FLUOR DE LYS® HDAC fluorometric activity assay kit

First-to-market by the leader in Epigenetics research tools

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.

Citations: 65 View Online »

- 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

Ordering Information

Order Online »

BML-AK500-0001	96 wells

Manuals, SDS & CofA

View Online »

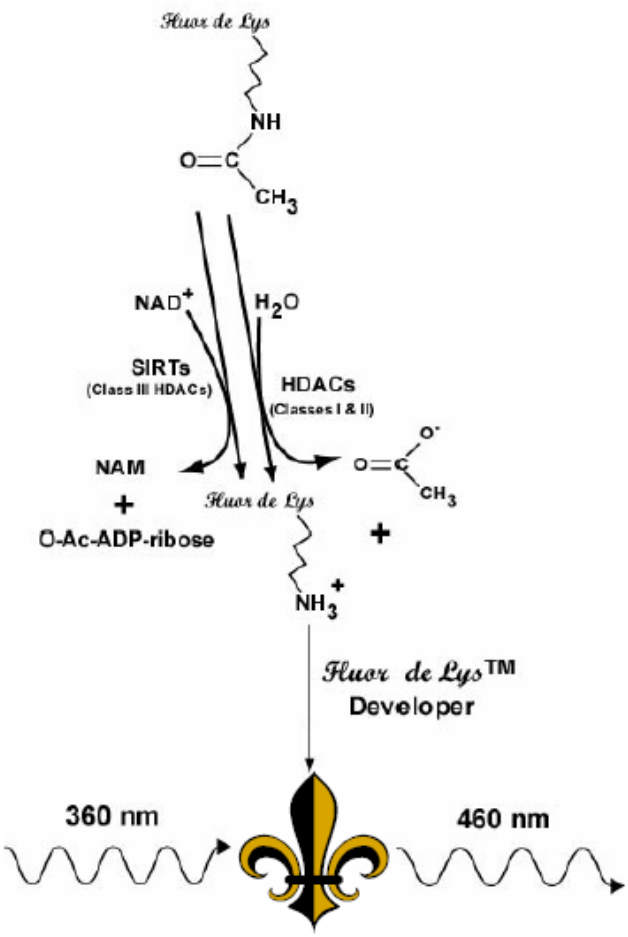


Figure 1: Reaction Scheme of the HDAC Fluorescent Activity Assay. Deacetylation of the substrate sensitizes it to the developer, which then generates a fluorophore (symbol). The fluorophore is excited with 360 nm light and the emitted light (460 nm) is detected on a fluorometric plate reader.

Handling & Storage

Use/Stability	Store all components, except the microtiter plate, at -80°C for the highest stability. The HeLa Nuclear Extract, BML-KI140, 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, 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	Histone deacetylase fluorescent assay kit
Application	Activity assay, Fluorescent detection, HTS

Contents

Nuclear Extract from HeLa Cells (human cervical cancer cell line) (Prod. No. BML-KI140-0100)
(100 µl; In 0.1M KCl, 20mM HEPES/NaOH, pH 7.9, 20% (v/v) glycerol, 0.2mM EDTA, 0.5mM DTT, 0.5Mm PMSF, prepared according to a modification 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-KI104-0050)
(50 µl; 50mM in DMSO)
Storage: -80°C
FLUOR DE LYS® Developer Concentrate (20x) (Prod. No. BML-KI105-0300) (300 µl; 20x stock solution, dilute in assay buffer before use)
Storage: -80°C
Trichostatin A (HDAC Inhibitor) (Prod. No. BML-GR309-9090)
(100 µl; 0.2mM in DMSO)
Storage: -80°C
FLUOR DE LYS® Deacetylated Standard (Prod. No. BML-KI142-0030)
(30 µl; 10mM in DMSO)
Storage: -80°C
HDAC Assay Buffer (Prod. No. BML-KI143-0020)
(20 ml; 50mM TRIS/Cl, pH 8.0, 137mM NaCl, 2.7mM KCl, 1mM MgCl2)
Storage: -20°C
1/2 volume microplate (Prod. No. BML-KI101)
Storage: Ambient
1/2 volume white microplate (Prod. No. BML-K571)
Storage: Ambient

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