PRODUCT DATA SHEET

FLUOR DE LYS® SIRT1 fluorometric drug discovery assay kit

BML-AK555
First-to-market by the leader in Epigenetics research tools

Product Number/Sizes
BML-AK555-0001 96 wells

- 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)

A FLUOR DE LYS® fluorometric 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(As)). Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the FLUOR DE LYS® Developer II produces a fluorophore.

Product Specifications
ALTERNATIVE NAME: Sirtuin 1 fluorescent assay kit
APPLICATIONS: Fluorescent detection, HTS
USE/STABILITY: Activity assay. Cell-based assays
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.

SHIPPING: Shipped on Dry Ice
LONG TERM STORAGE: -80°C

KIT/SET CONTAINS:
SIRT1 (Sirtuin 1, hSir2SIRT1 (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-K104), 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

For Research Use Only, Not for Human
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

SCIENTIFIC BACKGROUND:
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.

TECHNICAL INFO/PRODUCT NOTES:
Cited example:
HTS application. Use of 384-well plates with this kit.

UNIPROT ID:
Q96EB6
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.

Product Literature References


The peroxisome proliferator-activated receptor γ agonist pioglitazone prevents NF-κB activation in cisplatin nephrotoxicity through the reduction of p65 acetylation via the AMPK-SIRT1/p300 pathway J. Zhang, et al. Biochem. Pharmacol. 101 100 (2016)


Leucine Amplifies the Effects of Metformin on Insulin Sensitivity and Glycemic Control in Diet-Induced Obese Mice L. Fu, et al. Metabolism 64 845 (2015)


Modulation of the AMPK/Sirt1 axis during neuronal infection by herpes simplex virus type 1 C. Martin, et al. J. Alzheimers Dis. 42 301 (2014)


Synergistic effects of metformin, resveratrol, and hydroxymethylbutyrate on insulin sensitivity A. Bruckbauer & M.B. Zemel Diabetes Metab. Syndr. Obes. 6 93 (2013)


Effects of dietary consumption on SIRT1 and mitochondrial biogenesis in adipocytes and muscle cells A. Bruckbauer & M.B. Zemel Nutr. Metab. (Lond.) 8 91 (2011)


SIRT inhibitors induce cell death and p53 acetylation through targeting both SIRT1 and SIRT2 B. Peck, et al. Mol. Cancer Ther. 9 844 (2010)


Revised 17-Aug-17