Product Manual

HDAC/SIRT Chemiluminescent Drug Discovery Kit

Catalog #: BML-AK532

A *Chemilum de Lys*® Chemiluminescent Assay System

Enzo®

Product Manual

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

TABLE OF CONTENTS

Background 2

References 3

Reaction Scheme 4

Description 5

Materials Supplied 6

Other Materials Needed 7

Assay Procedure 8

Application Examples 14

Troubleshooting Guide 17

Related Products 18

Contact Information 20

BACKGROUND

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[1]. 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[2-4].

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[1-4]. Conversely, HDACs are found to associate with transcriptional repression complexes such as NuRD or those including Sin3[1-7].

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[8-16,20]. 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[17]. Human, archaeal and eubacterial Sir2 homologs also display NAD^+-dependent histone deacetylase activity[21]. These enzymes apparently function via a unique mechanism, which consumes NAD^+ and couples lysine deacetylation to formation of nicotinamide and O-acetyl-ADP-ribose[22-24]. 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[8,18,19,25]. Unlabeled, acetylated histone peptides have been used as substrates, but reactions then require resolution by HPLC17. 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

signal, avoids interference by fluorescence from compounds absorbing and/or emitting in the near UV and blue. In addition, the reaction is luciferase-free, thus avoiding compound interference with luciferase activity. The assay has been used successfully with class I, class IIb, class III (sirtuins) and class IV recombinant HDACs.

Recent studies[26] have indicated that some compounds shown to activate sirtuins with the fluorescent substrate do not activate deacetylation of the native peptide. The *Chemilum de Lys*® substrate appears to more closely mimic the natural substrate and does not show this substrate specific activation by resveratrol and other drugs that affect other HDAC or Sirtuin assays.

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PLEASE READ ENTIRE BOOKLET BEFORE PROCEEDING WITH THE ASSAY. CAREFULLY NOTE THE HANDLING AND STORAGE CONDITIONS OF EACH KIT COMPONENT. PLEASE CONTACT ENZO LIFE SCIENCES TECHNICAL SERVICES FOR ASSISTANCE IF NECESSARY.

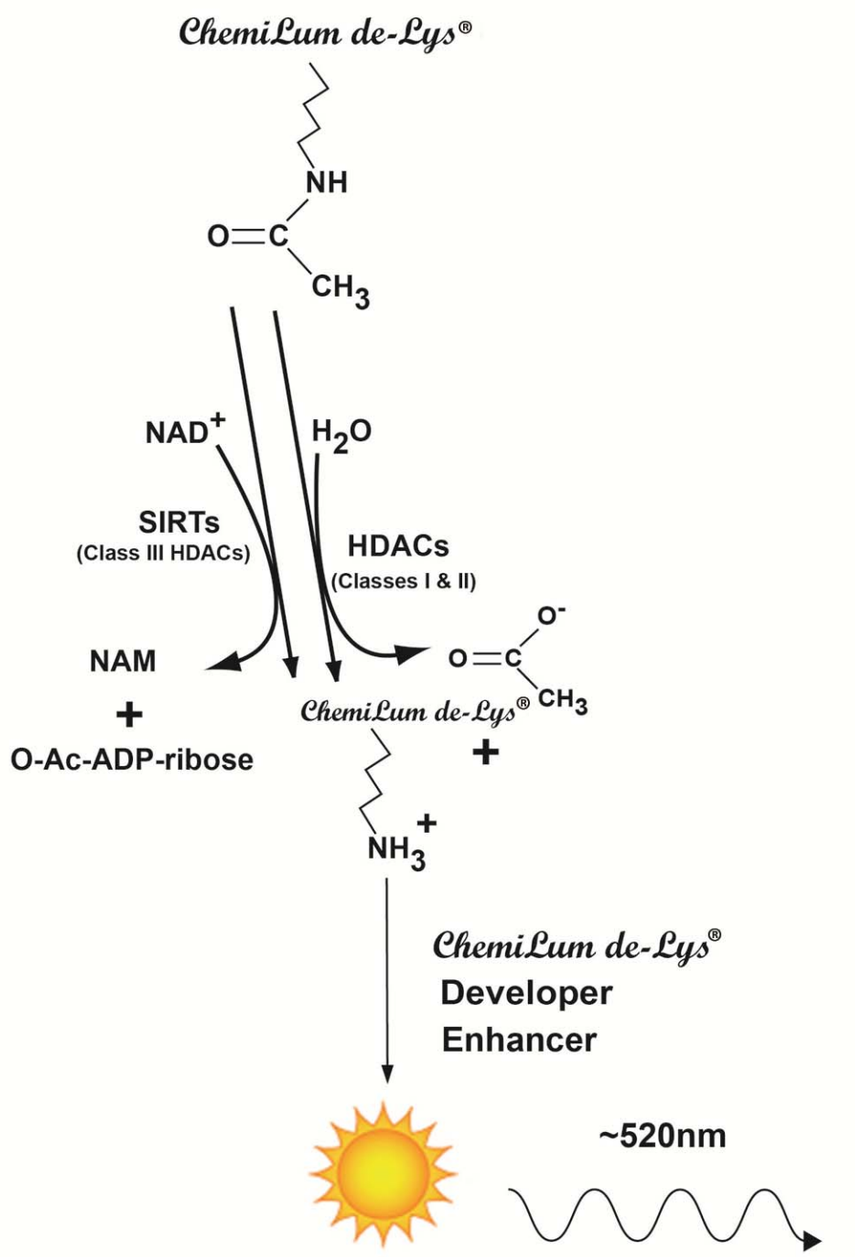

Figure 1. Reaction Scheme of the HDAC/SIRT Chemiluminescent Activity Assay*. Deacetylation of the substrate sensitizes it to the developer, which then generates a chemiluminescent compound (symbol). The chemiluminescent compound then emits light around 520 nm and is detected on a luminescent plate reader.

*Patent Pending.

DESCRIPTION

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 fluorescent 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.

MATERIALS SUPPLIED

1. **BML-KI140-0100 Nuclear Extract from HeLa Cells:**

   FORM: 0.1 M KCl, 20mM HEPES/NaOH, pH 7.9, 20% (v/v) glycerol, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF; Prepared according to a modification of J.D. Dignam et al. Nuc. Acids Res. 1983 11 1475 and S.M. Abmayr et al. Genes Dev. 1988 2 542.

   STORAGE: -70°C; AVOID FREEZE/THAW CYCLES!
   QUANTITY: 1 x 100µl vial

2. **BML-KI598-0125** *Chemilum de Lys*® **Substrate:**

   FORM: 10mM in DMSO
   STORAGE: -70°C
   QUANTITY: 1 x 125µl vial

3. **BML-KI599-0300** *Chemilum de Lys*® **Developer Concentrate (5x):**

   FORM: 5x Stock Solution; Dilute in Developer Buffer before use.

   STORAGE: -70°C

   QUANTITY: 4 x 300µl vials

4. **BML-GR309-9090 Trichostatin A (HDAC Inhibitor):**

   FORM: 0.2mM in DMSO

   STORAGE: -70°C

   QUANTITY: 1 x 100µl vial

5. **BML-KI282-0500 NAD+ (Sirtuin Substrate):**

   FORM: 50mM β-Nicotinamide adenine dinucleotide (oxidized form) in 50mM Tris/HCl, pH 8.0, 137mM NaCl, 2.7mM KCl, 1mM MgCl2.

   STORAGE: -70°C

   QUANTITY: 1 x 500µl vial

6. **BML-KI283-0500 Nicotinamide (Sirtuin Inhibitor):**

   FORM: 50mM Nicotinamide in 50mM Tris/HCl, pH 8.0, 137mM NaCl, 2.7mM KCl, 1mM MgCl2.

   STORAGE: -70°C

   QUANTITY: 1 x 500µl vial

7. **BML-KI286-0020 SIRT ASSAY BUFFER:**
   (50mM Tris/HCl, pH 8.0, 137mM NaCl, 2.7mM KCl, 1mM MgCl2, 0.1% BSA)
   STORAGE: -70°C
   QUANTITY: 1 x 20ml bottle
8. **BML-KI600-0010 DEVELOPER BUFFER**
   (50mM MES, pH 6, 40% DMSO)
   STORAGE: -70°C
   QUANTITY: 1 x 10ml bottle
9. **BML-KI601-0012** *Chemilum de Lys*® **ENHANCER part A**
   STORAGE: -70°C
   QUANTITY: 2 tubes, 1.2ml each
10. **BML-KI602-0007** *Chemilum de Lys*® **ENHANCER part B**
    STORAGE: -70°C
    QUANTITY: 1 x 0.7ml vial
11. **ADI-80-2406 WHITE ½ VOLUME NBS MICROPLATE**
    STORAGE: Room temperature
    QUANTITY: 1x 96 well plate

***PLEASE NOTE THAT ALL COMPONENTS, WITH THE EXCEPTION OF THE HELA CELL EXTRACT (KI140), CAN BE STORED AT EITHER -70°C OR -20°C. THE HELA EXTRACT MUST BE STORED AT -70°C.**

OTHER MATERIALS NEEDED

1. Microplate reading luminometer or fluorometer capable of reading luminescence.
2. Pipetman or multi-channel pipetman capable of pipetting 2-100 µl accurately.
3. Ice bucket to keep reagents cold until use.
4. Microplate warmer or other temperature control device (optional).

ASSAY PROCEDURE

***Notes on Storage**: 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.*

1. The assay is performed in three stages. Substrate deacetylation occurs first, in a total volume of 50µl. The second stage, which is initiated by the addition of 50µl of Developer, stops HDAC activity and produces the chemiluminescent compound. The final stage is the addition of 25µl of Enhancer for visualization of the chemiluminescent product. See "Preparing Reagents For Assay" and Table 1. A white ½ volume nonbinding surface 96-well microplate is provided with the kit.
2. Should it be necessary, there is some leeway for change in the reaction volumes. The wells of the microplates provided can accommodate 150µl. If planning a change in the volume of the Developer, it should be noted that it is important to try and maintain the ratios of the components as closely to those outlined in this manual and to maintain the concentration of Trichostatin (GR309) in the final mix at 1µM.
3. Assays should include a "time-zero" (sample for which substrate is added after addition of the Developer with TSA or other inhibitor) and/or a negative control (no enzyme). The measure of a sample's deacetylation is the difference between its luminescence and the luminescence of a time-zero or no enzyme sample with the same substrate concentration.
4. When 0.3µl/well of the HeLa Nuclear Extract is used as the source of HDAC activity reaction progress curves for a broad range of substrate concentrations (6-200µM Chemilum de Lys® Substrate) remain linear for at least 15 min. at 37°C and longer at lower temperatures (e.g., 25°C). This will not necessarily be true if a different source of HDAC activity, a different amount of extract, or a different assay temperature is used. A time course experiment will aid in the selection of

an incubation time, which yields a signal that is both sufficiently large and proportional to enzyme rate. The amount of HeLa extract per well may also be varied.

The HeLa nuclear extract is not a significant source of SIRT activity. Purified sirtuins should be used for sirtuin assays.

5. The apparent K_m of the HDAC activity in the HeLa Nuclear Extract (BML-KI140) for the *Chemilum de Lys*® Substrate is ~33µM (Fig. 3). Use of a substrate concentration at or below the K_m will help avoid substrate competition effects, which could mask the effectiveness of a potential inhibitor. If a different source of HDAC activity is to be used, a rate vs. concentration experiment should be performed before selecting a substrate concentration for the screening experiments.
6. When screening cell lysates for HDAC or Sirt activity, a substrate concentration higher than the K_m of the enzyme is recommended. In this type of assay, we recommend a final concentration of the substrate to be 200µM. If screening impure preparations for Sirtuins, Trichostatin A must be added to 1µM to inhibit endogenous HDAC activity.
7. When cell lysates are used as a source for HDAC, the use of 0.1% CHAPS is recommended to solubilize the enzyme from the cell.
8. It is conceivable that some compounds being screened for inhibition of HDACs may interfere with the action of the *Chemilum de Lys*® Developer. Assuming the concentration of the test compound(s) does not exceed 0.15mM, any interference will in all likelihood result in development slowing down rather than stopping. It is recommended to run a control by adding inhibitor after the HDAC reaction, during the development stage to control for any possible affects not related to HDAC activity.
9. Note that sirtuins (Sir2 and Sir2-like NAD^+-dependent HDACs) are insensitive to Trichostatin A (TSA). Therefore, Developer prepared as described below with added TSA will not completely block further deacetylation by these enzymes. If the kit is to be used to assay a sirtuin, 2 mM nicotinamide (BML-KI283, a Sirtuin Inhibitor) in 1x Developer should be used in place of TSA.

Preparing Reagents for Assay

1. Defrost all kit components and keep these and all dilutions described below, on ice until use. All undiluted kit components are stable for several hours on ice. *Chemilum de Lys®* Enhancer part A may require warming to room temperature and vortexing to solubilize.
2. Prepare a sufficient amount of HeLa Nuclear Extract (BML-KI140) or other HDAC source diluted in Assay Buffer to provide for the assays to be performed (# of wells x 15µl). Dilutions of HeLa Extract ranging from 150-fold to 50-fold will provide 0.1 to 0.3µl of the undiluted Extract per 15µl (Table 1).
3. Prepare dilution(s) of Trichostatin A and/or Test Inhibitors in Assay Buffer. Since 10µl will be used per well (figure 2), and since the final volume of the HDAC reaction is 50µl, these inhibitor dilutions will be 5x their final concentration.
4. Prepare dilution(s) of the *Chemilum de Lys®* Substrate (10mM) in Assay Buffer that will be 2x the desired final concentration(s), if needed, an intermediate dilution of substrate can be made in DMSO. For inhibitor screening, substrate concentrations at or below the K_m are recommended. Sirtuin assays should also contain NAD^+ (BML-KI282; usually 4mM final concentration) in the 2x substrate mix. Twenty-five µl will be used per well (Table 1). Substrate concentrations above 500µM in ice cold Assay Buffer may be difficult to solubilize. Rapid mixing and dilution into room temperature buffer will help prevent precipitation at high substrate concentration. **NOTE**: **Freezing/thawing of *Chemilum de Lys®* Substrate solutions in Assay Buffer may cause precipitation of the Substrate. Dilute only amount necessary for one day's experiment.** If preparing enough for an entire plate, 2.5ml of substrate is needed. To make a 200µM substrate (100µM in final reaction), add 50µl substrate to 2.45ml assay buffer.
5. Shortly before use (<30 min.), prepare sufficient *Chemilum de Lys®* Developer for the assays to be performed (50µl per well). First, dilute the *Chemilum de Lys®* Developer Concentrate 5-fold (e.g., 300µl plus 1200µl Developer Buffer) in cold Developer Buffer to create a 1X Developer Solution. Second, dilute the 0.2mM Trichostatin A 100-fold in the 1X Developer Solution just prepared (e.g., 10µl in 1ml; final Trichostatin A concentration in the 1x Developer = 2µM; final concentration after addition to HDAC/Substrate reaction = 1µM). Addition of Trichostatin A to the Developer insures that HDAC activity

stops when the Developer is added. Keep Developer on ice until use. If preparing enough for an entire plate, 5 ml is required. To make 5 ml Developer with Trichostatin A; mix 4.95 ml Developer Solution 1X (as in step 5 above) with 50µl Trichostatin A.

Alternatively, Sirtuin enzymes are insensitive to Trichostatin A and should be stopped with Nicotinamide (BML-KI283). In place of Trichostatin A, the Nicotinamide should be diluted 25 fold in the 1x Developer just prepared (e.g., 40µl in 1ml; final concentration after addition to HDAC/Substrate reaction = 1mM). If preparing enough for an entire plate, 5ml is required. To make 5 ml Developer with Nicotinamde; mix 4.8 ml Developer Solution 1X (as in step 5 above) with 200µl Nicotinamide.

6. Shortly before use (<30 min.), prepare sufficient *Chemilum de Lys*® Enhancer mix for the assays to be performed (25µl per well, plus some overage). Mix 3 parts Enhancer part A with 1 part Enhancer part B and mix thoroughly (for 1ml: add 0.75ml Enhancer part A with 0.25ml enhancer part B). Keep enhancer protected from light at room temperature. Enhancer part A may have to be warmed at 37°-50°C to clear the solution prior to mixing with Enhancer part B. If preparing enough for an entire plate, 2.5ml is required. To make 2.5ml Enhancer mix, add 1.875ml Enhancer part A with 0.625ml Enhancer part B and mix well.

Performing the Assay:

1. Add Assay buffer, diluted Trichostatin A or test inhibitor to appropriate wells of the microplate. (Table 1 lists examples of various assay types and the additions required for each. Fig. 2 depicts a schematic of the procedure.)
2. Add diluted HeLa extract or other HDAC sample to all wells except those that are to be “No-Enzyme Controls.”
3. Allow diluted *Chemilum de Lys*® Substrate and the samples in the microplate to equilibrate to assay temperature (e.g., 25° or 37°C).
4. Initiate HDAC reactions by adding diluted substrate (25µl) to each well and mixing thoroughly.
5. Allow HDAC reactions to proceed for desired length of time and then stop them by addition of *Chemilum de Lys*® Developer (50µl). Incubate plate at 37° for 1 hour.
6. Add 25µl of Enhancer to each well and mix just prior to reading chemiluminescence.

7. Immediately read samples in a microplate-reading luminometer capable of detection of emitted light in the range 500-550nm (a filter is not needed). The length of each reading should be set so each well can be read 5 times in a ten minute period (1 second for a 96 well plate or 0.2 seconds for a 384 well plate)

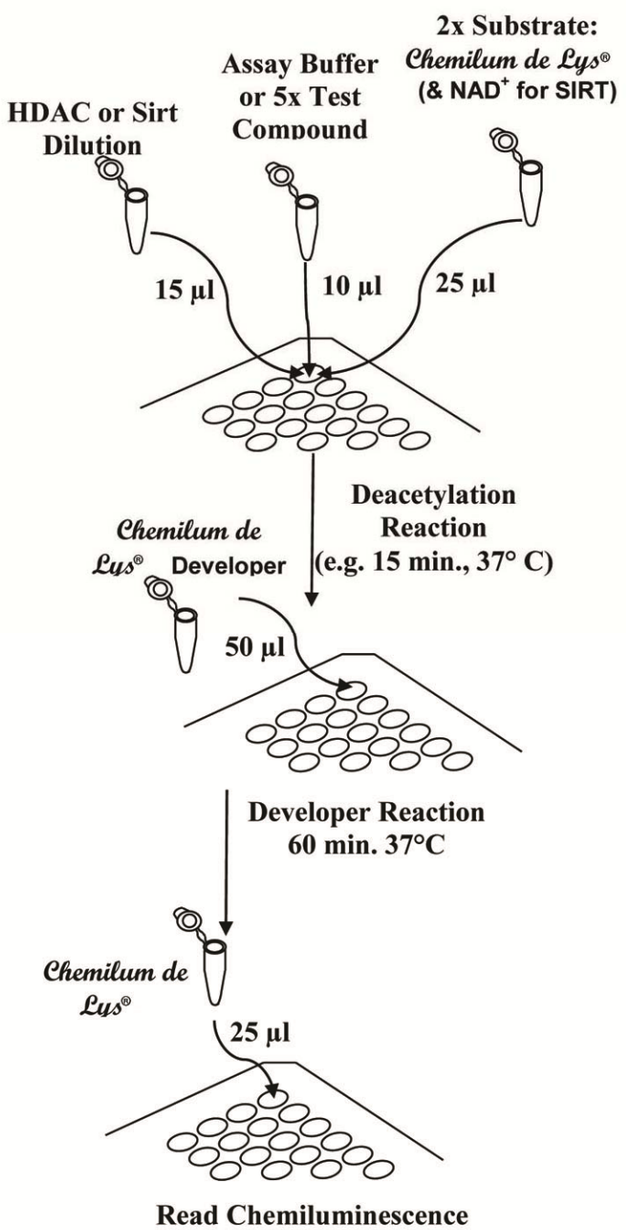

Figure 2. Performing the *Chemilum de Lys*® HDAC Activity Assay. The procedure is done in three stages. First, the components of the deacetylation reaction (HeLa extract, buffer or test compound, substrate) are mixed. Following an incubation in which substrate deacetylation takes place, Developer is 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; see Table 1 and text for other sample types and more details. When performing the assay on 384 well plates, all volumes should be cut in half.

TABLE 1. ASSAY MIXTURE EXAMPLES[6]

Sample	Assay Buffer	HeLa Extract (Dilution)	Inhibitor (5X)	*Chemilum de Lys*® Substrate (2X)
Blank (No Enzyme)	25 µl	0	0	25 µl
Control	10 µl	15 µl	0	25 µl
Trichostatin A	0	15 µl	10 µl[§]	25 µl
Test Sample	0	15 µl	10 µl[Ж]	25 µl

[6]HDAC reaction mixtures, prior to addition of *Chemilum de Lys*® Developer.

[§]Refers to dilution in Assay Buffer, which will be 5x the final concentration. Examples: 1) As a measure of non-HDAC background, 5 µM would yield final 1 µM concentration and essentially complete HDAC inhibition; 2) As a model inhibitor "hit", 2.5 nM would yield final 0.5 nM and ~50% inhibition at 20 µM substrate.

[Ж]Refers to potential inhibitor in Assay Buffer, which will be 5x its final concentration.

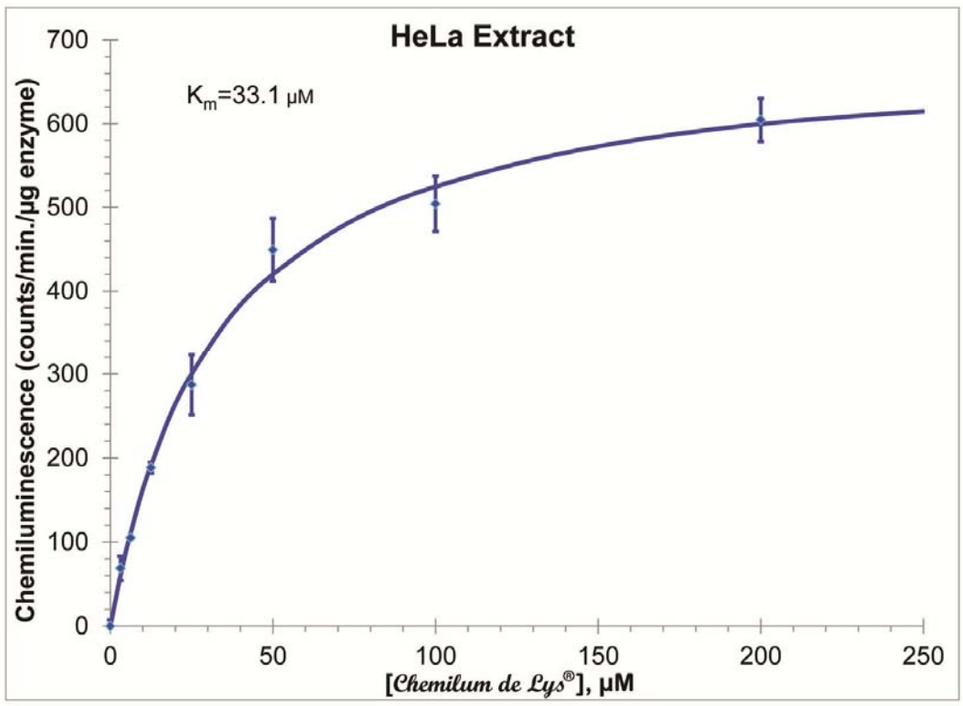

Figure 3. **Kinetics of *Chemilum de Lys*® Substrate Deacetylation by HeLa HDAC Activity**. HeLa Nuclear extract (0.35µl/7 µg per well) was incubated (37°C) with indicated concentrations of substrate. Reactions were stopped after 10 min. with *Chemilum de Lys*® Developer, incubated for 120 minutes, then 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)

Quenching or other interference from the test compounds.

1. The *Chemilum de Lys*® substrate avoids most instances of interference by compound fluorescence or quenching, because it is a chemiluminescent assay that emits at about 520 nM. Test compounds might absorb some of the emitted light.
2. If a compound is suspected of interfering with the assay, either by absorbing the emitted light, or by slowing the rate of the Developer, perform the reaction by adding the compound of interest (diluted in Developer buffer) after the deacetylation reaction, but before the Developer reaction. The ratio of activity when the test compound is with the deacetylase reaction versus when the test compound is only with the Developer reaction can show if the compound specifically affects HDAC.

APPLICATION EXAMPLES

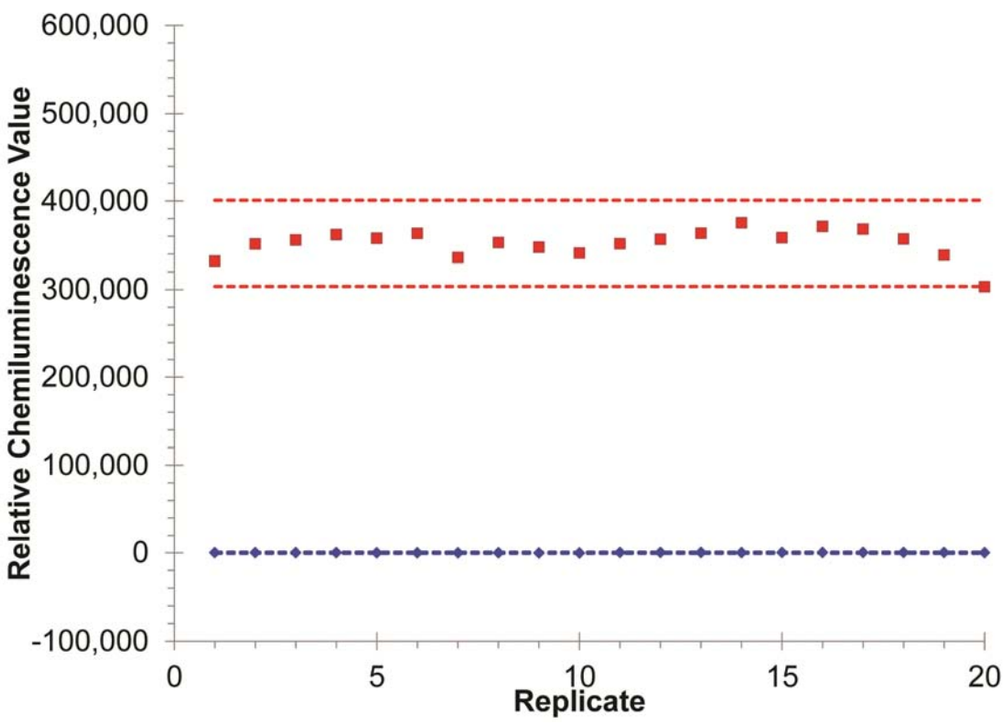

Fig. 4 Z-factor analysis. HeLa nuclear extract (4µg) (red squares) or buffer (blue diamonds) was incubated for 120 minutes at 24°C with 25µM *Chemilum de Lys*®. Reactions were stopped with *Chemilum de Lys*®□□Developer/2 mM Trichostatin A (1µM), Enhancer was added and chemiluminescence was read. The Z' factor for this assay was 0.86, (Z-factor = 1-((3SD^{positive} + 3SD^{negative})/(mean^{positive} –mean^{negative})). Dashed lines indicate the 3*Standard deviation range.

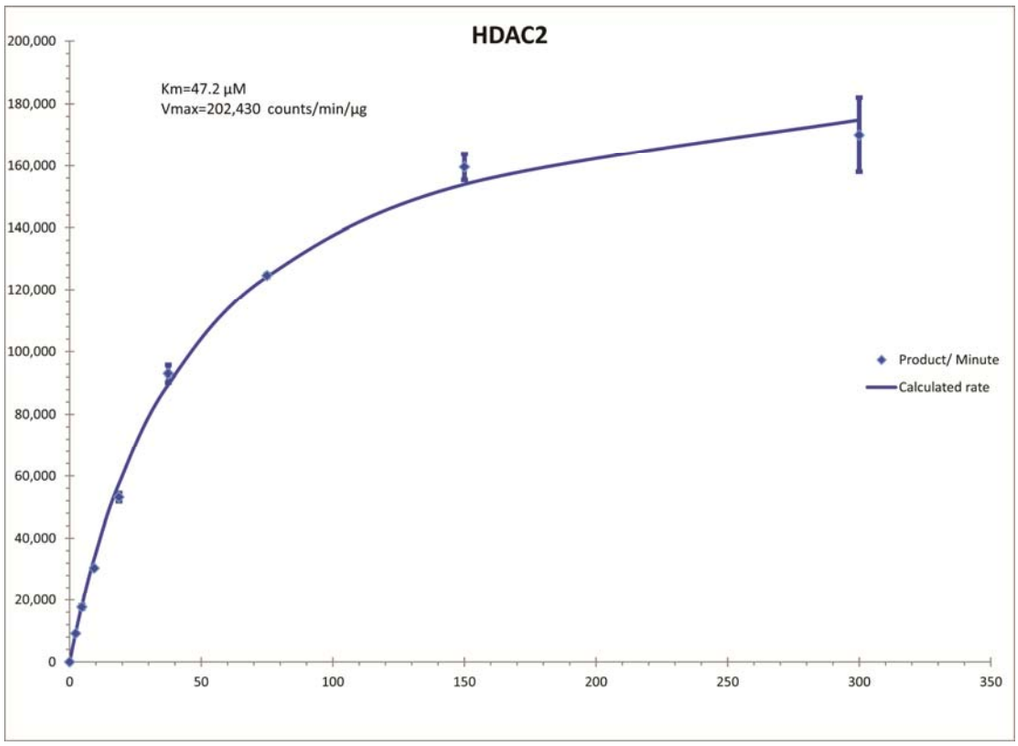

Fig. 5 HDAC2 kinetics. Initial rates using purified HDAC2 (10 ng) were determined with 10 minute incubations (37°C) in the presence of the indicated concentrations of *Chemilum de Lys*® substrate. Reactions were stopped with *Chemilum de Lys*® developer/1µM Trichostatin A. *Chemilum de Lys*® Enhancer was added, and chemiluminescence determined. Each point represents the mean of three determinations and the error bars are standard deviations. The line is a non-linear least-squares fit to the Michaelis-Menten equation (Microsoft Excel Solver tool). The K_m was determined to be 47.2µM.

Table 2 Observed K_m Values of *Chemilum de Lys*® with various enzymes*.

	K_m
HeLa extract	**33.1 µM**
HDAC1	**40.8 µM**
HDAC2	**47.2 µM**
HDAC3/NCOR1	**63.0 µM**
HDAC6	**40.0 µM**
HDAC8	**190.0 µM**
HDAC10	**77.0 µM**
HDAC11	**115.0 µM**
SIRT1	**67.4 µM**
SIRT2	**25.6 µM**
SIRT3	**109.4 µM**

***Reactions were performed as described in figure 2. Reactions with Sirtuins had 2 mM NAD^+ and the reactions were stopped with *Chemilum de Lys*® Developer/ 2mM nicotinamide.

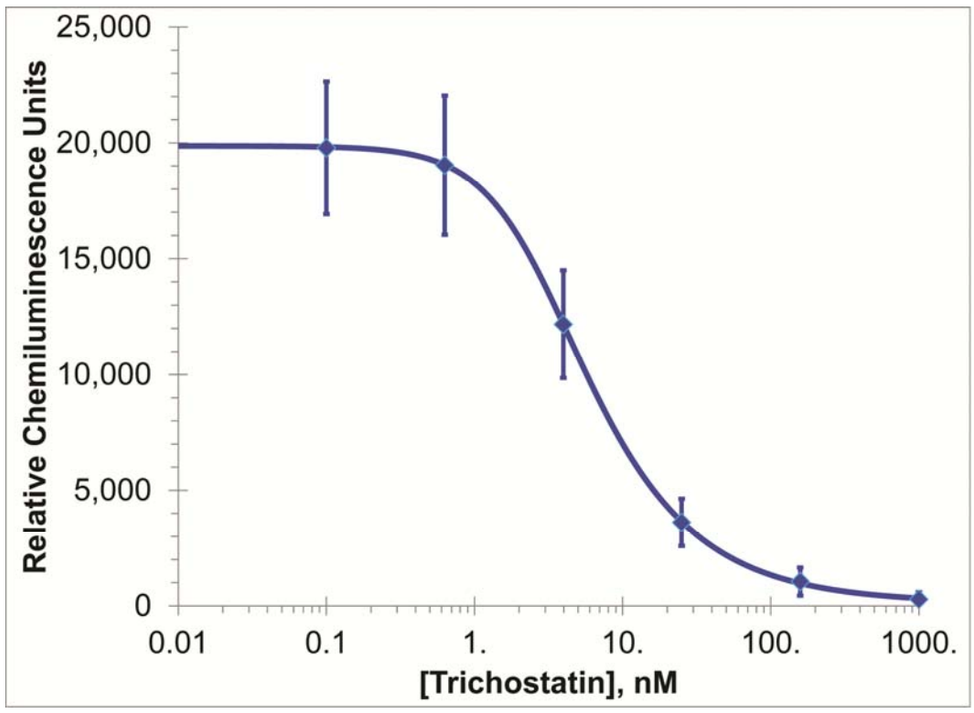

Fig. 6 Trichostatin A inhibition of HDAC. HeLa nuclear extract (4 µg) was used as a source of HDAC in reactions with 25 µM *Chemilum de Lys*® substrate for 20 minutes at 37°C with the indicated concentration of Trichostatin A. Reactions were stopped by the addition of *Chemilum de Lys*® Developer/1 µM Trichostatin A. *Chemilum de Lys*® Enhancer was added, and chemiluminescence determined. The IC50 for Trichostatin A was determined to be 5.7 nM.

NOTE: THE APPLICATION EXAMPLES DESCRIBED HEREIN ARE INTENDED ONLY AS GUIDELINES. THE OPTIMAL CONCENTRATIONS OF SUBSTRATES AND INHIBITORS, ASSAY VOLUMES, BUFFER COMPOSITION, AND OTHER EXPERIMENTAL CONDITIONS MUST BE DETERMINED BY THE INDIVIDUAL USER. NO WARRANTY OR GUARANTEE OF PARTICULAR RESULTS, THROUGH THE USE OF THESE PROCEDURES, IS MADE OR IMPLIED.

TROUBLESHOOTING GUIDE

Problem	Potential Cause	Suggestion
Little or no signal produced	Plates were allowed to sit too long after adding Enhancer.	Read the plates within 10 minutes of adding Enhancer mix.
	Enzyme has lost activity.	Store HeLa Nuclear extract at -70°C. Try to minimize freeze-thaw cycles. These recommendations are also important for other sources of enzyme.
	Not enough enzyme was added.	Titrate any new enzyme source to verify that the reaction occurs and the reaction is linear.
The reaction is not linear with enzyme concentration or time	Too much enzyme was added.	Titrate any new enzyme source to verify that the reaction occurs and the reaction is linear.
Chemilum de Lys® Enhancer mix does not go into solution	Not given enough time after thawing.	The *Chemilum de Lys*® Enhancer part A may be warmed to 50°C to clear the solution prior to adding part B.
Plate reader cannot read entire plate in the time expected	Plate reader adds extra steps.	If plate reader has a delay setting or shake before reading, set these to 0.
Chemilum de Lys® Substrate does not go into solution	Attempted to make a solution above 500 µM.	Keep the concentration of the stock below 500 µM. Pre-warm the dilution buffer to room temperature or 37°C if there is a problem.

ALSO AVAILABLE

PRODUCT	CATALOG #
SIRT1 Fluorometric Activity Assay	BML-AK555
SIRT2 Fluorometric Activity Assay	BML-AK556
SIRT3 Fluorometric Activity Assay Kit	BML-AK557
HDAC Fluorometric Activity Assay	BML-AK500
HDAC Colorimetric Assay Kit	BML-AK501
HDAC Green Fluorometric Assay Kit	BML-AK530
Fluor de Lys® Substrate	BML-KI104
Fluor de Lys® Developer	BML-KI105
Fluor de Lys®-SIRT1 Substrate	BML-KI177
Fluor de Lys®-SIRT2 Substrate	BML-KI179
Fluor de Lys®-H4-AcK16 Substrate	BML-KI174
Fluor de Lys®-HDAC8 Substrate	BML-KI178
Fluor de Lys® Developer II	BML-KI176
HeLa Nuclear Extract	BML-KI140
HDAC1 (recombinant, human)	BML-SE456
HDAC2 (full-length, recomb., human)	BML-SE533
HDAC2 (aa1-488, recomb., human)	BML-SE500
HDAC3 (recombinant, human)	BML-SE507
HDAC3/NCOR Complex (recomb., human)	BML-SE515
HDAC6 (recombinant, human)	BML-SE508
HDAC8 (recombinant, human)	BML-SE145
HDAC10 (recombinant, human)	BML-SE559
HDAC11 (recombinant, human)	BML-SE560
SIRT1 (recombinant, human)	BML-SE239
SIRT2 (recombinant, human)	BML-SE251
SIRT3 (recombinant, human)	BML-SE270
SIRT5 (recombinant, human)	BML-SE555
Resveratrol (SIRT1 Activator)	BML-FR104
Piceatannol (SIRT1 Activator)	BML-GR323
Suramin sodium (Sirtuin Inhibitor)	BML-G430
Trichostatin A (Class I/II HDAC Inhibitor)	BML-GR309
Anti-HDAC1 (polyclonal Ab)	BML-SA401
Anti-HDAC2 (polyclonal Ab)	BML-SA402
Anti-HDAC3 (polyclonal Ab)	BML-SA403
Anti-HDAC4 (polyclonal Ab)	BML-SA404

NOTES

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