



HDAC/SIRT Chemiluminescent drug discovery Kit

Product Information

Cat.No.

Kit-0412

Product Overview

High Specificity assay eliminates false positives or negatives Superior Signal-to-Noise ratio with no interference from cell extract detergents Delivers consistent results from a validated system

Size

96 wells

Description

The HDAC/SIRT Chemiluminescent Drug Discovery Kit is a complete assay system designed to measure histone deacetylase (HDAC) and sirtuin activity in cell or nuclear extracts, immunoprecipitates or purified enzymes. It comes in a convenient 96-well format, with all reagents necessary for chemiluminescent HDAC activity measurements and calibration of the assay. In addition, a HeLa nuclear extract, rich in HDAC activity, is included with the kit. The extract is useful as either a positive control or as the source of HDAC activity for inhibitor/drug screening. Also included are Trichostatin A and Nicotinamide, which may be used as model inhibitors for HDACs and sirtuins, respectively. The HDAC Chemiluminescent Activity Assay is based on the unique CHEMILUM DE LYS Substrate and Developer combination. The CHEMILUM DE LYS system (Chemiluminescent Histone deAcetylase Lysyl Substrate/Developer) is a highly sensitive and convenient alternative to radiolabeled, acetylated histones or peptide/HPLC methods for the assay of histone deacetylases. The assay procedure has three steps (Fig. 1). First, the CHEMILUM DE LYS Substrate, which comprises an acetylated lysine side chain, is incubated with a sample containing HDAC activity (HeLa nuclear or other extract, purified enzyme, bead-bound immunocomplex, etc.). Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the CHEMILUM DE LYS Developer followed by Enhancer produces light. The reaction is luciferase-free

Applications

Chemiluminescence, HTS



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Storage

-80°C

Kit Components

Nuclear Extract from HeLa Cells (human cervical cancer cell line) (100 µl; In 0.1M potassium chloride, 20mM HEPES/sodium hydroxide, pH 7.9, 20% (v/v) glycerol, 0.2mM ethylenediaminetetraacetic acid, 0.5mM dithiothreitol, 0.5mM PMSF, prepared according to a modification of J.D. Dignam et al. (1983) and S.M. Abmayr et al. (1988)). Storage: -70°C, avoid freeze/thaw cycles
CHEMILUM DE LYS Substrate (125 µl; 10mM in DMSO) Storage: -70°C
CHEMILUM DE LYS Developer Concentrate (20x) (300 µl; 20x stock solution, dilute in developer buffer before use) Storage: -70°C
Trichostatin A (HDAC Inhibitor) (100 µl; 0.2mM in DMSO) Storage: -70°C
NAD (Sirtuin Substrate) (500 µl; 50mM β-Nicotinamide adenine dinucleotide (oxidized form) in 50mM TRIS-HCl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride) Storage: -70°C
Nicotinamide (Sirtuin Inhibitor) (500µl; 50mM Nicotinamide in 50mM TRIS-HCl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride) Storage: -70°C
HDAC Assay Buffer (20 ml; 50mM TRIS-HCl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride) Storage: -70°C
Developer Buffer (10ml; 50mM MES, pH 6.0, 40% DMSO) Storage: -70°C
CHEMILUM DE LYS Enhancer part A (2 x 1.2ml) Storage: -70°C
CHEMILUM DE LYS Enhancer part B (0.7ml) Storage: -70°C
1/2 volume white microplate Storage: Room temperature
