CREATIVE BIOMART®
Assay Kit

Green SIRT5 fluorometric drug discovery assay Kit

Product Information

Cat.No.

Kit-0784

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 easyWhile the FLUOR DE LYS-Succinyl substrate contains an AMC ("blue") fluorophore that uses commonly-used wavelengths, FLUOR DE LYS-Succinyl Green substrate has longer excitation/emission wavelengths, avoiding interference often seen with screening compounds at shorter wavelengths.

Size

100 tests

Description

The FLUOR DE LYSGreen SIRT5 Fluorometric Drug Discovery Kitis 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 LYSsystem have also been successfully employed in other formats, including cuvettes and 384-well plates. The Green SIRT5 Fluorescent Activity Assay is based on the unique FLUOR DE LYS-Succinyl GreenSubstrate/Developer combination. The assay procedure has two steps. The FLUOR DE LYS-Succinyl GreenSubstrate, which comprises a lysine residue, Nε-succinylated on its side-chains, is first incubated with human recombinant SIRT5 together with the cosubstrate NAD+. Desuccinylation of FLUOR DE LYS-Succinyl Green sensitizes it so that, in the second step, treatment with the FLUOR DE LYSDeveloper 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

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Applications

Fluorescence microscopy, HTS

Storage

-80°C

Kit Components

SIRT5 (Sirtuin 5) (human, recombinant)FORM: Dissolved in 25 mM TRIS, pH 7.5, 100 mM NaCl, 5 mM DTT, 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 µMFLUOR DE LYS-Succinyl Green, Desuccinylase, 2000 µM NAD+.FLUOR DE LYS-Succinyl Green, Desuccinylase SubstrateFORM: 5 mM solution in DMSO (dimethylsulfoxide)STORAGE: -70°CQUANTITY: 50 µl FLUOR DE LYSDeveloper Concentrate (20x)FORM: 20x Stock Solution; Dilute in Assay Buffer before use.STORAGE: -70°CQUANTITY: 300 µlNAD+ (Sirtuin Substrate)FORM: 50 mM b-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 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 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) FLUOR DE LYS-Green Desuccinylated Standard FORM: 1 mM in DMSO (dimethylsulfoxide)STORAGE: -70°CQUANTITY: 30 µlSirtuin Assay Buffer(50 mM TRIS-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2, 1 mg/ml BSA)STORAGE: -70°CQUANTITY: 20 ml 1/2 VOLUME BLACK NBS MICROPLATESTORAGE: Room temperature.

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