhibitor) (Prod. No. BML-KI283) (500µl; 5 mM Nicotinamide in 50mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride) Storage: -70°C **Suramin sodium** (Sirtuin Inhibitor) (Prod. No. BML-KI285) (10 mg; Solid MW: 1429.2, soluble in water or assay buffer (to 25mM)) Storage: -70°C **FLUOR DE LYS® Deacetylated Standard** (Prod. No. BML-KI142) (30 µl; 10mM in DMSO) Storage: -70°C **Sirtuin Assay Buffer** (Prod. No. BML-KI286) (20ml; 50mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride, 1 mg/ml bovine serum albumin) Storage: -70°C **1/2 volume microplate** (Prod. No. BML-KI101) Storage: Room temperature **1/2 volume white microplate** (Prod. No. BML-KI110) Storage: Room temperature
UniProt ID	Q9NTG7

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