FLUOR DE LYS® HDAC fluorometric cellular activity assay kit

Monitor HDAC activity in cell culture using cell-permeable FLUOR DE LYS® substrate

The HDAC fluorometric cellular activity assay allows the determination of deacetylase activity within an undisturbed cellular environment and provides accurate activity information that is reflective of endogenous regulation. It also allows the study of the effects of upstream regulators on deacetylase activity and the detection of inhibitors or activators that act indirectly to affect deacetylase activity. The FLUOR DE LYS® HDAC substrate is cell permeable. It is deacetylated by cellular HDACs and thus suitable for high-throughput cell-based deacetylase assays. This kit provides the reagents and protocols needed for determining rates of intracellular deacetylase activity with cultured cells. Deacetylation can be quantitated by addition of developer to the media and lysed cells.

HDACs are typically found in multiprotein complexes and are tightly regulated by subcellular localization, phosphorylation, and likely by other mechanisms.

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Ordering Information

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BML-AK503-0001	96 wells

Manuals, SDS & CofA

View Online »

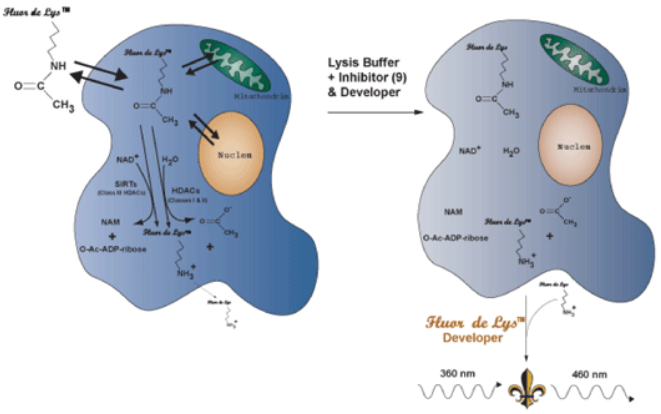


Figure: Method for Assaying Intracellular HDAC Activity with the Fluorogenic, Cell-permeable Substrate, FLUOR DE LYS® (Prod. No. BML-KI104). FLUOR DE LYS® is added to cell growth medium and enters the cells. Deacetylation by class I/II HDACs or sirtuins yields the deacetylated form of FLUOR DE LYS®, some of which may exit the cell. Lysis of the cells with detergent allows contact between the non-cell permeable Developer (Prod. No. BML-KI105) and both intra- and extracellular deacetylated substrate, thus producing a fluorescent signal (symbol). A strong class I/II HDAC inhibitor (e.g. trichostatin A) is added along with the lysis buffer containing detergent to insure that no deacetylation occurs after cell lysis.

Handling & Storage

Use/Stability	Store all components except the microplate at -80°C for the highest stability. The HeLa Nuclear Extract, Prod. No. BML-KI345, 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 -80°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 -80°C. The FLUOR DE LYS® Substrate, Prod. No. BML-KI104, 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.
Long Term Storage	-80°C
Shipping	Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	Cellular Histone deacetylase fluorescent assay kit
Application	Activity assay, Cell-based assays, Fluorescent detection, HTS

Contents

Nuclear Extracts from Hela Cells (human cervical cancer cell line) (Prod. No. BML-KI345)
(50 µl; 2 mg protein/ml in 0.1M KCl, 20mM HEPES/NaOH, pH 7.9, 20% (v/v) glycerol, 0.2mM EDTAacid, 0.5M DTT, 0.1mM PMSF; Prepared according to a midification of J.D. Dignam et al (1983) and S.M. Abmayr et al. (1988))
Storage: -80°C, avoid freeze/thaw cycles!
FLUOR DE LYS® Substrate (Prod. No. BML-KI104)
(50 µl; 50mM in DMSO)
Storage: -80°C
FLUOR DE LYS® Developer Concentrate (20x) (Prod. No. BML-KI105)
(300 µl; 20x stock solution, dilute in assay buffer before use)
Storage: -80°C
Trichostatin A (HDAC Inhibitor) (Prod. No. BML-GR-309-9090)
(100 µl; 0.2mM in DMSO)
Storage: -80°C
Nicotinamide (Sirtuin Inhibitor) (Prod. No. BML-KI283)
(500 µl; 50mM nicotinamide in 50mM TRIS/Cl, pH 8.0, 137mM NaCl, 2.7mM KCl, 1mM MgCl2)
Storage: -80°C
FLUOR DE LYS® Deacetylated Standard (Prod. No. BML-KI142)
(30 µl; 10mM in DMSO)
Storage: -80°C
HDAC Assay Buffer (Prod. No. BML-KI143)
(50mM TRIS/Cl, pH 8.0, 137mM NaCl, 2.7 mM KCl, 1mM MgCL2)
(20 ml)
Storage: -20°C
Cell Lysis Buffer (Prod. No. BML-KI346
(20 ml; 1.0% NP-40 in HDAC Assay Buffer)
Storage: -80°C
1/2 volume clear microplate (Prod. No. 80-2404)
Storage: Ambient
1/2 volume clear microplate, sterile (Prod. No. 80-2430)
Storage: Ambient

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