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Enabling Discovery in Life Science®

HDAC1 Fluorimetric Drug Discovery Assay Kit

A *Fluor de Lys*® Fluorescent Assay System

Instruction Manual
BML-AK511

For research use only

✦HDAC1 Fluorimetric Drug Discovery Kit - BML-AK511✦

⚜ A *Fluor de Lys*® Fluorescent Assay System* ⚜

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[1]. The best studied of these modifications, ε-amino acetylations of specific histone lysine residues, are catalyzed by histone acetyltransferases (HATs). Histone deacetylases (HDACs) are responsible for removal of these acetyl groups[2,3,4]. Histone hyperacetylation correlates with an open, decondensed chromatin structure and gene activation, while hypoacetylation correlates with chromatin condensation and transcriptional repression[1-7].

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[8-17]. HDACs can associate with transcription repression complexes such as NuRD, Sin3A or N-CoR/SMRT[1-7,18].

Human HDAC1 (HD1) was the first protein to be linked to histone deacetylase activity[8]. It is homologous to the yeast protein Rpd3[8], a relationship which has since come to define the "class I HDACs"[11]. HDAC1 promotes transcriptional repression by deacetylating lysine ε-amino groups in histone N-terminal tails, a function frequently carried out in association with multi-protein transcription repression complexes such as NuRD[19], Sin3[20] and CoREST[21,22]. Ubiquitously expressed in human tissues[10,23], HDAC1-containing complexes appear to contribute the greater part of (at least class I) deacetylase activity in HeLa nuclear extracts[10]. (See also Fig. 6 in datasheet for HDAC Fluorimetric Assay/Drug Discovery Kit Cat. # BML-AK500). Aside from its interaction with co-repressors, HDAC1 activity may be regulated by post-translation modifications such as phosphorylation[24] and sumoylation[25] or binding to the inhibitor maspin, a tumor-suppressive serpin homolog[26]. Although originally described as a "histone deacetylase", HDAC1 has been shown to catalyze the regulatory deacetylation of non-histone proteins (see review[27]), including p53[28,29]. Overexpression of HDAC1 has been found in various cancer types[30-33]. HDAC inhibitors (HDACi) have shown considerable promise as anti-cancer agents and HDACi compounds from multiple chemical classes are in stages of drug development ranging from preclinical to phase III trials (see review[34]).

HDAC inhibitors have shown promise as anti-tumor agents and naturally this has stimulated interest in the screening of compounds for HDAC1 inhibition. Unfortunately, standard techniques for HDAC assay involve the use of [^{3}H]acetyl-histone or [^{3}H]acetyl-histone peptide substrates and a cumbersome acid/ethyl acetate extraction step prior to scintillation counting[8,35,36]. Enzo Life Sciences' *HDAC1 Fluorimetric 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 (Fig. 1).

REFERENCES

1. B.D. Strahl and C.D. Allis *Nature.* 2000 **403** 41
2. M. Grunstein *Nature.* 1997 **389** 349
3. H. H. Ng and A. Bird *Trends Biochem. Sci.* 2000 **25** 121
4. W. L. Cheung *et al. Curr. Opin. Cell Biol.* 2000 **12** 326
5. D. Kadosh and K. Struhl *Mol. Cell. Biol.* 1998 **18** 5121
6. S.E.C. Rundlett *et al. Nature* 1998 **392** 831
7. Y. Zhang *et al. Mol. Cell* 1998 **1** 1021
8. J. Taunton *et al. Science* 1996 **272** 408
9. W. M. Yang *et al. Proc. Natl. Acad. Sci. USA* 1996 **93** 12845
10. W. M. Yang *et al. J. Biol. Chem.* 1997 **272** 28001
11. C.M. Grozinger *et al. Proc. Natl. Acad. Sci. USA* 1999 **96** 4868
12. W. Fischle *et al. J. Biol. Chem.* 1999 **274** 11713
13. A. Verdel and S. Khochbin *J. Biol. Chem.* 1999 **274** 24440
14. A.H. Wang *et al. Mol. Cell. Biol.* 1999 **19** 7816
15. H.-Y. Kao *et al. Genes Dev.* 2000 **14** 55
16. E. Hu *et al. J. Biol. Chem.* 2000 **275** 15254
17. X. Zhou *et al. Proc. Natl. Acad. Sci. USA* 2001 **98** 10572
18. P.L. Jones and Y.B. Shi *Curr. Top. Microbiol. Immunol.* 2003 **274** 237
19. Y. Zhang *et al. Genes Dev* 1999 **13** 192
20. C.D. Laherty *et al. Cell* 1997 **89** 349
21. A. You *et al. Proc Natl Acad Sci U S A* 2001 **98** 1454
22. G.W. Humphrey *et al. J Biol Chem* 2001 **276** 6817
23. A.J. de Ruijter *et al. Biochem J* 2003 **370** 737
24. M.K. Pflum *et al. J Biol Chem* 2001 **276** 47733
25. G. David *et al. J Biol Chem* 2002 **277** 23658
26. X. Li *et al. Cancer Res* 2006 **66** 9323
27. M.A. Glozak *et al. Gene* 2005 **363** 15
28. L.J. Juan *et al. J Biol Chem* 2000 **275** 20436
29. J. Luo *et al. Nature* 2000 **408** 377
30. J.H. Choi *et al. Jpn J Cancer Res* 2001 **92** 1300
31. K. Halkidou *et al. Prostate* 2004 **59** 177
32. Z. Zhang *et al. Breast Cancer Res Treat* 2005 **94** 11
33. A.J. Wilson *et al. J Biol Chem* 2006 **281** 13548
34. W. S. Xu *et al. Oncogene* 2007 **26** 5541
35. A. Inoue and D. Fujimoto *Biochem. Biophys. Res. Commun.* 1969 **36** 146
36. P.A. Wade *et al. Methods Enzymol.* 1999 **304** 715

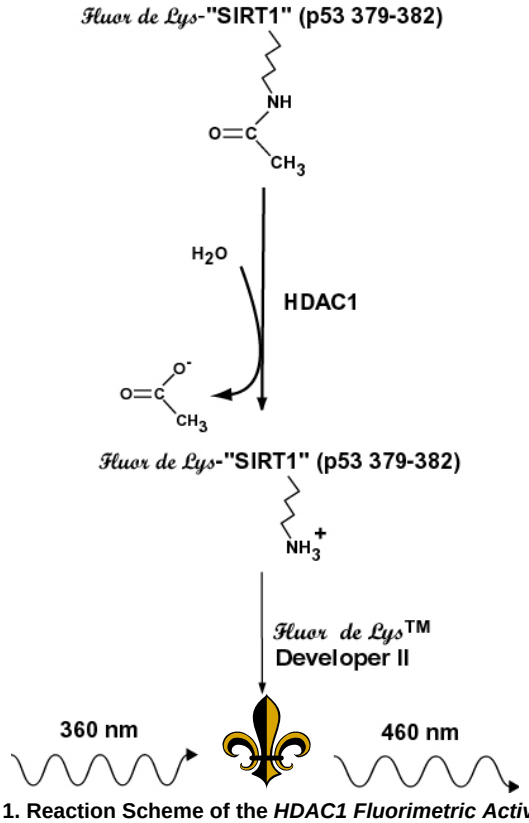


Figure 1. Reaction Scheme of the *HDAC1 Fluorimetric Activity Assay.** Deacetylation of the substrate sensitizes it to the Developer II, which then generates a fluorophore (symbol). The fluorophore is excited with 360 nm light and the emitted light (460 nm) is detected on a fluorometric plate reader.

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.

DESCRIPTION

The *HDAC1 Fluorimetric Drug Discovery Kit* is based on the ***Fluor de Lys®***-"SIRT1" (p53 379-382) Substrate (Cat. # BML-KI177) and Developer II (BML-KI176) 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 (Fig. 1). First, the ***Fluor de Lys®***-**"**SIRT1" Substrate, which comprises an acetylated lysine side chain, is incubated with HDAC1 (BML-SE456). Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the ***Fluor de Lys®*** Developer II produces a fluorophore. Despite the Substrate's name (it was first developed as a SIRT1 substrate), ***Fluor de Lys®***-"SIRT1" (BML-KI177) is an excellent substrate for HDAC1 (K_m= 19.3 µM, see Fig. 4)

COMPONENTS OF BML-AK511

BML-SE456-0050 HDAC1 (Histone Deacetylase 1) (human, recombinant)
FORM: 10 mM Tris, pH 7.5, 100 mM NaCl, 3mM $MgCl_2$, and 10% glycerol
STORAGE: -70°C; AVOID FREEZE/THAW CYCLES!
QUANTITY: 50 µg

BML-KI177-0005 ***Fluor de Lys®***–SIRT1, Deacetylase Substrate
FORM: 5 mM solution in 50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$
STORAGE: -70°C
QUANTITY: 100 µl

BML-KI176-1250 ***Fluor de Lys®*** Developer II Concentrate (5x)
FORM: 5x Stock Solution; Dilute in Assay Buffer before use.
STORAGE: -70°C
QUANTITY: 5 x 250 µl

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

BML-KI142-0030 ***Fluor de Lys®*** Deacetylated Standard
FORM: 10 mM in DMSO (dimethylsulfoxide)
STORAGE: -70°C
QUANTITY: 30 µl

BML-KI422-0020 HDAC ASSAY BUFFER II
(50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1 mg/ml BSA)
STORAGE: -70°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 $MgCl_2$)
STORAGE: -70°C
QUANTITY: 20 ml

80-2407 ½ VOLUME MICROPLATES
1 clear and 1 white, 96-well
STORAGE: Room temperature

OTHER MATERIALS REQUIRED

Microplate reading fluorimeter capable of excitation at a wavelength in the range 350-380 nm and detection of emitted light in the range 440-460 nm.
Pipetman or multi-channel pipetman capable of pipetting 2-100 µl accurately
Ice bucket to keep reagents cold until use.
Microplate warmer and/or other temperature control device (optional)

ASSAY PROCEDURES

Notes On Storage: *Store all components except the microplate and instruction booklet at -70°C for the highest stability. The HDAC1 Enzyme, BML-SE456, 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 -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 enzyme into separate tubes and store at -70°C.*

Some Things To Consider When Planning Assays:

1. The assay is performed in two stages. The first stage, during which the HDAC1 acts on the Substrate, is done in a total volume of 50 µl. The second stage, which is initiated by the addition of 50 µl of Developer II, stops HDAC activity and produces the fluorescent signal. See "Preparing Reagents For Assay" and Table 1 (p. 3).

   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 fluorimeter 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-KI110) 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, (p. 3).
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 HDAC1 for the ***Fluor de Lys®***-SIRT1 Substrate has been measured at 19.3 µM (Fig. 4). Use of substrate concentrations at or below K_m will help avoid substrate competition effects, which could mask the effectiveness of competitive inhibitors. For inhibition studies a final substrate concentration of 5-20 µM would be appropriate (see Fig. 5).
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—HDAC Assay Buffer II (BML-KI422) and HDAC Assay Buffer (BML-KI143). The first of these, BML-KI422, is for running the first phase of the assay, the HDAC1 deacetylation reaction itself. It should therefore be used for preparing all working dilutions of HDAC1 (BML-SE456), substrate (BML-KI177) and any compounds being screened for effects on HDAC1. 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®***- Developer II. It is therefore important to confirm that apparent HDAC1 inhibitor "hits" are in fact acting only via HDAC1 inhibition. One approach to this involves retesting the candidate inhibitor in a reaction with the ***Fluor de Lys®***-Deacetylated Standard (BML-KI142) plus the ***Fluor de Lys®***-Developer II. A detailed retesting procedure is described below, in the section "Uses Of The ***Fluor de Lys®***- Deacetylated Standard" (p. 3-5). In some cases, it may be possible to avoid this retesting by means of measurements taken during the fluorescence development phase of the initial HDAC1 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 HDAC1 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 HDAC1 Enzyme (BML-SE456) will be diluted in HDAC Assay Buffer II (BML-KI422). Dilutions of the HDAC1 in which 15 µl (volume used per well) contains 0.1-0.5 µg of the enzyme are appropriate (see Table 1, Figs. 3-5). Volume of diluted enzyme required to provide for the assays to be performed = # of wells x 15 µl. 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 HDAC Assay Buffer II (BML-KI422). Since 10 µl will be used per well (Table 1), 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®***-SIRT1 Substrate (BML-KI177; 5 mM) in HDAC Assay Buffer II (BML-KI422) that will be 2x the desired final concentration(s). For inhibitor screening, final substrate concentrations in the range of 5 µM-20 µM are recommended. Twenty-five µl will be used per well (Table 1).
5. Shortly before use (<30 min.), prepare sufficient ***Fluor de Lys®*** Developer II for the assays to be performed (50 µl per well). First, dilute the ***Fluor de Lys®*** Developer II Concentrate 5-fold (e.g. 250 µl plus 1000 µl 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 µl in 1.25 ml; final Trichostatin A concentration in the 1x Developer II = 2 µM; final concentration after addition to HDAC/Substrate reaction = 1 µM). 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 HDAC Assay Buffer II, 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 HDAC Assay Buffer II for diluting the enzyme to assay temperature. Add chilled, undiluted HDAC1 to the warmed buffer.
3. Add diluted HDAC1 to all wells except those that are to be "No Enzyme Controls."
4. Initiate HDAC1 reactions by adding diluted substrate (25 µl) to each well and mixing thoroughly.
5. Allow HDAC1 reactions to proceed for desired length of time and then stop them by addition of ***Fluor de Lys®*** Developer II (50 µl) prepared in step #5 (p.3). Incubate plate at room temperature (or 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 fluorimeter 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[6]

Sample	HDAC Assay Buffer II	HDAC1 (Dilution)	Inhibitor (5x)	*Fluor de Lys®* -SIRT1 Substrate (BML-KI177) (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 II.

‡ Refers to dilution of Trichostatin A in HDAC Assay Buffer II, 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 HDAC1 inhibition; 2) As a model inhibitor "hit", 10 nM would produce final 2 nM and ~50% inhibition.

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

USES OF THE *Fluor de Lys®* DEACETYLATED STANDARD (BML-KI142)

Preparation of a Standard Curve:

1. The exact concentration range of the ***Fluor de Lys®*** Deacetylated Standard (BML-KI142) that will be useful for preparing a standard curve will vary depending on the fluorimeter 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 HDAC Assay Buffer II (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®*** Deacetylated Standard dilutions that span this range. Pipet 50 µl of each of these dilutions, and 50 µl of HDAC Assay Buffer II 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 II for the standard wells (50 µl per well).
4. Mix 50 µl of the Developer II with the 50 µl in each standard well and incubate 5-10 min. at room temperature (or 30°C).

5. Read samples in a microplate reading fluorimeter 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®*** Deacetylated Standard (x-axis). Determine slope as AFU /µM. See example in Fig. 2.

Testing of Potential HDAC1 Inhibitors for Interference with the ***Fluor de Lys®*** Developer II or the Fluorescence Signal:

1. The ***Fluor de Lys®*** 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 HDAC1 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®*** Deacetylated Standard is added (e.g. amount equivalent to 5 µM in the 50 µl HDAC1 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/µM, 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 HDAC1 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®*** 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 HDAC1, 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®*** 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) HDAC1 reaction. Mix 40 µl of the diluted Standard with 10 µl inhibitor or 10 µl HDAC Assay Buffer II (see Table 2). Initiate development by adding 50 µl 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 µl) and diluting the Developer II Concentrate 2.5-fold, rather 5-fold. All three of these approaches may be used separately or in combination.

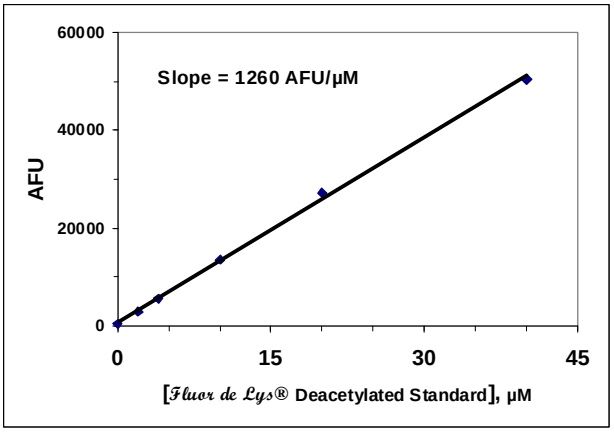


Figure 2. Fluorescence Standard Curve. Fifty µl aliquots of ***Fluor de Lys®*** Deacetylated Standard, in Assay Buffer at the indicated concentrations, were mixed with 50 µl Developer II and incubated 10 min., 30°C. Fluorescence was then measured in the wells of the clear microtiter-plate (BML-KI101) 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	HDAC Assay Buffer II	Inhibitor (5x)	Diluted[6] *Fluor de Lys*® deAc. Standard (1.25x)	DEVELOPER II (1x).
Control	10 µl	0	40 µl	50 µl
Tricho-statin A‡	0	10 µl	40 µl‡	50 µl
Test Inhibitor⌘	0	10 µl	40 µl⌘	50 µl

[6]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 HDAC1 assay. The dilution in HDAC Assay Buffer II 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.

‡Refers to dilution of Trichostatin A in HDAC Assay Buffer II, 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.

⌘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 *HDAC1 Fluorescent Activity Assay/Drug Discovery Kit* has been used to investigate the kinetics of *Fluor de Lys*®–SIRT1 (BML-KI177) deacetylation by HDAC1 enzyme (Figures 3 & 4) and the inhibition of HDAC1 by the inhibitors trichostatin A and BML-210.

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.

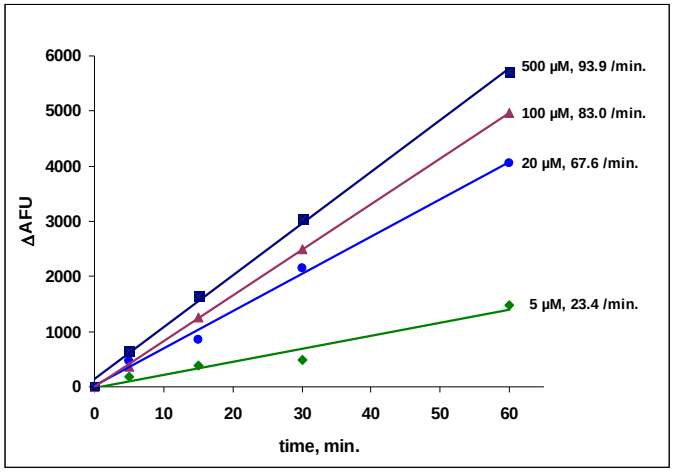


Figure 3. Time Courses of *Fluor de Lys*®-SIRT1 Substrate (BML-KI177) Deacetylation by HDAC1. HDAC1 Enzyme (250 ng/well) was incubated (37°C) with indicated concentrations of substrate. Reactions were stopped at indicated times with *Fluor de Lys*® Developer II and fluorescence measured (CytoFluor™ II, PerSeptive Biosystems, Ex. 360 nm, Em. 460 nm, gain=70). Data labels include deacetylation rates (AFU/min.) determined from linear best-fits to the data for each concentration of BML-KI177.

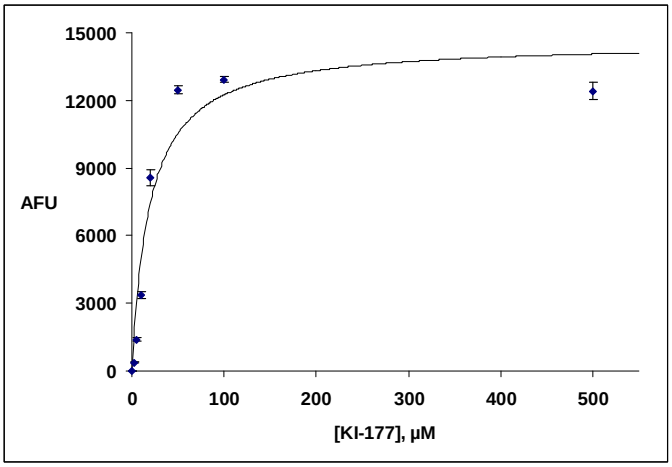
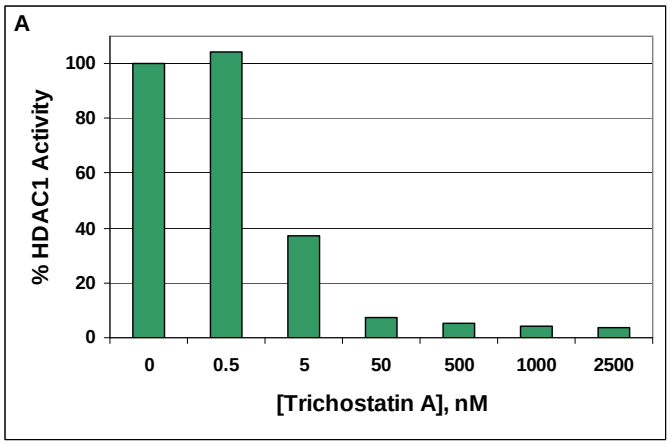


Figure 4. Kinetics of *Fluor de Lys*®–SIRT1 (BML-KI177) Substrate Deacetylation by HDAC1. HDAC1 Enzyme (100 ng/well) was incubated (37°C) with indicated concentrations of substrate. Reactions were stopped after 60 min. with *Fluor de Lys*® Developer II and fluorescence measured (CytoFluor™ II, PerSeptive Biosystems, Ex. 360 nm, Em. 460 nm, gain=70). 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.). The best-fit K_m for BML-KI177 was 19.3 µM and the V_{max} 14,600 AFU/hr.



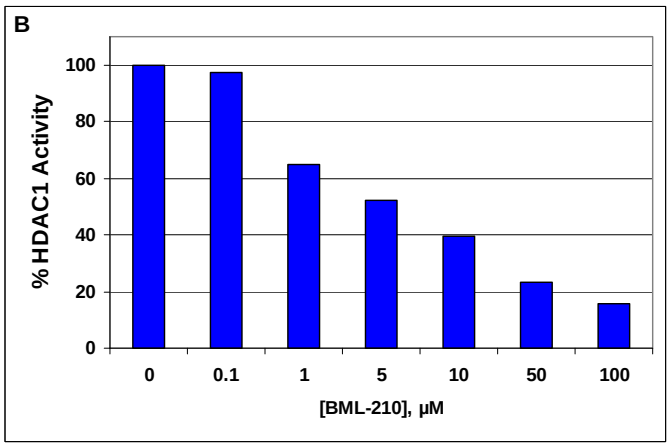


Fig. 5 Trichostatin A and BML-210 (Cat. #BML-GR330) Inhibition of HDAC1 Determined by *Fluor de Lys*®-SIRT1 (BML-KI177) Substrate Deacetylation. HDAC1 Enzyme (250 ng/well) was incubated (37°C) with 5 µM substrate at indicated concentrations of Trichostatin A (Panel **A**) and BML-210 (Panel **B**). Reactions were stopped after 60 min. with *Fluor de Lys*® Developer II and fluorescence measured (CytoFluor™ II, PerSeptive Biosystems, Ex. 360 nm, Em. 460 nm, gain=70).

LITERATURE CITATIONS OF *Fluor de Lys*® PRODUCTS

X. Zhou *et al. Proc. Natl. Acad. Sci. USA* 2001 **98** 10572
B. Heltweg and M. Jung *Anal. Biochem.* 2002 **302** 175
S. Milutinovic *et al. J. Biol. Chem.* 2002 **277** 20974
K. Ito *et al. Proc. Natl. Acad. Sci. USA* 2002 **99** 8921
K.J. Bitterman *et al. J. Biol. Chem.* 2002 **277** 45099
G.V. Kapustin *et al. Org. Lett.* 2003 **5** 3053
K.T. Howitz *et al. Nature* 2003 **425** 191
K. Zhao *et al. Nat. Struct. Biol.* 2003 **10** 864
D.-K. Kim *et al. J. Med. Chem.* 2003 **46** 5745
R.M. Anderson *et al. Science* 2003 **302** 2124
T. Suzuki *et al. Bioorg. Med. Chem. Lett.* 2003 **13** 4321
L.H. Wang *et al. Nature Medicine* 2004 **10** 40
C.M. Gallo *et al. Mol. Cell. Biol.* 2004 **24** 1301
N. Gurvich *et al. Cancer Res.* 2004 **64** 1079
F. Yeung *et al. EMBO J.* 2004 **23** 2369
J.G. Wood *et al. Nature* 2004 **430** 686
B.G. Cosío *et al. Am. J. Respir. Crit. Care Med.* 2004 **170** 141
J.L. Avalos *et al. Mol. Cell* 2005 **17** 855
T. Suzuki *et al. J. Med. Chem.* 2005 **48** 1019
K. Ito *et al. N. Engl. J. Med.* 2005 **352** 1967
A. Mai *et al. J. Med. Chem.* 2005 **48** 7789
A. D. Napper *et al. J. Med. Chem.* 2005 **48** 8045
V.C. de Boer *et al. Mech. Ageing Dev.* 2006 **127** 618
S.L. Gantt *et al. Biochemistry* 2006 **45** 6170
W. Gu *et al. Bioorg. Med. Chem.* 2006 **14** 3320
D. Herman *et al. Nature Chem. Biol.* 2006 **10** 551
X. Li et al. Cancer Res. 2006 66 9323
P. Aksoy et al. Biochem. Biophys. Res. Commun. 2006 349 353
J.M. Solomon et al. Mol. Cell. Biol. 2006 26 28
V.M. Nayagam et al. J. Biomol. Screen. 2006 doi:10.1177/1087057106294710
P.H. Kiviranta et al. Bioorg. Med. Chem. Lett. 2007 17 2448
D.H. Kim et al. Biochem. Biophys. Res. Commun. 2007 356 233
T.F. Outeiro et al. Science 2007 **317** 516
S. Lain *et al. Cancer Cell* 2008 **13** 454
X. Hou *et al. J. Biol. Chem.* 2008 **283** 20015
Y. Nakahata *et al. Cell* 2008 **134** 329
S. Rashid *et al. J. Biol. Chem.* 2009 **284** 18115
Y. Chung *et al. Carcinogenesis* 2009 **30** 1387
H. Nian *et al. Carcinogenesis* 2009 **30** 1416
S. Agbor-Enoh *et al. Antimicrob. Agents Chemother.* 2009 **53** 1727
B.G. Cosio *et al. Thorax* 2009 **64** 424
P.D. N'Guessan *et al. Arterioscler. Thromb. Vasc. Biol.* 2009 **29** 380
J. Chen *et al. Blood* 2009 **113** 4038

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