

SIRT3 fluorometric drug discovery assay Kit

Product Information

Cat.No.

Kit-0787

Product Overview

Useful for inhibitor screening or characterizing enzyme kineticsIncludes optimal substrate selected from a panel of acetylated sites in p53 and histonesSupplied with enough recombinant enzyme for 96 assays (1 x 96-well plate)

Size

96 wells

Description

A FLUOR DE LYS fluorescent assay system. The SIRT3 Fluorescent Activity Assay/Drug Discovery Kit is a complete assay system designed to measure the lysyl deacetylase activity of the recombinant human SIRT3 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 SIRT3 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 SIRT3. Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the FLUOR DE LYS Developer II produces a fluorophore.

Applications

Fluorescence microscopy, HTS

Storage

-80°C

Kit Components

SIRT3 (Sirtuin 3) (human, recombinant)(500 U, one U=1 pmol/min at 37°C, 500µM, FLUOR DE LYS-SIRT2 substrate, 500µM NAD ; Recombinant enzyme dissolved in 25mM TRIS, pH 7.5, 100mM sodium

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chloride, 5mM dithiothreitol and 10% glycerol. See vial label for activity and protein concentrations)Storage: -70°C, avoid freeze/thaw cycles!FLUOR DE LYS-SIRT2, Deacetylase substrate(100µl; 5mM solution in 25mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride).Note: this SIRT2 substrate is also hydrolyzed by SIRT3.FLUOR DE LYS Developer II Concentrate (5x)(5 x 250 µl; 5x Stock Solution; Dilute in Assay Buffer before use)Storage: -70°CNAD (Sirtuin Substrate)(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: -70°CNicotinamide (Sirtuin Inhibitor)(500µl; 5 mM Nicotinamide in 50mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride)Storage: -70°CSuramin sodium (Sirtuin Inhibitor)(10 mg; Solid MW: 1429.2, soluble in water or assay buffer (to 25mM))Storage: -70°CFLUOR DE LYS Deacetylated Standard(30 µl; 10mM in DMSO)Storage: -70°C Sirtuin Assay Buffer(20ml; 50mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride, 1 mg/ml bovine serum albumin)Storage: -70°C1/2 volume microplateStorage: Room temperature1/2 volume white microplateStorage: Room temperature
