CREATIVE BIOMART®
Assay Kit

SIRT5 fluorometric drug discovery assay Kit

Product Information

Cat.No. Kit-0788

Product Overview

Easy-to-use kits (two-step) for screening SIRT5 inhibitors/activators; includes enough active enzyme for entire plateIncludes optimal substrate selected from a panel of succinylated sites96-well plate included, but can be adapted to higher well formatControl inhibitors includedSuitable for high-throughput screening (Z"-factors >0.73)Optimal, specific SIRT5 substrate means low enzyme concentration, making "hit" validation easyThe SIRT5 Fluorometric Drug Discovery Kit is a complete assay system designed to measure the lysyl desuccinylase activity of the recombinant human SIRT5 included in the kit. A black 96-well microplate is packaged with the kit, but it should be noted that reagents of the FLUOR DE LYS system have also been successfully employed in other formats, including cuvettes and 384-well plates. The SIRT5 Fluorometric Activity Assay is based on the unique FLUOR DE LYS-Succinyl Substrate/Developer combination. The assay procedure has two steps. The FLUOR DE LYS-Succinyl Substrate, which comprises a single lysine residue, Nε-succinylated on its side-chain, is first incubated with human recombinant SIRT5 together with the cosubstrate NAD+. Desuccinylation of FLUOR DE LYS-Succinyl sensitizes it so that, in the second step, treatment with the FLUOR DE LYS Developer produces a fluorophore. Use of a succinylated, rather than acetylated substrate with SIRT5 results in readily observed saturation kinetics and a greater than 1000-fold increase in assay sensitivity.

Size

100 tests

Description

The SIRT5 Fluorometric Drug Discovery Kit is a complete assay system designed to measure the lysyl desuccinylase activity of the recombinant human SIRT5 included in the kit. A black 96-well microplate is packaged with the kit, but it should be noted that reagents of the FLUOR DE LYS system have also been successfully employed in other formats, including cuvettes and 384-well plates. The SIRT5 Fluorometric Activity Assay is based on the unique FLUOR DE LYS-Succinyl Substrate/Developer combination. The assay procedure has two steps. The FLUOR DE LYS-Succinyl

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.org
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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Substrate, which comprises a single lysine residue, Nε-succinylated on its side-chain, is first incubated with human recombinant SIRT5 together with the cosubstrate NAD+. Desuccinylation of FLUOR DE LYS-Succinyl sensitizes it so that, in the second step, treatment with the FLUOR DE LYS Developer produces a fluorophore. Use of a succinylated, rather than acetylated substrate with SIRT5 results in readily observed saturation kinetics and a greater than 1000-fold increase in assay sensitivity.

Applications

Fluorescence microscopy, HTS

Storage

-80°C

Kit Components

SIRT5 (Sirtuin 5) (human, rec.)Form: Dissolved in 25 mM TRIS, pH 7.5, 100 mM NaCl, 5 mMDTT, 1 mg/mL BSA and 10% glycerol.STORAGE: -70°C; AVOID FREEZE/THAW CYCLES!QUANTITY: 1200 U; See vial label for specific activity and protein concentration. One U = 1 pmol/min at 37°C, 250 μM FLUOR DE LYSSuccinyl, Desuccinylase, 2000 μM NAD+. BML-KI590-0050 FLUOR DE LYSSuccinyl, Desuccinylase SubstrateFORM: 5 mM solution in DMSO (dimethylsulfoxide)STORAGE: -70°CQUANTITY: 50 μLBML-KI105-0300 FLUOR DE LYS Developer Concentrate (20x)FORM: 20x Stock Solution; Dilute in Assay Buffer before use.STORAGE: -70°CQUANTITY: 300 μL BML-KI282-0500 NAD+ (Sirtuin Substrate)FORM: 50 mM β-Nicotinamide adenine dinucleotide (oxidized form) in 50 mM TRIS-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2.STORAGE: -70°CQUANTITY: 500 μL BML-KI283-0500 Nicotinamide (Sirtuin Inhibitor)FORM: 50 mM Nicotinamide in 50 mM TRIS-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2.STORAGE: -70°CQUANTITY: 500 μL BML-KI285-0010 Suramin sodium (Sirtuin Inhibitor)FORM: SolidMW: 1429.2STORAGE: -70°CQUANTITY: 10 mgSOLUBILITY: Water or Assay Buffer to 25 mM (10 mg in 0.27 ml) BML-KI592-0030 FLUOR DE LYS Desuccinylated StandardFORM: 10 mM in DMSO (dimethylsulfoxide)STORAGE: -70°CQUANTITY: 30 μL BML-KI286-0020 Sirtuin Assay Buffer(50 mM TRIS-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mMMgCl2, 1 mg/ml BSA)STORAGE: -70°CQUANTITY: 20 ml 80-2409 1/2 Volume Black NBS MicroplateSTORAGE: Room temperature.

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