FLUOR DE LYS® SIRT2 fluorometric drug discovery assay kit

A FLUOR DE LYS® fluorescent assay system. 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. 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® SIRT2 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 SIRT2. Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the FLUOR DE LYS®Developer II produces a fluorophore.

Due to their roles in gene silencing, aging and oxidative stress responses, the sirtuins (NAD -dependent deacetylases related to yeast Sir2), are currently the subject of intense research interest. Like Sir2, human SIRT2 is a class I sirtuin, although not as closely related to Sir2 as human SIRT1. The three-dimensional structure of SIRT2 has been determined, and its catalytic core has the same basic two-domain architecture and central groove as other sirtuins. SIRT2 is a cytoplasmic protein, although a lesser amount of nuclear localization has also been reported and an interaction with the homeobox transcription factor HOXA10 have also been reported. A complex comprising HDAC6 and SIRT2 colocalizes with the microtubule network and both enzymes can deacetylate a-tubulin lysine-40 in purified tubulin heterodimers or microtubules. SIRT2 protein levels rise and it becomes phosphorylated during mitosis . Overexpression of SIRT2 delays exit of cells from mitosis, suggesting that it may play a role in cell cycle regulation.

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- 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

Order Online »

BML-AK556-0001	96 wells

Manuals, SDS & CofA

View Online »

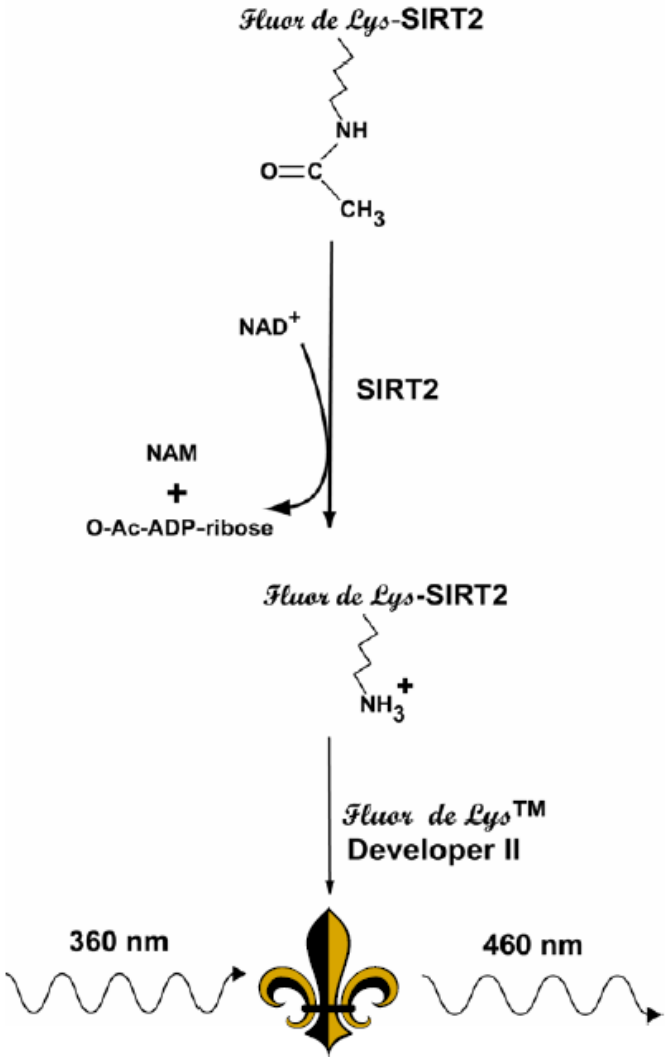

Figure 1: Reaction Scheme of the SIRT2 Fluorescent Activity Assay. NAD^+-dependent deacetylation of the substrate by recombinant human SIRT2 sensitizes it to Developer II, which then generates a fluorophore (symbol). The fluorophore is excited with 360 nm light and the emitted light (460 nm) is detected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

Handling & Storage

Use/Stability	Store all components except the microplates and instruction booklet at -80°C for the highest stability. The SIRT2 enzyme, Prod. No. 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 extract 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 (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 2 fluorescent assay kit
Application	Activity assay, Cell-based assays, Fluorescent detection, HTS

Contents

SIRT2 (Sirtuin 2, hSir2) (human, recombinant) (Prod. No. BML-SE25)
(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: -80°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 potassion chloride, 1mM magnesium chloride)
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: -80°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: -80°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: -80°C
Suramin sodium (Sirtuin Inhibitor) (Prod. No. BML-KI285)
(10 mg; Solid MW: 1429.2, soluble in water or assay buffer (to 25mM))
Storage: -80°C
FLUOR DE LYS® Deacetylated Standard (Prod. No. BML-KI142) (30 µl; 10mM in DMSO)
Storage: -80°C
Sirtuin Assay Buffer (Prod. No. BML-KI286)
(20 ml; 50mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride, 1 mg/ml bovine serum albumin)
Storage: -80°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 Q8IXJ6

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ENZO LIFE SCIENCES, INC.
Phone: 800.942.0430
info-usa@enzolifesciences.com

European Sales Office
ENZO LIFE SCIENCES (ELS) AG
Phone: +41 61 926 8989
info-eu@enzolifesciences.com

Belgium, The Netherlands & Luxembourg
Phone: +32 3 466 0420
info-be@enzolifesciences.com

France
Phone: +33 472 440 655
info-fr@enzolifesciences.com

Germany
Phone: +49 7621 5500 526
info-de@enzolifesciences.com

UK & Ireland
Phone (UK customers): 0845 601 1488
Phone: +44 1392 825900
info-uk@enzolifesciences.com

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