

Green HDAC fluorometric activity assay Kit

Product Information

Cat.No.

Kit-0420

Size

96 wells

Description

An improved FLUOR DE LYS HDAC assay with FLUOR DE LYS-Green, a new substrate offering higher sensitivity and an excitation and emission (485/530nm) that avoids quenching and fluorescent interference from compounds absorbing in the near UV and blue range. The FLUOR DE LYS-Green HDAC assay is a complete kit for measuring histone deacetylase (HDAC) activity in cell or nuclear extracts, immunoprecipitates or purified enzymes. It comes in a convenient 96-well format, with all reagents necessary for fluorescent HDAC or sirtuin activity measurements and calibration of the assay. The FLUOR DE LYS-Green HDAC assay is based on the FLUOR DE LYS-Green substrate and FLUOR DE LYS developer combination. The FLUOR DE LYS system (Fluorogenic 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 two steps. First, the FLUOR DE LYS-Green 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 FLUOR DE LYS developer produces a fluorophore.

Applications

Fluorescence microscopy, HTS

Storage

-80°C

Kit Components

Nuclear Extract from HeLa Cells (human cervical cancer cell line) (100µl in 0.1M potassium chloride,

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20mM HEPES/NaOH, 20% (v/v) glycerol, 0.2mM EDTA, 0.5mM DTT, 0.5mM PMSF)Storage: -70°C.FLUOR DE LYS-Green Substrate (50µl 50mM in DMSO)Storage: -70°C.FLUOR DE LYS Developer Concentrate (20X) (300µl 20x stock solution; dilute in assay buffer before use)Storage: -70°C.Trichostatin A (100µl 0.2mM in DMSO)Storage: -70°C.FLUOR DE LYS-Green Standard (30µl 1mM in DMSO)Storage: -70°C.Note: BML-KI605 is an improved replacement of BML-KI573.NAD⁺ (Sirtuin Substrate) (500µl; 50mM in 50mM TRIS, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride)Storage: -70°C.Nicotinamide (500µl; 50mM in 50mM TRIS, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride)Storage: -70°C.HDAC Assay Buffer (20ml; 50mM TRIS, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride)Storage: -70°C.96 Well PlatesStorage: Room temperatureWhite NBS MicroplateStorage: Room temperature