FLUOR DE LYS® SIRT1 fluorometric drug discovery assay kit

First-to-market by the leader in Epigenetics research tools

A FLUOR DE LYS® fluorescent assay system. The SIRT1 Fluorescent Activity Assay/Drug Discovery Kit is a complete assay system designed to measure the lysyl deacetylase activity of the recombinant human SIRT1 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® SIRT1 assay is based on the FLUOR DE LYS® SIRT1 Substrate and FLUOR DE LYS® Developer II combination. The assay procedure has two steps. First, the FLUOR DE LYS® SIRT1 Substrate, which contains a peptide comprising amino acids 379-382 of human p53 (Arg-His-Lys-Lys(Ac)). Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the FLUOR DE LYS®Developer II produces a fluorophore.

Yeast Sir2 (Silent information regulator 2) is the founding exemplar of the 'sirtuins', an apparently ancient group of enzymes that occurs in eukaryotes, the archaea and eubacteria. In yeast and *C. elegans*, added copies of sirtuin genes extend lifespan and Sir2 is required for the lifespan extension conferred by caloric restriction in yeast. There are seven human sirtuins, which have been designated SIRT1-SIRT7. SIRT1, which is located in the nucleus, is the human sirtuin with the greatest homology to Sir2 and has been shown to exert a regulatory effect on p53 by deacetylation of lysine-382. Dr. Konrad Howitz at Enzo Life Sciences carried out a screen for modulators of SIRT1 activity which yielded a number of small molecule activators, all of which were plant polyphenols. Several of these Sirtuin Activating Compounds (STACs) extended yeast lifespan in a way that mimicked caloric restriction. Resveratrol, the most potent of these STACs activated SIRT1 in human cells and enhanced the survival rate of cells stressed by irradiation.

Citations: 95 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

Order Online »

BML-AK555-0001	96 wells

Manuals, SDS & CofA

View Online »

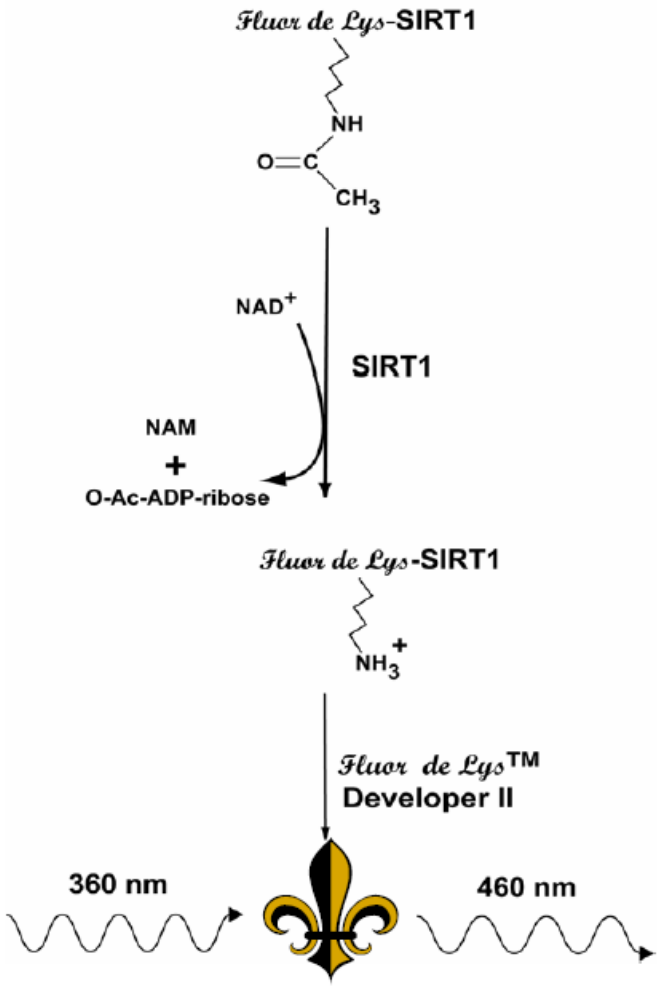

Figure: Reaction Scheme of the SIRT1 Fluorescent Activity Assay*. NAD+-dependent deacetylation of the substrate by recombinant human SIRT1 sensitizes it to Developer II, which then generates a fluorophore (symbol). The fluorophore is excited with 360 nm light and the emitted light (460 nm) is detected 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 SIRT1 enzyme, (Prod. No. BML-SE239), 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 (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 1 fluorescent assay kit
Application	Activity assay, Cell-based assays, Fluorescent detection, HTS

Contents

SIRT1 (Sirtuin 1, hSir2^{SIRT1}) (human, recombinant) (Prod. No. BML-SE239)
(100 U; One U=1 pmol/min at 37°C, 250 µM, FLUOR DE LYS® Substrate (Prod. No. BML-KI104), 500 µM 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® SIRT1, Deacetylase Substrate (Prod. No. BML-KI177)
(100µl; 5mM solution in 50mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride)
Storage: -80°C
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; 50 mM Nicotinamide in 50mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride)
Storage: -80°C
Resveratrol (Sirtuin Activator) (Prod. No. BML-KI284)
(10 mg; Solid MW: 228.2, soluble in DMSO or 100% ethanol (to 100mM)
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
(50mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride, 1 mg/ml bovine serum albumin) (Prod. No. BML-KI286) (20 ml)
Storage: -80°C
1/2 volume microplates (Prod. No. 80-2407)
1 clear and 1 white, 96-well
Storage: Room temperature

Technical Info / Product Notes

Cited example:

HTS application. Use of 384-well plates with this kit.

UniProt ID

Q96EB6

Last modified: May 29, 2024

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