CHEMILUM DE LYS® HDAC/SIRT Chemiluminescent drug discovery kit

All the Benefits of Chemiluminescent Detection Without Any of the Side Effects

The HDAC/SIRT Chemiluminescent Drug Discovery Kit is a complete assay system designed to measure histone deacetylase (HDAC) and sirtuin activity in cell or nuclear extracts, immunoprecipitates or purified enzymes. It comes in a convenient 96-well format, with all reagents necessary for chemilumnescent HDAC activity measurements and calibration of the assay. In addition, a HeLa nuclear extract, rich in HDAC activity, is included with the kit. The extract is useful as either a positive control or as the source of HDAC activity for inhibitor/drug screening. Also included are Trichostatin A and Nicotinamide, which may be used as model inhibitors for HDACs and sirtuins, respectively.

The HDAC Chemiluminescent Activity Assay is based on the unique CHEMILUM DE LYS® Substrate and Developer combination.

The CHEMILUM DE LYS® system (Chemiluminescent 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 three steps (Fig. 1). First, the CHEMILUM 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, treatment with the CHEMILUM DE LYS® Developer followed by Enhancer produces light. The reaction is luciferase-free

- High Specificity assay eliminates false positives or negatives
- Superior Signal-to-Noise ratio with no interference from cell extract detergents
- Delivers consistent results from a validated system

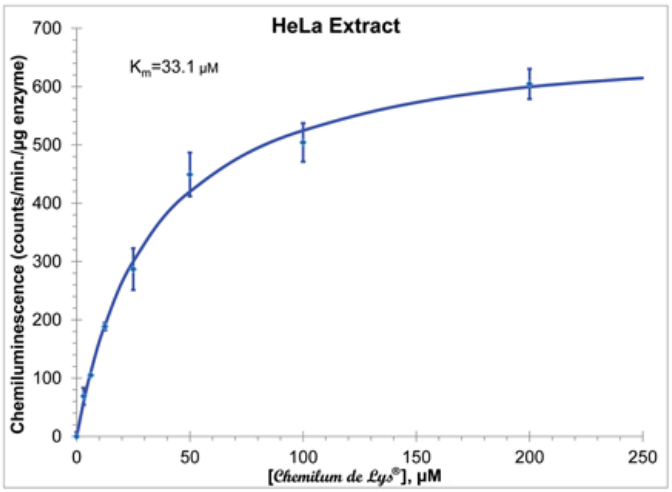
Ordering Information

Order Online »

BML-AK532-0001	96 wells

Manuals, SDS & CofA

View Online »

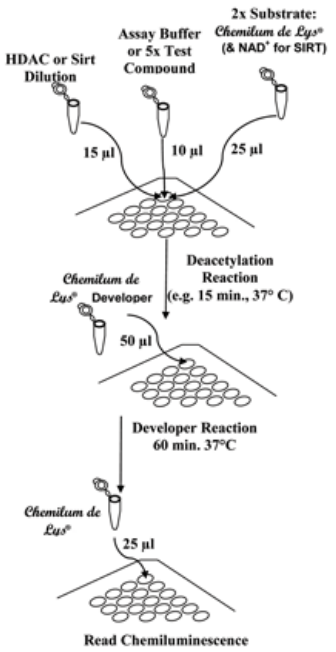


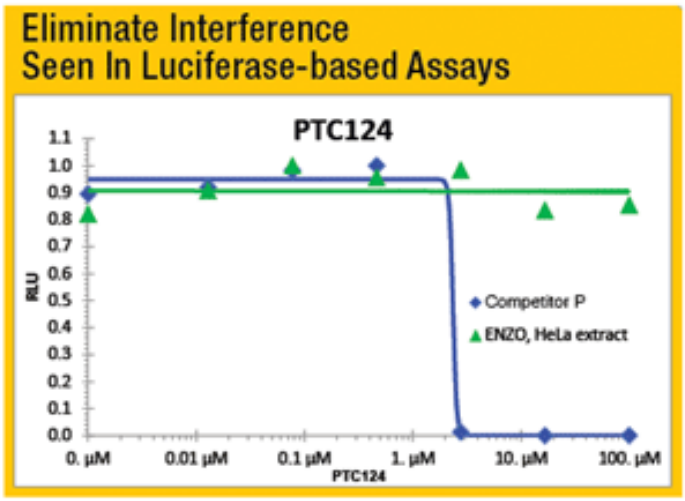
Kinetics of CHEMILUM DE LYS® Substrate Deacetylation by HeLa HDAC Activity. HeLa Nuclear extract (0.35 µl/7 µg per well) was incubated (37°C) with indicated concentrations of substrate. Reactions were stopped after 10 min. with CHEMILUM DE LYS® Developer, incubated for 120 minutes, then Enhancer was added and chemiluminescence measured (BMG Labtech, FluoStar Optima). Points are the mean of three determinations and error bars are standard errors of the means. Each determination is the average of at least five chemiluminescence readings taken in the first ten minutes. Kinetic parameters and the line derive from a non-linear least squares fit of the data to the Michaelis-Menten equation (Microsoft XL, Solver tool).

Eliminate False HDAC/SIRT Screening Hits with *Chemilum de Lys®*

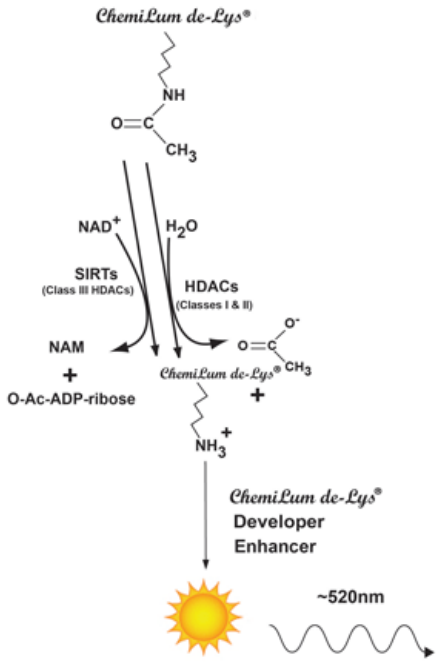
		Competitor HDAC Assays		
	Chemilum de Lys®	Luciferase-Based Chemiluminescent Assay	Colorimetric Assay	Fluorometric Assay
No Artifactual activation by resveratrol	✔	✔	X	X
Resistant to Protease Inhibitors	✔	X	✔	✔
Resistant to Kinase Inhibitors	✔	X	✔	✔

Performing the CHEMILUM DE LYS® HDAC Activity Assay. The procedure is done in three stages. First, the components of the deacetylation reaction (HeLa extract, buffer or test compound, substrate) are mixed. Following an incubation in which substrate deacetylation takes place, Developer is added and mixed. This stops the deacetylation and produces a product that will generate a chemiluminescent signal when enhancer is added. The signal can be read in 60 min, after the addition of Enhancer. The scheme depicts mixes for “Control” or “Test Sample” reactions. When performing the assay on 384 well plates, all volumes should be cut in half.





Performing the Chemilum de Lys HDAC Activity Assay. The procedure is done in three stages. First, the components of the deacetylation reaction (HeLa extract, buffer or test compound, substrate) are mixed. Following an incubation in which substrate deacetylation takes place, Developer is added and mixed. This stops the deacetylation and produces a product that will generate a chemiluminescent signal when enhancer is added. The signal can be read in 60 min, after the addition of Enhancer. The scheme depicts mixes for “Control” or “Test Sample” reactions. When performing the assay on 384 well plates, all volumes should be cut in half.



Handling & Storage

Use/Stability	Store all components except the microplate at -70°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 -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 CHEMILUM DE LYS® Substrate, 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, Chemiluminescence, HTS

Contents

Nuclear Extract from HeLa Cells (human cervical cancer cell line) (Prod. No. BML-KI140)
(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 cycles
***CHEMILUM DE LYS*® Substrate** (Prod. No. BML-KI598)
(125 µl; 10mM in DMSO)
Storage: -70°C
***CHEMILUM DE LYS*® Developer Concentrate** (20x) (Prod. No. BML-KI599)
(300 µl; 20x stock solution, dilute in developer buffer before use)
Storage: -70°C
Trichostatin A (HDAC Inhibitor) (Prod. No. BML-GR309-9090) (100 µl; 0.2mM in DMSO)
Storage: -70°C
NAD (Sirtuin Substrate) (Prod. No. BML-KI282)
(500 µl; 50mM β-Nicotinamide adenine dinucleotide (oxidized form) in 50mM TRIS-HCl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride)
Storage: -70°C
Nicotinamide (Sirtuin Inhibitor) (Prod. No. BML-KI283)
(500µl; 50mM Nicotinamide in 50mM TRIS-HCl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride)
Storage: -70°C
HDAC Assay Buffer (Prod. No. BML-KI143)
(20 ml; 50mM TRIS-HCl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chlroide)
Storage: -70°C
Developer Buffer (Prod. No. BML-KI600) (10ml; 50mM MES, pH 6.0, 40% DMSO)
Storage: -70°C
CHEMILUM DE LYS® Enhancer part A (Prod. No. BML-KI601)
(2 x 1.2ml)
Storage: -70°C
CHEMILUM DE LYS® Enhancer part B (Prod. No. BML-KI602) (0.7ml) Storage: -70°C
1/2 volume white microplate (Prod. No. ADI-80-2406)
Storage: Room temperature

Technical Info / Product Notes

Histones form the protein core of nucleosomes, the DNA/protein complexes that are the subunits of eukaryotic chromatin. The histones' N-terminal "tails" are subject to a variety of post-translational modifications, including phosphorylation, methylation, ubiquitination, ADP-ribosylation and acetylation. These modifications have been proposed to constitute a 'histone code' with profound regulatory functions in gene transcription. The best studied of these modifications, acetylation of the ε-amino groups of specific histone lysine residues, are catalyzed by histone acetyltransferases (HATs). Histone deacetylases (HDACs) are responsible for hydrolytic removal of these acetyl groups.

Histone hyperacetylation correlates with an open, decondensed chromatin structure and gene activation, while hypoacetylation correlates with chromatin condensation and transcriptional repression. Consistent with this, HATs have been shown to associate with several transcriptional activators and some transcriptional activators have been found to have intrinsic HAT activity. Conversely, HDACs are found to associate with transcriptional repression complexes such as NuRD or those including Sin3.

Thus far, eleven human HDACs have been identified, all trichostatin A-sensitive and all homologs of either RPD3 (Class I HDACs) or HDA1 (Class II HDACs), yeast histone deacetylases. Interestingly, Sir2, the yeast mother cell longevity factor, and its mouse homolog, mSir2α, have been shown to be trichostatin A-insensitive, NAD+-dependent histone deacetylases. Human, archaeal and eubacterial Sir2 homologs also display NAD^+-dependent histone deacetylase activity. These enzymes apparently function via a unique mechanism, which consumes NAD^+ and couples lysine deacetylation to formation of nicotinamide and O-acetyl-ADP-ribose. The Sir2 family (sirtuins) thus constitutes a third class of HDACs, but its members have not been included in the HDAC (Class I/Class II) numbering scheme.

Histone deacetylase inhibitors have shown promise as anti-tumor agents and naturally this has stimulated interest in the screening of compounds for HDAC inhibition. Unfortunately, the standard techniques for HDAC assay are cumbersome. Use of [^{3}H]acetyl-histone or [^{3}H]acetyl-histone peptides as substrates involves an acid/ethyl acetate extraction step prior to scintillation counting. Unlabeled, acetylated histone peptides have been used as substrates, but reactions then require resolution by HPLC. The original FLUOR DE LYS® HDAC assay addressed these problems by providing an assay that can be carried out in two simple mixing steps, all on the same 96-well plate (384-well plates may also be used, but are not included). The CHEMILUM DE LYS® assay has those same advantages, but also, due to chemiluminescent

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