FLUOR DE LYS® HDAC fluorometric activity assay kit
BML-AK500
First-to-market by the leader in Epigenetics research tools

Product Number/Sizes
BML-AK500-0001 96 wells

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

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 Enzo Life 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.

Product Specifications
ALTERNATIVE NAME: Histone deacetylase fluorescent assay kit
APPLICATIONS: Fluorescent detection, HTS
Activity assay
USE/STABILITY: Store all components except the microtiter plate and instruction booklet at -70°C for the highest stability. The HeLa Nuclear Extract, Prod. No. BML-K1140, must be handled with particular care in order to retain maximum enzymatic activity. Defrost it quickly in a RT water bath or by rubbing between fingers, then immediately store on an ice bath. The remaining unused extract should be refrozen quickly, by placing at -70°C. If possible, snap freeze in liquid nitrogen or a dry ice/ethanol bath. To minimize the number of freeze/thaw cycles, aliquot the extract into separate tubes and store at -70°C. The FLUOR DE LYS® Substrate, Prod. No. BML-K1104, when diluted in Assay Buffer, may precipitate after freezing and thawing. It is best, therefore, to dilute only the amount needed to perform the assays of that day.

SHIPPING: Shipped on Dry Ice
LONG TERM STORAGE: -80°C
KIT/SET CONTAINS: Nuclear Extract from HeLa Cells (human cervical cancer cell line) (Prod. No. BML-K1140) (100 µl): In 0.1M potassium chloride, 20mM HEPES/sodium hydroxide, pH 7.9, 20% (v/v) glycerol, 0.2M 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
FLUOR DE LYS® Substrate (Prod. No. BML-KI104)  
(50 µl; 50mM in DMSO)  
Storage: -70°C

FLUOR DE LYS® Developer Concentrate (20x) (Prod. No. BML-KI105) (300 µl; 20x stock solution, dilute in assay buffer before use)  
Storage: -70°C

Trichostatin A (HDAC Inhibitor) (Prod. No. BML-GR309-9090) (100 µl; 0.2mM in DMSO)  
Storage: -70°C

FLUOR DE LYS® Deacetylated Standard (Prod. No. BML-KI142)  
(30 µl; 10mM in DMSO)  
Storage: -70°C

HDAC Assay Buffer (Prod. No. BML-K1143)  
(20 ml; 50mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride)  
Storage: -70°C

1/2 volume microplate (Prod. No. BML-K1101)  
Storage: Room temperature

1/2 volume white microplate (Prod. No. BML-K571)  
Storage: Room temperature
Figure 1: Reaction Scheme of the HDAC Fluorescent Activity Assay. Deacetylation of the substrate sensitizes it to the developer, which then generates a fluorophore (symbol). The fluorophore is excited with 360 nm light and the emitted light (460 nm) is detected on a fluorometric plate reader.

Product Literature References
Activated microglia decrease histone acetylation and Nr2-inducible anti-oxidant defence in astrocytes: restoring effects of inhibitors of HDACs, p38 MAPK and GSK3B F. Correa, et al. Neurobiol. Dis. 44 142 (2011)
Histone deacetylase is a target of valproic acid-mediated cellular differentiation N. Gurvich et al. Cancer Res. 64 1079 (2004)
Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1 K.J. Bitterman et al. J. Biol. Chem. 277 45099 (2002)
Cloning and characterization of a histone deacetylase, HDAC9 X. Zhou et al. PNAS 98 10572 (2001)

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