



## HDAC8 Fluorometric Drug Discovery Kit

Catalog # BML-AK518

**A FLUOR DE LYS® Fluorescent Assay System**

**NOTE:** This version contains a change to reagent storage temperatures.

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Please read entire booklet before proceeding with the assay.



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

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## BACKGROUND

Histones form the core of nucleosomes, the DNA/protein complexes that are the subunits of eukaryotic chromatin. 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<sup>1</sup>. The best studied of these modifications,  $\epsilon$ -amino acetylations of specific histone lysine residues, are catalyzed by histone acetyltransferases (HATs). Histone deacetylases (HDACs) are responsible for removal of these acetyl groups<sup>2,3,4</sup>. Histone hyperacetylation correlates with an open, decondensed chromatin structure and gene activation, while hypoacetylation correlates with chromatin condensation and transcriptional repression<sup>1-7</sup>.

Eleven human class I and class II HDACs (hydrolytic deacetylases) have been identified, all trichostatin A-sensitive and homologs of either RPD3 (class I) or HDA1 (class II), yeast HDACs<sup>8-17</sup>. HDACs can associate with transcription repression complexes such as NuRD, Sin3A or N-CoR/SMRT<sup>1-7,18</sup>.

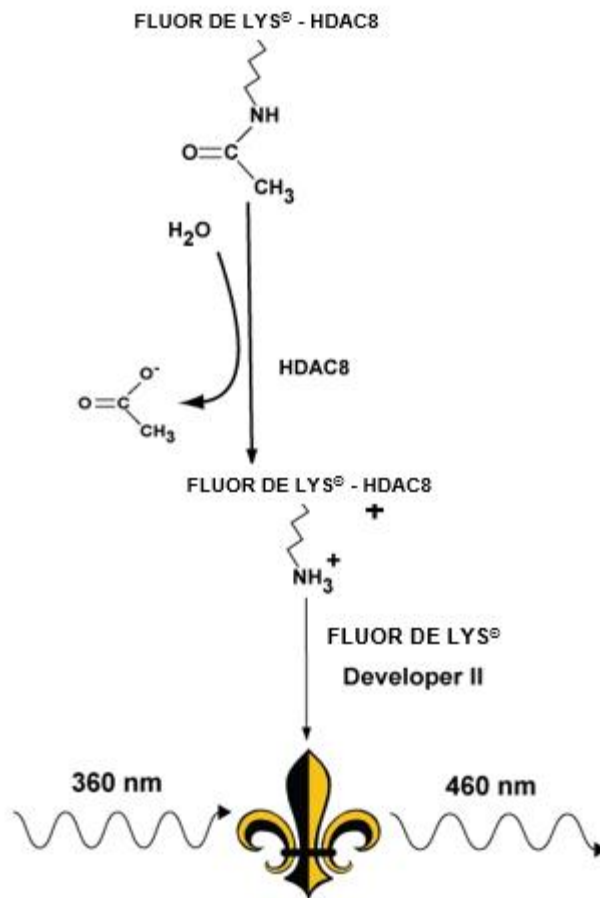
Human HDAC8 is a 377 residue, class I HDAC, *i.e.* a member of the same homology group as yeast RPD3 and human HDACs 1, 2, and 3<sup>19-21</sup>. Although early studies suggested that HDAC8 localized to the nucleus<sup>19,21</sup> and was ubiquitously expressed<sup>19,20</sup>, recent work indicates that in normal tissue it is primarily cytosolic and expressed in smooth muscle cells<sup>27,28</sup>. However, like other class I HDACs, HDAC8 exhibits trichostatin A-inhibitable histone deacetylase activity<sup>19-21</sup> and can mediate transcription repression<sup>19,22</sup>. A crystal structure has been obtained for HDAC8 complexed with another, more potent inhibitor in the trichostatin class (hydroxamic acids)<sup>29</sup>.

HDAC8 localizes to stress-fibers, along with smooth muscle  $\alpha$ -actin<sup>27</sup>, and its expression appears to be essential for smooth muscle contractility<sup>28</sup>. This affinity for cytoskeletal proteins may underlie HDAC8's binding to the core binding factor  $\beta$ /smooth muscle myosin heavy chain fusion protein (CBF $\beta$ /SMMHC), which is created by the inversion(16) chromosomal translocation (*inv*(16))<sup>23</sup>. CBF $\beta$ /SMMHC can associate with the mSin3A corepressor<sup>23</sup> and may thereby act as a dominant repressor of genes regulated by CBF $\beta$ -AML1<sup>23,24</sup>. Repression of these genes, which in the case of *inv*(16) would appear to be mediated by HDAC8 activity<sup>23</sup>, is implicated in the genesis of acute myeloid leukemia (AML)<sup>22-24</sup>. siRNA-knockdown of HDAC8 expression has been shown to inhibit the growth of several human tumor cell lines<sup>29</sup>, suggesting that HDAC8 activity may be significant in the pathogenesis of solid<sup>29</sup> as well as hematologic tumors<sup>22-24</sup>.

HDAC inhibitors have shown promise as anti-tumor agents and naturally this has stimulated interest in the screening of compounds for HDAC8 inhibition. Unfortunately, standard techniques for HDAC assay involve the use of [<sup>3</sup>H]acetyl-histone or [<sup>3</sup>H]acetyl-histone peptide substrates and a cumbersome acid/ethyl acetate extraction step prior to scintillation counting<sup>8,25,26</sup>. Enzo Life Sciences' *HDAC8 Fluorometric Drug Discovery Kit* addresses these problems by providing an assay that can be carried out in two simple mixing steps, all on the same 96-well plate (Figure 1).

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**Figure 1. Reaction Scheme of the HDAC8 Fluorometric Activity Assay.** Deacetylation of the substrate sensitizes it to the Developer II, 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.

## DESCRIPTION

The *HDAC8 Fluorometric Drug Discovery Kit* is based on the unique FLUOR DE LYS<sup>®</sup>-HDAC8 Substrate and Developer II combination. The FLUOR DE LYS<sup>®</sup> 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 (Figure 1). First, the FLUOR DE LYS<sup>®</sup>-HDAC8 Substrate, which comprises an acetylated lysine side chain, is incubated with HDAC8 (BML-SE145). Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the FLUOR DE LYS<sup>®</sup> Developer II produces a fluorophore.

**MATERIALS SUPPLIED****BML-SE145-0100 Histone Deacetylase 8 (HDAC8) (human, recombinant)**FORM: 10 mM Tris, pH 7.5, 100 mM NaCl, 3mM MgCl<sub>2</sub>, and 10% glycerol

STORAGE: -80°C; AVOID FREEZE/THAW CYCLES!

QUANTITY: 100 U, One U=1 pmol/min at 37°C, 250 µM, FLUOR DE LYS<sup>®</sup>  
Substrate (BML-KI104)**BML-KI178-0005 FLUOR DE LYS<sup>®</sup>-HDAC8, Deacetylase Substrate**FORM: 5 mM solution in 50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl,  
1 mM MgCl<sub>2</sub>

STORAGE: -80°C

QUANTITY: 100 µL

**BML-KI176-1250 FLUOR DE LYS<sup>®</sup> Developer II Concentrate (5x)**

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

STORAGE: -80°C

QUANTITY: 5 x 250 µL

**BML-GR309-9090 Trichostatin A (HDAC Inhibitor)**

FORM: 0.2 mM in DMSO

STORAGE: -80°C

QUANTITY: 100 µL

**BML-KI142-0030 FLUOR DE LYS<sup>®</sup> Deacetylated Standard**

FORM: 10 mM in DMSO

STORAGE: -80°C

QUANTITY: 30 µL

**BML-KI311-0020 HDAC8 ASSAY BUFFER**(50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl<sub>2</sub>, 10% PEG  
(avg. MW 8000 Da), 1 mg/mL BSA

STORAGE: -80°C

QUANTITY: 20 mL

**BML-KI143-0020 HDAC ASSAY BUFFER**(50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl<sub>2</sub>)

STORAGE: -20°C

QUANTITY: 20 mL

**80-2407 1/2 VOLUME MICROPLATES**

1 clear and 1 white, 96-well

STORAGE: Ambient

## STORAGE

Store all components except the microplate at  $-80^{\circ}\text{C}$  for the highest stability. The HDAC8 Enzyme, BML-SE145, 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 enzyme should be refrozen quickly, by placing at  $-80^{\circ}\text{C}$ . If possible, snap freeze in liquid nitrogen or a dry ice/ethanol bath. To minimize the number of freeze/thaw cycles, aliquot the enzyme into separate tubes and store at  $-80^{\circ}\text{C}$ .

## ADDITIONAL MATERIALS NEEDED

- Microplate reading fluorometer capable of excitation at a wavelength in the range 350-380 nm and detection of emitted light in the range 440-460 nm
- Pipette or multi-channel pipette capable of pipetting 2-100  $\mu\text{L}$  accurately
- Ice bucket to keep reagents cold until use
- Microplate warmer and/or other temperature control device (optional)

## SAFETY WARNINGS & PRECAUTIONS

1. Wear appropriate personnel protective apparel. Avoid contact with clothes and exposed skin. In case of accidental skin exposure, flush with water immediately. Consult a physician if required.
2. Use a safety pipetting device for all pipetting. Never pipette by mouth.
3. Interpretation of the results is the sole responsibility of the user.

## PROCEDURES

### Some Things To Consider When Planning Assays:

1. The assay is performed in two stages. The first stage, during which the HDAC8 acts on the Substrate, is done in a total volume of 50  $\mu\text{l}$ . The second stage, which is initiated by the addition of 50  $\mu\text{l}$  of Developer II, stops HDAC activity and



produces the fluorescent signal. See “[Preparing Reagents For Assay](#)” and Table 1.

Two types of ½-volume, 96-well microplates are provided with the kit. The signal obtained with the opaque, white plate can be ~5-fold greater than that obtained with the clear plate (BML-KI101). As long as the fluorometer to be used is configured so that excitation and emission detection occur from above the well, the white plate should significantly increase assay sensitivity.

Should it be necessary, for convenience in adding or mixing reagents, there is some leeway for change in the reaction volumes. The wells of the microplates provided (BML-KI101 or BML-KI584) can readily accommodate 150 µL. If planning a change to the volume of the Developer II, 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) 10 µL/well amount of Developer II Concentrate (BML-KI176). See “[Preparing Reagents For Assay](#)”, Step #5.

2. Experimental samples should be compared to a “time zero” (sample for which Developer II is added immediately after mixing of the HDAC with substrate) and/or a negative control (no enzyme).
3. The  $K_m$  of HDAC8 for the FLUOR DE LYS<sup>®</sup>- HDAC8 Substrate has been measured at 1.5 mM (Figure 4). Use of substrate concentrations well below  $K_m$  will help avoid substrate competition effects, which could mask the effectiveness of competitive inhibitors. For inhibition studies it is not recommended to exceed a final substrate concentration of 100 µM.
4. Best results will be obtained by adding the chilled, undiluted enzyme directly to pre-warmed buffer and proceeding immediately to the addition of pre-warmed substrate. Plan the timing of the preparation and warming of enzyme dilutions, 2x substrate solutions and inhibitor solutions accordingly. (See “[Preparing Reagents for Assay](#)”.)
5. Two buffers are provided with the kit—HDAC8 Assay Buffer (BML-KI311) and HDAC Assay Buffer (BML-KI143). The first of these, BML-KI311, is for running the first phase of the assay, the HDAC8 deacetylation reaction itself. It should therefore be used for preparing all working dilutions of HDAC8 (BML-SE145), substrate (BML-KI178) and any compounds being screened for effects on HDAC8. The other buffer, BML-KI143, should be used for diluting the Developer II Concentrate (BML-KI176) and Trichostatin A in preparation of 1x Developer.

6. It is conceivable that some compounds being screened for inhibition of HDACs may interfere with the action of the FLUOR DE LYS<sup>®</sup> - Developer II. It is therefore important to confirm that apparent HDAC8 inhibitor “hits” are in fact acting only via HDAC8 inhibition. One approach to this involves retesting the candidate inhibitor in a reaction with the FLUOR DE LYS<sup>®</sup>-Deacetylated Standard (BML-KI142) plus the FLUOR DE LYS<sup>®</sup>- Developer II. A detailed retesting procedure is described below, in the section “Uses Of The FLUOR DE LYS<sup>®</sup>- Deacetylated Standard”. In some cases, it may be possible to avoid this retesting by means of measurements taken during the fluorescence development phase of the initial HDAC8 assay. This is also discussed in that section.

## PREPARING REAGENTS FOR ASSAY

1. Defrost all kit components and keep these, and all dilutions described below, on ice until use. With the exception of the HDAC8 enzyme, undiluted kit components are stable for several hours on ice. The enzyme is stable on ice for the time typically required to set up an experiment (30-60 min.), but may lose activity with dilution and/or prolonged storage on ice. It is recommended that the enzyme be thawed and placed on ice as shortly before its use as practical.
2. The HDAC8 Enzyme (BML-SE145) will be diluted in HDAC8 Assay Buffer (BML-KI311). A dilution of the HDAC8 in which 15  $\mu$ L contains 1U of the enzyme is an appropriate amount to use per well (Table 1). Volume of diluted enzyme required to provide for the assays to be performed = # of wells x 15  $\mu$ L. Prewarm the buffer to assay temperature, add chilled, undiluted enzyme and proceed immediately to the aliquoting of enzyme to assay wells and the addition of substrate.
3. Prepare dilution(s) of Trichostatin A and/or Test Inhibitors in HDAC8 Assay Buffer (BML-KI311). Since 10  $\mu$ L will be used per well (Table 1), and since the final volume of the HDAC reaction is 50  $\mu$ L, these inhibitor dilutions will be 5x their final concentration.
4. Prepare dilution(s) of the FLUOR DE LYS<sup>®</sup> - HDAC8 Substrate (BML-KI178; 5 mM) in HDAC8 Assay Buffer (BML-KI311) that will be 2x the desired final concentration(s). For inhibitor screening, final substrate concentrations in the range of 25  $\mu$ M-100  $\mu$ M are recommended. 25  $\mu$ L will be used per well (Table 1).

5. Shortly before use (<30 min.), prepare sufficient FLUOR DE LYS<sup>®</sup> Developer II for the assays to be performed (50  $\mu$ L per well). First, dilute the FLUOR DE LYS<sup>®</sup> Developer II Concentrate 5-fold (e.g. 250  $\mu$ L plus 1000  $\mu$ L Assay Buffer) in cold HDAC Assay Buffer (BML-KI143). Second, dilute the 0.2 mM Trichostatin A (BML-GR309-9090) 100-fold in the 1x Developer II just prepared (e.g. 12.5  $\mu$ L in 1.25 mL; final Trichostatin A concentration in the 1x Developer II = 2  $\mu$ M; final concentration after addition to HDAC/Substrate reaction = 1  $\mu$ M). Addition of Trichostatin A to the Developer II insures that HDAC activity stops when the Developer II is added. Keep Developer II on ice until use.

## PERFORMING THE ASSAY

1. Add HDAC8 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.
2. Warm 2x Substrate solution and the HDAC8 Assay Buffer for diluting the enzyme to assay temperature. Add chilled, undiluted HDAC8 to the warmed buffer.
3. Add diluted HDAC8 to all wells except those that are to be “No Enzyme Controls.”
4. Initiate HDAC8 reactions by adding diluted substrate (25  $\mu$ L) to each well and mixing thoroughly.
5. Allow HDAC8 reactions to proceed for desired length of time and then stop them by addition of FLUOR DE LYS<sup>®</sup> Developer II (50  $\mu$ L) prepared in step #5. Incubate plate at room temperature (30°C) for at least 45 min. Signal is stable for at least 60 min. beyond this time.
6. Read samples in a microplate reading fluorometer capable of excitation at a wavelength in the range 350-380 nm and detection of emitted light in the range 440-460 nm.

**TABLE 1. ASSAY MIXTURE EXAMPLES<sup>6</sup>**

Sample	HDAC8 Assay Buffer	HDAC8 (Dilution)	Inhibitor (5x)	FLUOR DE LYS <sup>®</sup> - HDAC8 Substrate (2x)
Blank (No Enzyme)	25µl	0	0	25µl
Control	10µl	15µl	0	25µl
Trichostatin A <sup>‡</sup>	0	15µl	10µl <sup>‡</sup>	25µl
Test Sample <sup>⌘</sup>	0	15µl	10µl <sup>⌘</sup>	25µl

<sup>6</sup> HDAC reaction mixtures, prior to addition of FLUOR DE LYS<sup>®</sup> Developer II.

<sup>‡</sup> Refers to dilution of Trichostatin A in HDAC8 Assay Buffer, which will be 5x the final concentration. Examples: 1) As a measure of non-HDAC background, 10 µM would produce final 2 µM concentration and essentially complete HDAC8 inhibition; 2) As a model inhibitor “hit”, 25 nM would produce final 5 nM and ~50% inhibition.

<sup>⌘</sup> Refers to dilution of potential inhibitor in HDAC8 Assay Buffer, which will be 5x its final concentration.

## PREPARATION OF A STANDARD CURVE (USES OF THE FLUOR DE LYS<sup>®</sup> DEACETYLATED STANDARD (BML-KI142))

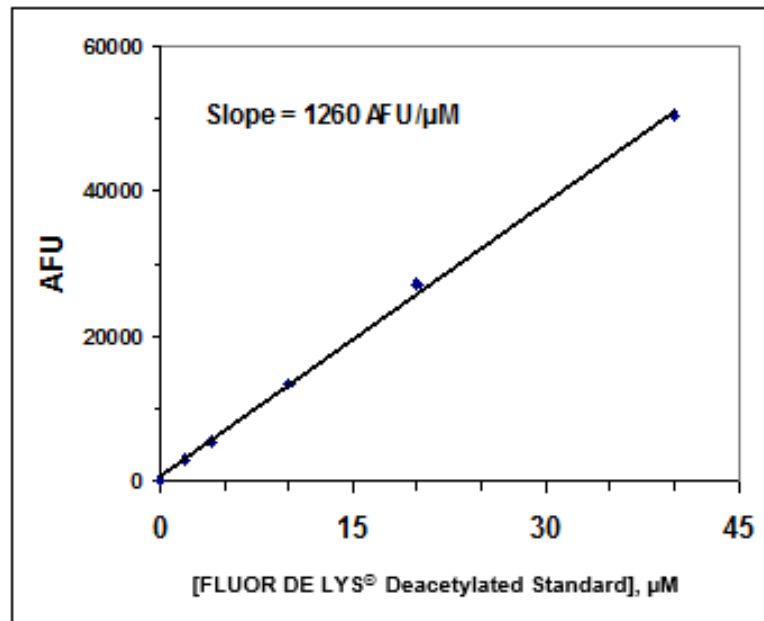
1. The exact concentration range of the FLUOR DE LYS<sup>®</sup> Deacetylated Standard (BML-KI142) that will be useful for preparing a standard curve will vary depending on the fluorometer model, the gain setting and the exact excitation and emission wavelengths used. We recommend diluting some of the standard to a relatively low concentration with HDAC8 Assay Buffer (1 to 5 µM). The fluorescence signal should then be determined, as described below, after mixing 50 µL of the diluted standard with 50 µL of Developer II. The estimate of AFU(arbitrary fluorescence units)/µM obtained with this measurement, together with the observed range of values obtained in the enzyme assays can then be used to plan an appropriate series of dilutions for a standard curve. 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<sup>®</sup> Deacetylated Standard dilutions that span this range. Pipet 50 µL of each of these dilutions, and 50 µL of HDAC8 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., sufficient FLUOR DE LYS<sup>®</sup> Developer II for the standard wells (50  $\mu$ L per well).
4. Mix 50  $\mu$ L of the Developer II with the 50  $\mu$ L in each standard well and incubate 5-10 min. at room temperature (30°C).
5. Read samples in a microplate reading fluorometer capable of excitation at a wavelength in the range 350-380 nm and detection of emitted light in the range 440-460 nm.
6. Plot fluorescence signal (y-axis) versus concentration of the FLUOR DE LYS<sup>®</sup> Deacetylated Standard (x-axis). Determine slope as AFU/ $\mu$ M. See example in Figure 2.

Testing of Potential HDAC8 Inhibitors for Interference with the FLUOR DE LYS<sup>®</sup> Developer II or the Fluorescence Signal:

1. The FLUOR DE LYS<sup>®</sup> Developer II is formulated so that, under normal circumstances, the reaction goes to completion in less than 5 min. at 30°C. That, together with the recommended 45 min. reaction time, should help insure that in most cases, even when some retardation of the development reaction occurs, the signal will fully develop prior to the reading of the plate.
2. A convenient step to control for substances that interfere with the Developer II reaction or the fluorescence signal itself may be built directly into an inhibitor screening protocol. After waiting for the signal from the HDAC8 reaction to fully develop and stabilize (usually less than 45 min., see 1. above), the fluorescence is recorded and a ‘spike’ of FLUOR DE LYS<sup>®</sup> Deacetylated Standard is added (e.g. amount equivalent to 5  $\mu$ M in the 50  $\mu$ L HDAC8 reaction). Sufficient Developer II reactivity should remain to produce a full signal from this ‘spike’. When the new, increased fluorescence level has fully developed (<15 min.), the fluorescence is read and the difference between this reading and the first one can provide an internal standard, in terms of AFU/ $\mu$ M, for appropriate quantitation of each well. This is particularly useful in cases, for example with highly colored potential inhibitors, where the development reaction itself is not compromised but the fluorescence signal is diminished. As discussed further below (see 3.), interference with the development reaction *per se* will be reflected in the kinetics of signal development, both that of the initial HDAC8 reaction and that of the Deacetylated Standard ‘spike’.

3. It should be possible to identify many cases in which there is interference with the development reaction by taking a series of fluorescence readings immediately following addition of the FLUOR DE LYS<sup>®</sup> Developer II (e.g. readings at 5 min. intervals for 60 min.). The fluorescence of control samples (no inhibitor) will change very little after the first or second reading. Samples containing compounds which inhibit HDAC8, but which do not interfere with the Developer II, will display similarly rapid kinetics, although a lower final fluorescence. Trichostatin A (5 nM) provides a good model of this behavior. Any sample in which the approach to the final fluorescence is substantially slower than in the above examples should be suspected of interference with the development reaction. For samples in which little or no fluorescence has developed, it may be impossible to assess the development kinetics.
4. Absolute certainty regarding interference with the Developer II can only be obtained through an assay in which the compound in question is tested for its effect on the reaction of FLUOR DE LYS<sup>®</sup> Deacetylated Standard with the Developer II. Using a standard curve such as that described in the previous section, determine the concentration of Deacetylated Standard that will yield a signal similar to that produced after development of a control (no inhibitor) HDAC8 reaction. Mix 40  $\mu$ L of the diluted Standard with 10  $\mu$ L inhibitor or 10  $\mu$ L HDAC8 Assay Buffer (see Table 2). Initiate development by adding 50  $\mu$ L of 1x Developer II to each well. Follow fluorescence development by reading at 1 or 2 min. intervals for 30 min. If a test inhibitor sample reaches its final fluorescence more slowly than the control or if the final value is significantly below that of the control, then there is interference with the Developer II reaction.
5. Once it is determined that a particular substance does interfere with the Developer II reaction, it may be possible to adjust reaction conditions to eliminate this effect. In cases where the same final fluorescence is achieved, but more slowly than the control (e.g. 25 min. rather than 1 min.), simply extending the incubation time after addition of the Developer II would be sufficient. Other possible adjustments include increasing the volume of Developer II used per well (e.g. to 100  $\mu$ L) and diluting the Developer II Concentrate 2.5-fold, rather 5-fold. All three of these approaches may be used separately or in combination.



**Figure 2. Fluorescence Standard Curve.** 50 µL aliquots of FLUOR DE LYS® Deacetylated Standard, in Assay Buffer at the indicated concentrations, were mixed with 50 µL Developer II and incubated 10 min., 30°C. Fluorescence was then measured in the wells of the clear microtiter-plate (BML-K1101) with a CytoFluor II fluorescence plate reader (PerSeptive Biosystems, Ex. 360 nm, Em. 460 nm, gain=70).

**TABLE 2. ASSAY MIXTURES FOR INHIBITOR RETESTING WITH FLUOR DE LYS® DEACETYLATED STANDARD**

Sample	HDAC8 Assay Buffer	Inhibitor (5x)	Diluted <sup>6</sup> FLUOR DE LYS® deAc. Standard (1.25x)	DEVELOPER II (1x)
Control	10 µL	0	40 µL	50 µL
Trichostatin A <sup>‡</sup>	0	10 µL	40 µL <sup>‡</sup>	50 µL
Test Inhibitor <sup>⌘</sup>	0	10 µL	40 µL <sup>⌘</sup>	50 µL

<sup>6</sup> The appropriate dilution of the FLUOR DE LYS® Deacetylated Standard, may be determined from the standard curve and should be the concentration producing a fluorescent signal equal to that produced by control (no inhibitor) samples in the HDAC8 assay. The dilution in HDAC8 Assay Buffer is prepared at 1.25x this concentration to compensate for the 4/5 dilution due to addition of 10 µL of Assay Buffer or inhibitor.

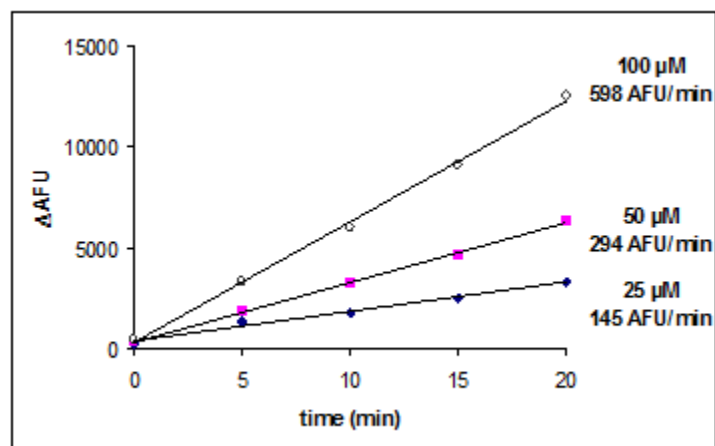
<sup>‡</sup> Refers to dilution of Trichostatin A in HDAC8 Assay Buffer, which will be 5x its final concentration in the 50 µL volume, prior to addition of Developer II. Example: As a model inhibitor that does not interfere with the Developer II, 25 nM Trichostatin A would produce a final 5 nM concentration.

<sup>⌘</sup> Refers to dilution of potential inhibitor in Assay Buffer, which will be 5x its final concentration in the 50 µL volume, prior to addition of Developer II.

## APPLICATION EXAMPLES

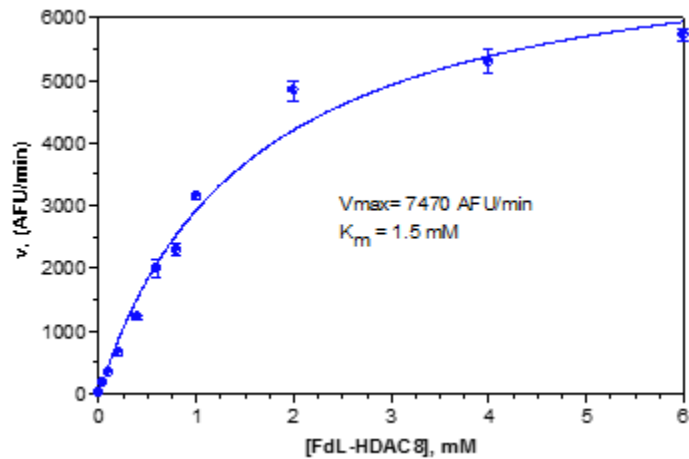
The HDAC8 Fluorescent Activity Assay/Drug Discovery Kit has been used to investigate the kinetics of FLUOR DE LYS<sup>®</sup> HDAC8 Substrate deacetylation by HDAC8 enzyme (Figures 3 & 4). This activity is nearly totally sensitive to the HDAC inhibitor Trichostatin A (Figure 5).

**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.

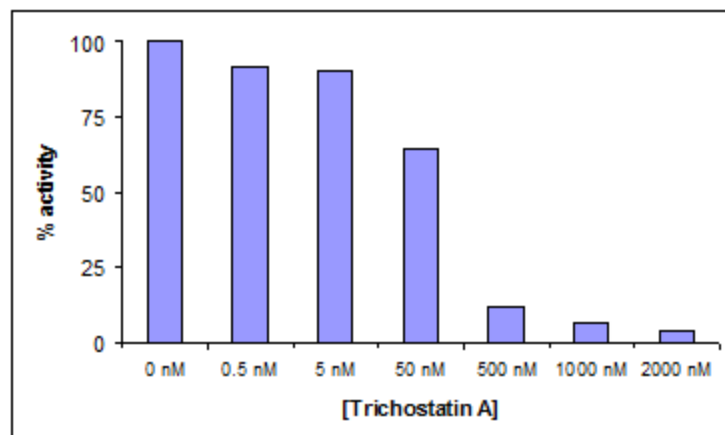


**Figure 3. Time Courses of FLUOR DE LYS<sup>®</sup> - HDAC8 Substrate Deacetylation by HDAC8.** HDAC8 Enzyme (1U/well) was incubated (30°C) with indicated concentrations of substrate. Reactions were stopped at indicated times with FLUOR DE LYS<sup>®</sup> Developer II and fluorescence measured (CytoFluor II, PerSeptive Biosystems, Ex. 360 nm, Em. 460 nm, gain=70).





**Figure 4. Kinetics of FLUOR DE LYS® HDAC8 Substrate Deacetylation by HDAC8 Activity.** HDAC8 Enzyme (1U/well) was incubated (30°C) with indicated concentrations of substrate. Reactions were stopped after 10 min. with FLUOR DE LYS® Developer II and fluorescence measured (CytoFluor II, PerSeptive Biosystems, Ex. 360 nm, Em. 460 nm, gain=65). Points are the mean of three determinations and error bars are standard deviations from the mean. Line is a non-linear least squares fit of the data to the Michaelis-Menton equation (Delta Graph 4.0, Deltapoint, Inc.).



**Figure 5. Trichostatin A Inhibition of FLUOR DE LYS®-HDAC8 Substrate Deacetylation by HDAC8 Enzyme.** HDAC8 Enzyme (1U/well) was incubated (30°C) with 50  $\mu$ M substrate and indicated concentrations of Trichostatin A. Reactions were stopped after 10 min. with FLUOR DE LYS® Developer II and fluorescence measured (CytoFluor II, PerSeptive Biosystems, Ex. 360 nm, Em. 460 nm, gain=70).

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HDAC8 (recombinant, human)	BML-SE145
Trichostatin A (HDAC Inhibitor)	BML-GR309
SIRT1 (recombinant, human)	BML-SE239
Suberoyl bis-hydroxamic acid (HDAC Inhibitor)	BML-GR323
Scriptaid (HDAC Inhibitor)	BML-GR326
HC Toxin (HDAC Inhibitor)	BML-GR320
Anti-HDAC1 (polyclonal Ab)	BML-SA401
Anti-HDAC2 (polyclonal Ab)	BML-SA402
Anti-HDAC3 (polyclonal Ab)	BML-SA403
Anti-HDAC4 (polyclonal Ab)	BML-SA404



# Product Manual

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