Fluor de Lys®-Green HDAC Assay Kit

A Fluorometric Assay/Drug Discovery Kit

Instruction Manual
BML-AK530

For research use only

Fluor de Lys®-Green HDAC Assay - *BML-AK530*

✦ *Fluorometric Assay/Drug Discovery Kit** ✦

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, recently 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 HPLC[17]. 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. The *Fluor de Lys®-Green* assay has those same advantages, but also, due to its higher wavelength excitation and emission, avoids interference by quenching or fluorescence from compounds absorbing and/or emitting in the near UV and blue. The assay has been used successfully with class I, class IIb class III (sirtuins) and class IV recombinant HDACs (see Figs. 7 & 8).

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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.

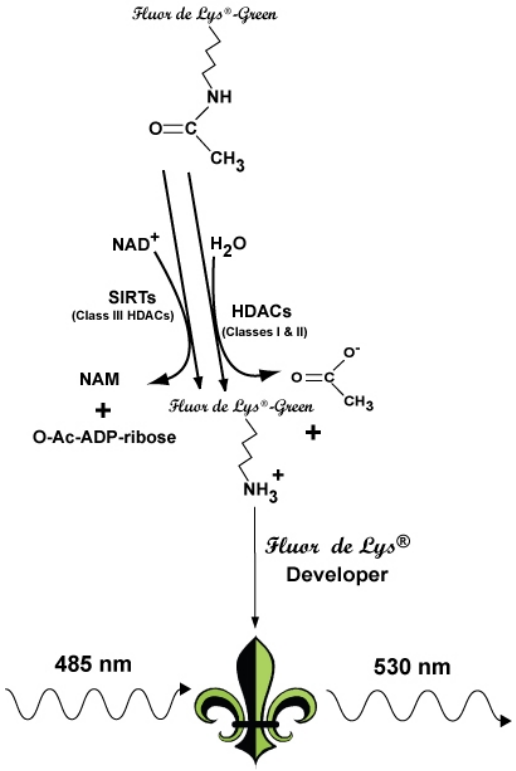

Figure 1. Reaction Scheme of the *Fluor de Lys®-Green* HDAC Activity Assay*. Deacetylation of the substrate sensitizes it to the developer, which then generates a fluorophore (symbol). The fluorophor is excited with 485 nm light (470-500 nm) and the emitted light (~530 nm) is detected on a fluorometric plate reader.

DESCRIPTION

The *Fluor de Lys®-Green HDAC Assay* is a complete kit for measuring histone deacetylase (HDAC) 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 or sirtuin activity measurements and calibration of the assay. In addition, a HeLa nuclear extract, rich in class I 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 the sirtuin co-substrate, NAD^+, the sirtuin inhibitor nicotinamide and the potent class I/II/IV HDAC inhibitor, Trichostatin A.

The *Fluor de Lys®-Green HDAC Assay* is based on the *Fluor de Lys®-Green* Substrate and *Fluor de Lys®* Developer combination. The *Fluor de Lys®* system (Fluorogenic 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 two steps (Figs. 1 & 2). First, the *Fluor de Lys®-Green* 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 *Fluor de Lys®* Developer produces a fluorophore.

COMPONENTS OF BML-AK530

BML-KI140-0100 Nuclear Extract from HeLa Cells (human cervical cancer cell line)
FORM: In 0.1 M KCl, 20 mM 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: 100 µl

BML-KI572-0050 *Fluor de Lys®-Green* Substrate
FORM: 50 mM in DMSO (dimethylsulfoxide)
STORAGE: -70°C
QUANTITY: 50 µl

BML-KI105-0300 *Fluor de Lys®* Developer Concentrate (20x)
FORM: 20x Stock Solution; Dilute in Assay Buffer before use.
STORAGE: -70°C
QUANTITY: 300 µl

BML-GR309-9090 Trichostatin A (HDAC Inhibitor)
FORM: 0.2 mM in DMSO (dimethylsulfoxide)
STORAGE: -70°C
QUANTITY: 100 µl

BML-KI605-0030 *Fluor de Lys®-Green* Standard
FORM: 1 mM in DMSO (dimethylsulfoxide)
STORAGE: -70°C
QUANTITY: 30 µl

BML-KI282-0500 NAD^+ (Sirtuin Substrate)
FORM: 50 mM β-Nicotinamide adenine dinucleotide (oxidized form) in 50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$.
STORAGE: -70°C
QUANTITY: 500 µl

BML-KI283-0500 Nicotinamide (Sirtuin Inhibitor)
FORM: 50 mM Nicotinamide in 50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$.
STORAGE: -70°C
QUANTITY: 500 µl

BML-KI143-0020 HDAC ASSAY BUFFER
(50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$)
STORAGE: -70°C
QUANTITY: 20 ml

80-2408 1/2 VOLUME NBS MICROPLATES
1 black and 1 white, 96-well, non-binding
STORAGE: Room temperature.

OTHER MATERIALS REQUIRED

Microplate reading fluorometer capable of excitation at a wavelength in the range 470-500 nm and detection of emitted light in the range 520-550 nm.
Pipetman or multi-channel pipetman capable of pipetting 2-100 µl accurately
Ice bucket to keep reagents cold until use.
Microtiter plate warmer or other temperature control device (optional)

ASSAY PROCEDURES

Notes On Storage: *Store all components except the microtiter plate and instruction booklet 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* ***Fluor de Lys®-Green*** Substrate, BML-KI572, *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.*

Some Things To Consider When Planning Assays:

1. The assay is performed in two stages. Substrate deacetylation occurs first, in a total volume of 50 µl. The second stage, which is initiated by the addition of 50 µl of Developer, stops HDAC activity and produces the fluorescent signal. See "Preparing Reagents For Assay" (p. 3), Fig. 2, (p. 4) and Table I (p. 5). .

   Two types of ½-volume, 96-well microplates are provided. The signal obtained with the opaque, white plate will be greater than that obtained with the black plate, but the black plate may reduce background and well-well cross-talk.

   If needed, there is some leeway for change in the reaction volumes. The wells of the microplates provided (80-2408BML) can readily accommodate 150 µl. If planning a

change to the volume of the Developer, it should be noted that it is important to keep two factors constant: 1) the 1 µM concentration of Trichostatin in the final mix; 2) a minimum 2.5 µl/well amount of Developer Concentrate (BML-KI105). See "Preparing Reagents For Assay", Step #5, (p. 3).

2..Assays should include a "time-zero" (sample for which enzyme 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 fluorescence and the fluorescence of a time-zero or no enzyme sample with the same substrate concentration.

3. When 0.3 µl/well of the HeLa Nuclear Extract is used as the source of HDAC activity, reaction progress curves, for a broad range of substrate concentrations (6-200 µM ***Fluor de Lys®-Green*** 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 (Fig. 4). The amount of HeLa extract per well may also be varied;

4. The apparent K_m of the HDAC activity in the HeLa Nuclear Extract (BML-KI140) for the ***Fluor de Lys®-Green*** Substrate is ~43 µM (Fig. 5). 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.

5. It is conceivable that some compounds being screened for inhibition of HDACs may interfere with the action of the ***Fluor de Lys®*** Developer. Assuming the concentration of the test compound(s) does not exceed 0.15 mM, any interference will in all likelihood result in fluorescence development slowing down rather than stopping. It is recommended that, after addition of Developer, fluorescence be read at regular intervals (e.g. every 5 min.) until fluorescence readings stabilize at a higher value. If a sample's fluorescence continues to increase significantly after 20 min. at 25°C, this may indicate interference. Nevertheless, a valid final reading may still be obtained by continuing to monitor the fluorescence until it does stabilize. A follow-up assay in which the amount of Developer Concentrate is increased (e.g. using 50 µl of a 10x Developer Concentrate dilution) should display faster kinetics of fluorescence development and may be useful for confirming the first reading.

6.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 (sirtuin inhibitor) in 1x Developer may 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.

2. Prepare a sufficient amount of HeLa Nuclear Extract (BML-KI140) or other HDAC source diluted in Assay Buffer (BML-KI143) to provide for the assays to be performed (# of wells x 15 µl). Dilutions of HeLa Extract ranging from 150-fold to 50-fold will provide 0.1 to 0.3 µl (0.9 to 2.7 µg protein) of the undiluted Extract per 15 µl (Table 1).

3. Prepare dilution(s) of Trichostatin A and/or Test Inhibitors in Assay Buffer (BML-KI143). Since 10 µl will be used per well (Table 1, Fig. 2), and since the final volume of the HDAC reaction is 50 µl, these inhibitor dilutions will be 5x their final concentration.

4. Prepare dilution(s) of the ***Fluor de Lys®-Green*** Substrate (BML-KI572; 50 mM) in Assay Buffer (BML-KI143) that will be 2x the desired final concentration(s). For inhibitor screening, substrate concentrations at or below the K_m are recommended. Twenty-five µl will be used per well (Table 1). Initial dilutions of 50-fold or greater in Assay Buffer (1.0 mM or less) yield stable solutions (see NOTE on freezing and thawing below). Rapid mixing and dilution into room temperature buffer will help prevent precipitation at high substrate concentration. NOTE: Freezing/thawing of ***Fluor de Lys®-Green*** Substrate solutions in Assay Buffer may cause precipitation of the Substrate. Dilute only amount necessary for one day's experiment.

5. Shortly before use (<30 min.), prepare sufficient *Fluor de Lys®* Developer for the assays to be performed (50 µl per well). First, dilute the *Fluor de Lys®* Developer Concentrate 20-fold (e.g. 50 µl plus 950 µl Assay Buffer) in cold Assay Buffer (BML-KI143). Second, dilute the 0.2 mM Trichostatin A (BML-GR309-9090) 100-fold in the 1x Developer just prepared (e.g. 10 µl in 1 ml; final Trichostatin A concentration in the 1x Developer = 2 µM; final concentration after addition to HDAC/Substrate reaction = 1 µM). Addition of Trichostatin A to the Developer insures that HDAC activity stops when the Developer is added. Keep Developer on ice until use.

Performing the Assay:

1. Add Assay buffer, diluted trichostatin A or test inhibitor to appropriate wells of the microtiter plate. (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 ***Fluor de Lys®-Green*** Substrate and the samples in the microtiter plate to equilibrate to assay temperature (e.g. 25 or 37°C).

4. Initiate HDAC reactions by adding diluted substrate (25 µl) to each well and mixing thoroughly.

5. Allow HDAC reactions to proceed for desired length of time and then stop them by addition of *Fluor de Lys®* Developer (50 µl). Incubate plate at room temperature (25°C) for 10-15 min. or transfer to fluorometer to monitor signal development Once fully developed, the signal is stable and can even be read hours later.

6. Read samples in a microplate-reading fluorometer capable of excitation at a wavelength in the range 470-500 nm and detection of emitted light in the range 520-550 nm.

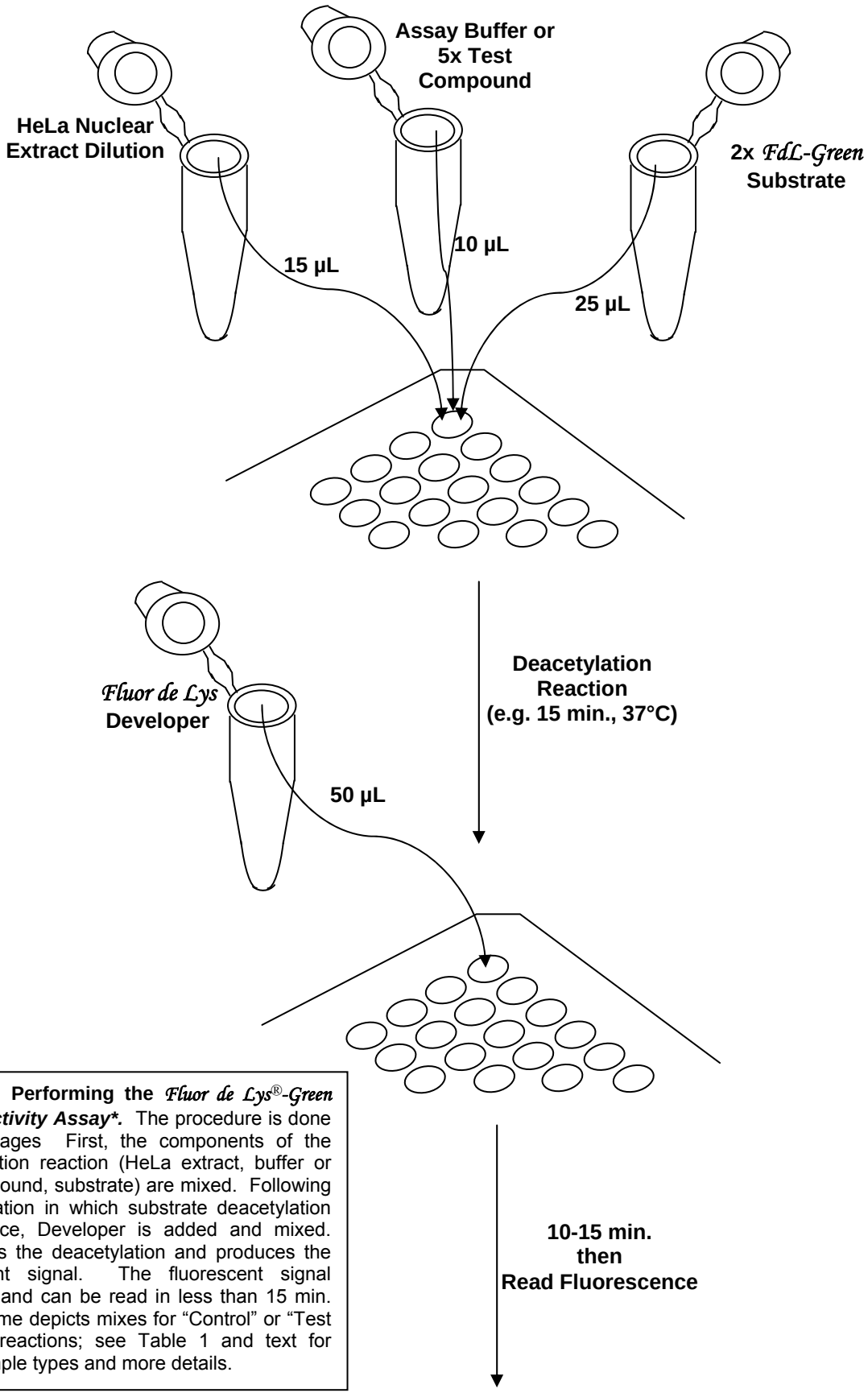

Figure 2. Performing the *Fluor de Lys®-Green* HDAC Activity Assay*. The procedure is done in two stages First, the components of the deacetylation reaction (HeLa extract, buffer or test compound, substrate) are mixed. Following an incubation in which substrate deacetylation takes place, Developer is added and mixed. This stops the deacetylation and produces the fluorescent signal. The fluorescent signal develops and can be read in less than 15 min. The scheme depicts mixes for "Control" or "Test Sample" reactions; see Table 1 and text for other sample types and more details.

TABLE 1. ASSAY MIXTURE EXAMPLES[6]

Sample	Assay Buffer	HeLa Extract (Dilution)	Inhibitor (5x)	*FdL.-Green* 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⌘	25 µl

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

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

⌘Refers to dilution of potential inhibitor in Assay Buffer, which will be 5x its final concentration.

USE OF THE *Fluor de Lys*®*-Green* STANDARD (BML-KI605)

Preparation of a Standard Curve:

1. The *Fluor de Lys*®*-Green* Standard (BML-KI605) will be used for preparing a standard curve. The best conditions will vary depending on the fluorimeter model, the gain setting, the plate chosen and the exact excitation and emission wavelengths used. We recommend making several dilutions of *Fluor de Lys*®*-Green* Standard (BML-KI605) in Assay Buffer (e.g. 1, 5 and 20 µM). The fluorescence signal should then be determined, as described below, after mixing 50 µl of the diluted standard with 50 µl of Developer. Readings taken with these samples may be used to adjust the gain, test excitation/emission wavelengths and cutoff filters and to plan an appropriate series of dilutions for a full standard curve. Under the conditions shown in Figure 3, fluorescence increases monotonically, although non-linearly, with concentration, up to about 20 µM. The relationship between Standard concentration and fluorescence remains linear to at least 0.4 µM. For most applications involving initial rate determinations, it should be possible to work with signals entirely within this linear range. Provided the same wavelength and gain settings are used each time, there should be no need to prepare a standard curve more than once.
2. After ascertaining an appropriate concentration range, prepare, in Assay Buffer, a series of *Fluor de Lys*®*-Green* Standard dilutions that span this range. Pipet 50 µl of each of these dilutions, and 50 µl of Assay Buffer as a 'zero', to a set of wells on the microtiter plate.
3. Prepare, as described in "Preparing Reagents For Assay", step #5. (p.3), sufficient *Fluor de Lys*® Developer for the standard wells (50 µl per well).
4. Mix 50 µl of the Developer with the 50 µl in each standard well and incubate 5-10 min. at room temperature (25°C).
5. Read samples in a microtiter-plate reading fluorimeter capable of excitation at a wavelength in the range 470-500 nm and detection of emitted light in the range 520-550.
6. Plots of pmol. *FdL-Green* Std. (y-axis) vs. fluorescence are shown in Fig. 3, **A** and **B.** A plot covering a relatively narrow range of Standard quantities, such as 0-20 pmol/well (0-0.4 µM in 50 µL), will provide a good linear fit (**A**). Plotting a broader range of Standard quantities, such as 0-1000 pmol/well (0-20 µM in 50 µL), will yield a curve that fits well to a second-order polynomial (**B**). The accuracy of the fluorescence to pmol conversion will be best if the range chosen is the minimum necessary to cover the full range of fluorescence increases likely to be seen in your experimental samples. [NOTE: "Fluorescence Increase" = Sample – Background, where Background Fluorescence is the fluorescence of a time-zero or no-enzyme sample of the same substrate concentration.]

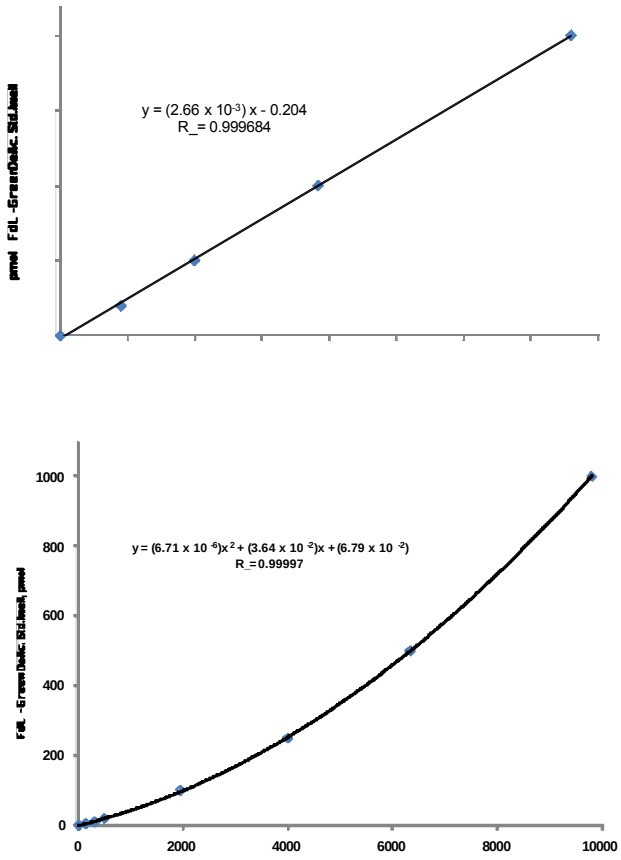

Figure 3. Fluorescence Standard Curves. *Fluor de Lys*®*-Green* Standard (*FdL-Green* Std.) was diluted in Assay Buffer, to the concentrations of 0-0.4 µM (**A**) or 0-20 µM (**B**) and 50 µl aliquots mixed with 50 µl Developer. Fluorescence was measured in a black microplate (**A**) with a Synergy2 reader (BioTek, Ex. 485 nm, Em. 528 nm, gain=60) or a white microplate (**B**) with a CytoFluor™ II fluorescence plate reader (PerSeptive Biosystems, Ex. 485 nm, Em. 530 nm, gain=37). Linear or second-order best-fit curves were obtained with Microsoft XL (equations). Depending on the concentration range of deacetylated substrate, an equation from one of these two plot types may be used to convert fluorescence increases due to deacetylation to the number of pmol of *Fluor de Lys*®*-Green* Substrate that have been deacetylated.

Fluorescence Quenching and/or Fluorescence from Test Compounds

1. The *Fluor de Lys®-Green* assay avoids most instances of interference by compound quenching or fluorescence because it is unaffected by compounds absorbing and/or emitting in the near UV and blue parts of the spectrum. Given the excitation and emission spectra of the *Fluor de Lys®-Green* fluorophor (peak Ex. 496 nm, Em. 520 nm), test compounds that might quench assay fluorescence or fluoresce at the assay wavelength are likely to be highly colored.
2. If a compound is suspected of interfering with the assay by fluorescence or fluorescence quenching, prepare a standard curve with *Fluor de Lys®-Green* Standard (BML-KI605) in the presence of the working concentration of that compound and compare to curves done in its absence. (Fig. 3). If quenching is not too severe, it may be possible to correct for quenching by converting deacetylation-induced fluorescence increases to acetylation rates (e.g. in pmol/min) using the standard curve obtained in the compound's presence.
3. Interference from a compound that fluoresces, but does not quench the fluorescence of the *Fluor de Lys®-Green* Standard, can be corrected with a no-enzyme blank or time-zero sample that includes the compound. Note that in all cases, whether a fluorescent compound is present or not, deacetylation is a function of the increase in a sample's fluorescence above that of a corresponding no-enzyme blank or time-zero sample.

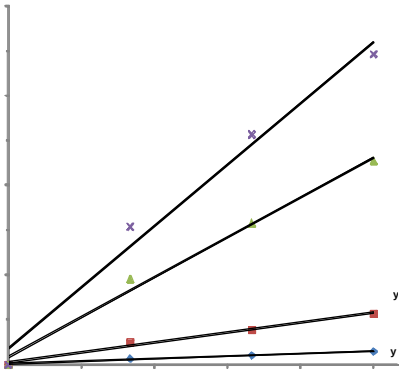

Figure 4. Time Courses of *Fluor de Lys®-Green* Substrate Deacetylation by HeLa Nuclear Extract HDAC Activity. HeLa Nuclear extract (0.3 µl /well) was incubated (37°C) with indicated concentrations of substrate. Reactions were stopped at indicated times with *Fluor de Lys®* Developer and fluorescence measured (CytoFluor™ II, PerSeptive Biosystems, Ex. 485 nm, Em. 530 nm, gain=37). Each point represents a single determination with background (0 min.) fluorescence subtracted. Equations of linear best-fit lines for each data set are shown underneath the substrate concentration.

APPLICATION EXAMPLES

The *Fluor de Lys®-Green* HDAC Assay has been used to investigate the kinetics of *Fluor de Lys®-Green* Substrate deacetylation by HeLa nuclear extract (Figures 4 & 5). This activity is totally sensitive to the HDAC inhibitor trichostatin A (Figure 6), with an IC50 of ~0.5 nM. Examples of *Fluor de Lys®-Green* Substrate deacetylation by HDACs from class I (HDACs 1, 3 and 8), class IIb (HDAC6), class III (the sirtuins; SIRT1) and class IV (HDAC11) are shown in Figs. 7 and 8.

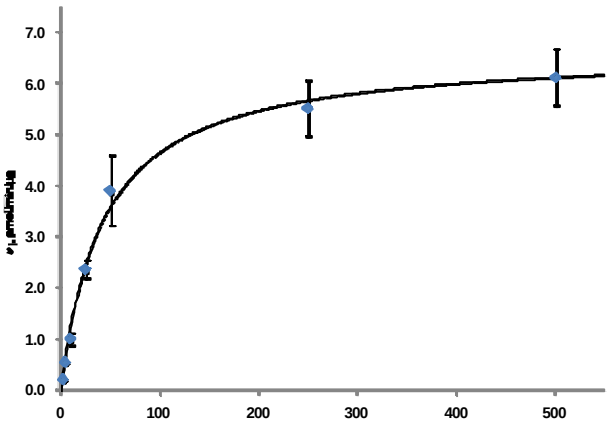

Figure 5. Kinetics of *Fluor de Lys®-Green* Substrate Deacetylation by HeLa HDAC Activity. HeLa Nuclear extract (0.1 µl/0.9 µg per well) was incubated (37°C) with indicated concentrations of substrate. Reactions were stopped after 15 min. with *Fluor de Lys®* Developer and fluorescence measured (CytoFluor™ II, Ex. 485 nm, Em. 530 nm, gain=37). Fluorescence increases were converted to pmol of deacetylated *Fluor de Lys®-Green*.Substrate by means of a best fit equation from a fluorescence standard curve (see Fig. 1B). Points are the mean of three determinations and error bars are standard errors of the means. 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).

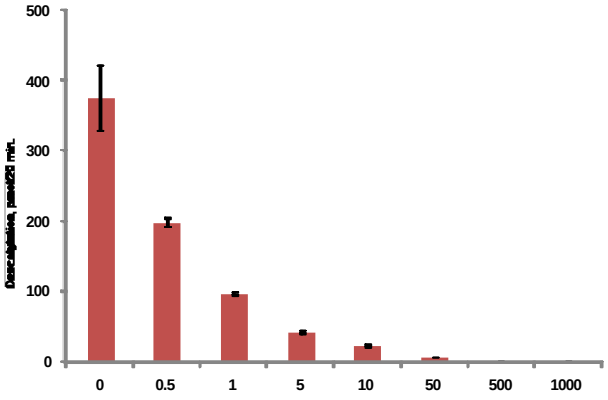

Figure 6. Trichostatin A Inhibition of *Fluor de Lys®-Green* Substrate Deacetylation by HeLa Nuclear Extract. HeLa Nuclear Extract (0.3 µl/well) was incubated (37°C) with 20 µM substrate and indicated concentrations of Trichostatin A (TSA). Reactions were stopped after 20 min. with *Fluor de Lys®* Developer and fluorescence measured (CytoFluor™ II, Ex. 485 nm, Em. 530 nm, gain=37). The TSA IC_{50}, derived from a two parameter fit (top and bottom fixed at 376 and 0 pmol/20 min.), was 0.49 nM. Error bars represent standard errors of the means of three determinations.

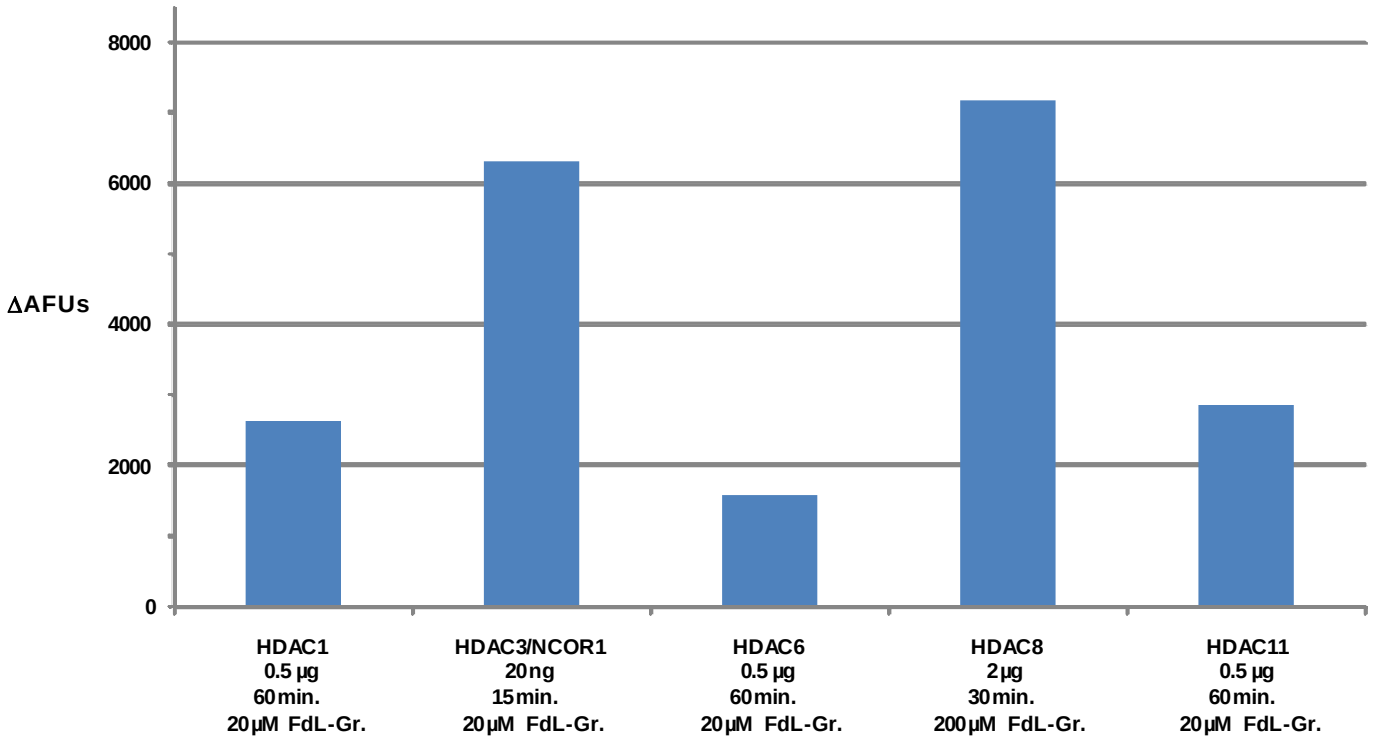

Figure 7. Class I, Class IIb and Class IV Recombinant HDACs Deacetylate *Fluor de Lys®-Green* Substrate. Recombinant human HDAC1 (Cat. # BML-SE456), HDAC3 (complex with NCOR1-DAD; Cat. # BML-SE515), HDAC6 (Cat. # BML-SE508), HDAC8 (Cat. # BML-SE145) and HDAC11 were incubated at 37°C with *Fluor de Lys®-Green Substrate* (*FdL-Gr.*) under the indicated conditions.. Reactions were stopped with *Fluor de Lys®* Developer and fluorescence measured (CytoFluor™ II, PerSeptive Biosystems, Ex. 485 nm, Em. 530 nm, gain=37). Fluorescence increases **(ΔAFUs**) were obtained by subtraction of corresponding 0 min. sample fluorescences (~560 AFU for 20 µM *FdL-Gr.*, ~2200 AFU for 200 µM *FdL-Gr.*)

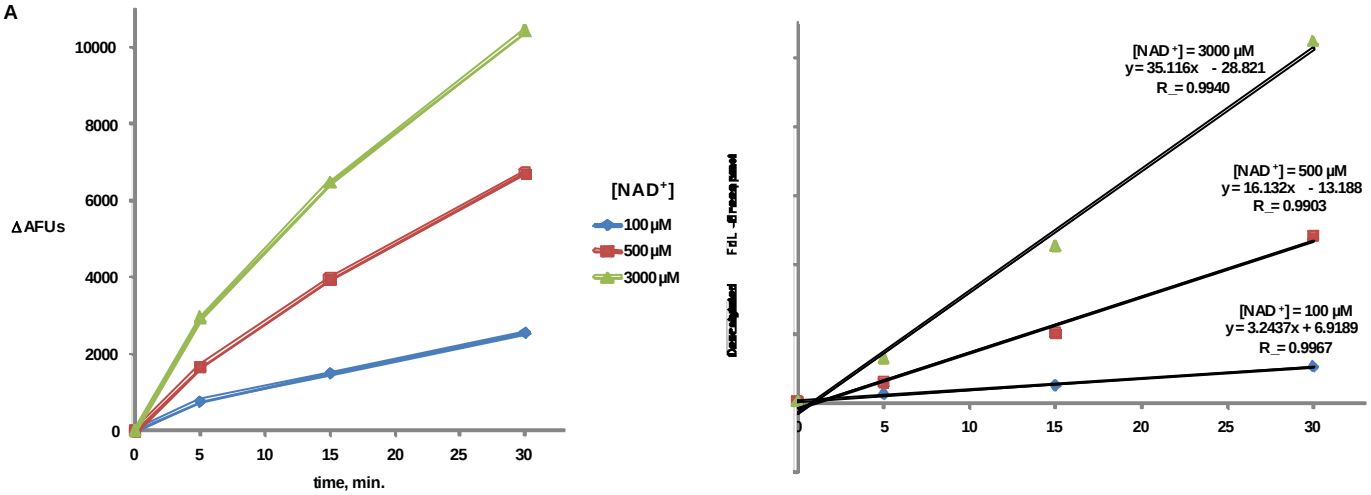

Figure 8. SIRT1 Deacetylates *Fluor de Lys®-Green* Substrate. Purified recombinant human SIRT1 (0.6 µg; Cat. # BML-SE239) was incubated at 37°C with 50 µM *Fluor de Lys®-Green* .Substrate and the indicated concentrations of NAD^+. Reactions were stopped at indicated times with *Fluor de Lys®* Developer and fluorescence measured (CytoFluor™ II, PerSeptive Biosystems, Ex. 485 nm, Em. 530 nm, gain=37). Net increases in fluorescence from 0 min. (ΔAFUs; **A**) were converted to pmol of deacetylated *Fluor de Lys®-Green* .Substrate (**B**) by means of a second-order best fit equation from a fluorescence standard curve (see **Fig. 1B**). Each point represents a single determination with background (0 min.) fluorescence subtracted (**A**).. Equations of linear best-fit lines for each data set are shown underneath the $[NAD^+]$ (**B**). .

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.

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TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending.

ALSO AVAILABLE ...

PRODUCT	CATALOG #
SIRT1 Fluorescent Activity Assay	BML-AK555
HDAC Fluorescent Activity Assay	BML-AK500
HDAC Colorimetric Assay Kit	BML-AK501
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
HDAC8 (recombinant, human)	BML-SE145
SIRT1 (recombinant, human)	BML-SE239
SIRT2 (recombinant, human)	BML-SE251
SIRT3 (recombinant, human)	BML-SE270
Resveratrol (SIRT1 Activator)	BML-FR104
Piceatannol (SIRT1 Activator)	BML-GR323
Suramin sodium (SIRT1 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

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