CREATIVE BIOMART®
Assay Kit

HDAC fluorometric activity assay Kit

Product Information

Cat.No. Kit-0414

Product Overview

Useful for assaying lysates, immunoprecipitates or inhibitor screening using the nuclear extract providedIncludes HeLa nuclear extract, a rich source of HDACs 1 & 2 for use as a positive control or as a source of HDAC activity for screeningCompatible with class I & IIb HDAC and sirtuins (with addition of NAD+)Includes enough reagent for 100-200 assays

Size

96 wells

Description

No radioactivity. No extractions. HTS friendly-mix and read on one 96-well plate. For class I and class II HDACs/sirtuins. Applications include cell-based assays and assay of immunoprecipitates.Histone deacetylase inhibitors have shown promise as anti-tumor agents and naturally this has stimulated interest in the screening of compounds for HDAC inhibition. The FLUOR DE LYS HDAC fluorometric activity assay kit is a sensitive and convenient alternative to protocols utilizing radiolabeled, acetylated histones or peptide/HPLC methods for the assay of histone deacetylases. It is based on the unique FLUOR DE LYS (Fluorimetric Histone deAcetylaseLysyl) substrate and developer combination and provides an assay that can be carried out in two simple mixing steps, all on the same 96-well plate. First, the FLUOR 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, mixing with the FLUOR DE LYS developer generates a fluorophore. The assay has been used successfully with preparations of all the known class I HDACs-HDAC1, HDAC2, HDAC3 and HDAC8 (see product data sheet) with class II HDACs 4-7, 9 and 10 and with the human Sir2 homolog, SIRT1 (see product data sheet). Work at Sciences has shown that the FLUOR DE LYS substrate is cell-permeable and is deacetylated in situ by cellular HDACs. The deacetylated substrate accumulates inside cells and may be quantified by addition of FLUOR DE LYS developer to a cell lysate.

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Applications

Fluorescence microscopy, HTS

Storage

-80°C

Kit Components

Nuclear Extract from HeLa Cells (human cerival 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 cyclesFLUOR DE LYS Substrate (50 µl; 50mM in DMSO)Storage: -70°CFLUOR DE LYS Developer Concentrate (20x) (300 µl; 20x stock solution, dilute in assay buffer before use)Storage: -70°CTrichostatin A (HDAC Inhibitor) (100 µl; 0.2mM in DMSO)Storage: -70°CFLUOR DE LYS Deacetylated Standard (30 µl; 10mM in DMSO)Storage: -70°CHDAC Assay Buffer (20 ml; 50mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chlroide)Storage: -70°C1/2 volume microplateStorage: Room temperature1/2 volume white microplateStorage: Room temperature

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