TECHNICAL MANUAL

HDAC-Glo™ Class IIa Assay

Instructions for Use of Product
G9560

Revised 5/15
TM407

HDAC-Glo™ Class IIa Assay

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The HDAC-Glo™ Class IIa Assay[a–c] is a single-reagent-addition, homogeneous, luminescence assay that selectively measures the relative activity of histone deacetylase (HDAC) class IIa enzymes from cells, extracts or recombinant sources. The assay uses an isoenzyme-selective, acetylated, live-cell permeant, luminogenic peptide substrate that can be deacetylated by HDAC class IIa enzymes. Deacetylation of the peptide substrate is measured using a coupled enzymatic system in which a protease in the Developer Reagent cleaves the peptide from amino-luciferin. The resulting luminescence is quantified in a reaction using Ultra-Glo™ Recombinant Luciferase (Figure 1).

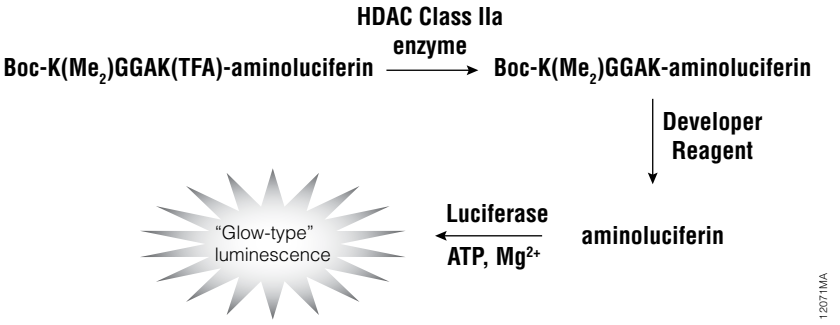

Figure 1. HDAC-Glo™ Class IIa Assay chemistry. HDAC Class IIa enzyme deacetylates the luminogenic substrate making the peptide sensitive to a specific proteolytic cleavage event that liberates aminoluciferin. Free aminoluciferin can then be measured using the firefly luciferase reaction to produce a stable, persistent emission of light.

Note: The three enzymatic events occur in a coupled, nearly simultaneous reaction that is proportional to deacetylase activity.

Assay Advantages

- **Simple Measurement of Deacetylating Activities:** Single-reagent-addition, homogeneous "add-mix-measure" protocol.
- **Sensitive:** The assay provides 100-fold or better sensitivity than comparable fluorescence methods.
- **Utility:** The assay may be used with recombinant enzyme sources or in a cell-based format.
- **Fast Data Acquisition:** Collect maximal signal in as few as 20 minutes with persistent, "glow-type" steady state signal half-life.

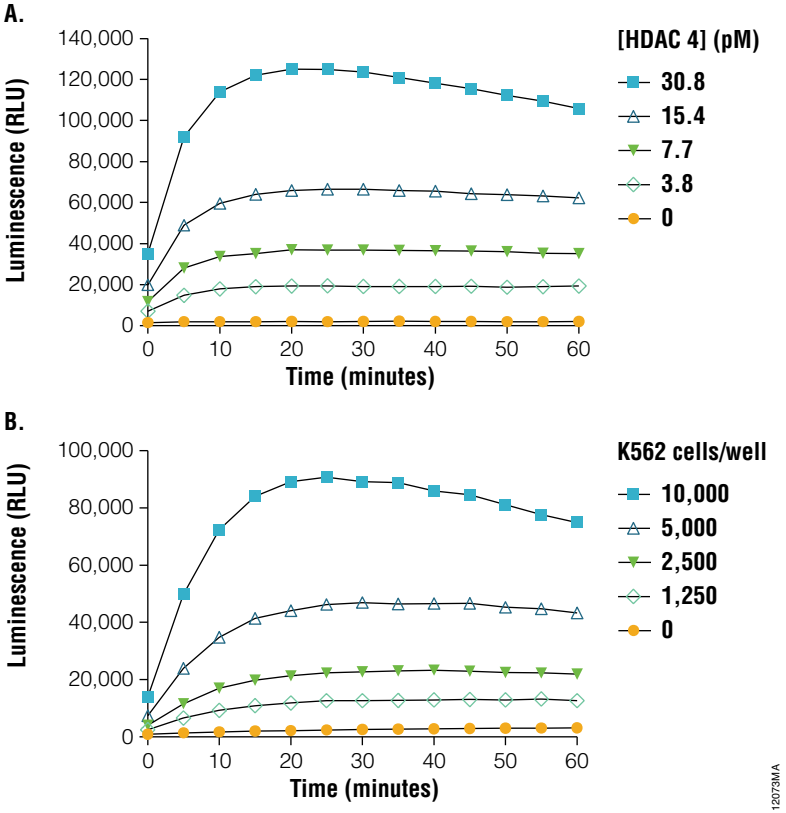

Figure 2. The HDAC-mediated luminescent signal is proportional to deacetylase activity. HDAC-mediated signal is persistent in a purified biochemical assay (**Panel A**) and in a cell-based lytic assay (**Panel B**). Enzymatic steady state (between deacetylase, developer enzyme and luciferase) is typically achieved within 10 minutes for biochemical assays and within 15–20 minutes for cell-based lytic assays, with a half-life of approximately 60–90 minutes after steady state is achieved.

2. Product Components and Storage Conditions

Product	Size	Cat.#
HDAC-Glo™ Class IIa Assay	**10ml**	**G9560**

This system contains sufficient reagents for 100 × 100µl assays in 96-well plates or 1,000 × 10µl biochemical-based assays in 384-well plates (500 × 20µl cell-based assays in 384-well plates). Includes:

- 25ml HDAC-Glo™ Class IIa Assay Buffer
- 70µl HDAC-Glo™ Class IIa Substrate, 10mM
- 10µl Developer Reagent
- 1 vial Luciferin Detection Reagent

Storage Conditions: Store the HDAC-Glo™ Class IIa Assay components at –20°C. For HDAC-Glo™ Class IIa Assay Buffer, HDAC-Glo™ Class IIa Substrate and Luciferin Detection Reagent, thaw and equilibrate to room temperature prior to use (see Section 3). For Developer Reagent, thaw and store on ice until addition to the substrate solution just prior to assay. Protect HDAC-Glo™ Class IIa Substrate from light exposure.

Additional Storage Considerations: Use HDAC-Glo™ Class IIa Final Detection Reagent on the day it is prepared. Prepare HDAC-Glo™ Class IIa Final Detection Reagent immediately prior to assaying samples. If HDAC-Glo™ Class IIa Final Detection Reagent cannot be used immediately for assay of HDAC activity, hold it on ice protected from light until use (with brief equilibration to room temperature before adding to sample). We do not recommend storing beyond 8 hours due to stability issues. In the event that an entire kit will not be used in one experiment, rehydrate Luciferin Detection Reagent with HDAC-Glo™ Class IIa Assay Buffer, add HDAC-Glo™ Class IIa Substrate (follow Step 1, Section 3) and divide HDAC-Glo™ Class IIa Substrate solution into two aliquots. Supplement the aliquot to be used immediately with a proportional volume of Developer Reagent to create HDAC-Glo™ Class IIa Final Detection Reagent, and freeze the remaining aliquot of HDAC-Glo™ Class IIa Substrate Solution.

3. Preparation of Reagents

Figure 3 provides a schematic diagram of the following detailed instructions.

1. Thaw HDAC-Glo™ Class IIa Assay Buffer and HDAC-Glo™ Class IIa Substrate. Add 10ml of HDAC-Glo™ Class IIa Assay Buffer to Luciferin Detection Reagent (add 9ml for lytic cell-based assays). Add 70µl of 10mM HDAC-Glo™ Class IIa Substrate to Luciferin Detection Reagent. Incubate at 37°C for 1 hour.
2. Thaw Developer Reagent on ice. Add 10µl of Developer Reagent to HDAC-Glo™ Class IIa Substrate solution prepared in Step 1 to make HDAC-Glo™ Final Detection Reagent. For lytic cell-based assays add 1ml of 10% Triton® X-100 in HDAC-Glo™ Class IIa Assay Buffer to make HDAC-Glo™ Final Detection Reagent. Incubate at room temperature for 15 minutes.

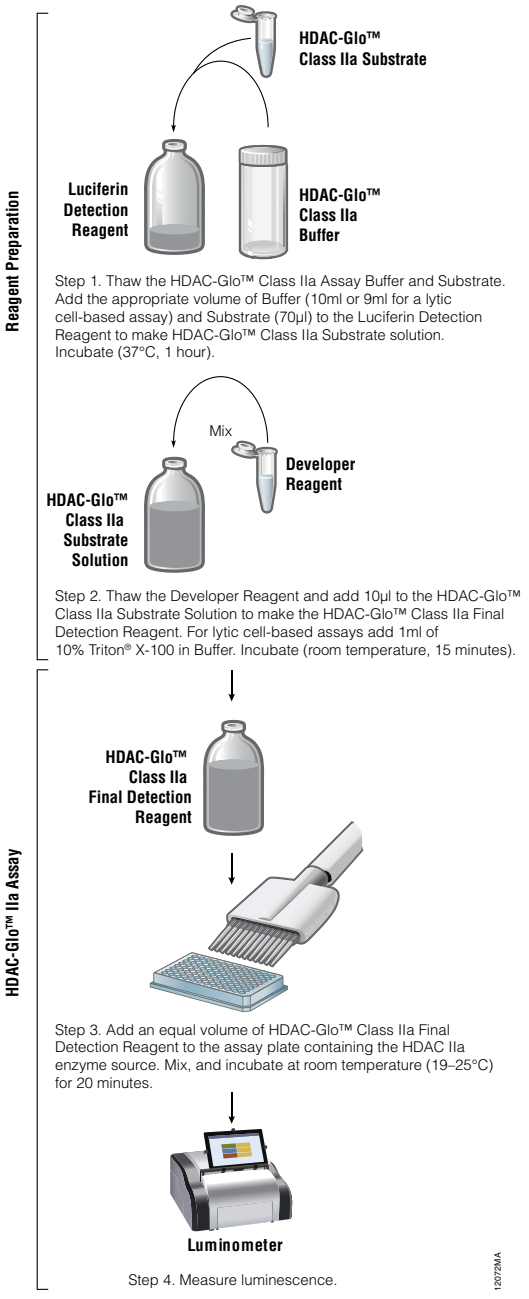

Figure 3. Reagent preparation and assay procedure. See Section 3 for detailed reagent preparation information.

4. Protocols

Materials to Be Supplied by the User

- 96-, 384- or 1536-well, white-walled tissue culture plates compatible with luminometer. Recommended 96-well plates: Corning Costar® Cat.# 3917 (white bottom) or Corning Costar® Cat.# 3903 (clear bottom); for 384-well plates: Corning Cat.# 3673 or 3674 (biochemical), Corning Cat.# 3570 (cell-based)
- multichannel pipettor or liquid-dispensing robot
- reagent reservoirs
- orbital shaker
- recombinant HDAC 4, HDAC 5, HDAC 7 or HDAC 9 enzyme source **or** cells that have HDAC class IIa activity (such as K562 cells)
- Triton® X-100
- DMSO
- control inhibitor compound (e.g., TMP269)

4.A. HDAC Class IIa Inhibitor Potency Determination using Recombinant Enzyme

See Figure 4, Panel A, for sample IC_{50} data from a biochemical assay.

1. Follow Step 1 of Figure 3 (see Section 3 for detailed reagent preparation information). During the 1-hour incubation at 37°C, prepare compound dilution series in a parallel plate as follows:
    a. Prepare threefold serial dilutions of the unknown compound or TMP269 control at 100X of the final assay concentration in 100% DMSO. Be sure to include a no-compound (DMSO-only) control.
    b. Transfer the 100X compound dilution series to HDAC-Glo™ Class IIa Assay Buffer to obtain a final 2X/2% DMSO concentration (i.e., 5µl of 100X compound + 245µl of HDAC-Glo™ Class IIa Assay Buffer). This is the master intermediate dilution series of compound.
    c. Transfer 50µl of each dilution from the 2X compound dilution series (Step1.b) to the white 96-well assay plate (5µl for a 384-well plate). Replicates should be prepared from the same master intermediate dilution series, and DMSO percentage should be equal across the inhibitor titration.
2. Dilute HDAC Class IIa enzyme source (to 2X final desired enzyme concentration) in HDAC-Glo™ Class IIa Assay Buffer and dispense into inhibitor dilutions and no-compound controls in 50µl volumes to the white 96-well assay plate (5µl for a 384-well plate). All assay components should now be at 1X concentration and 1% DMSO. **Note:** An HDAC IIa enzyme titration may be necessary before inhibitor titrations are made, to determine the optimal concentration of HDAC IIa enzyme to use per well.
3. Mix briefly using an orbital shaker at 500–700rpm to ensure homogeneity.
4. Incubate enzyme/inhibitor mixes for at least 30 minutes at room temperature (19–25°C).
5. Prepare HDAC-Glo™ Class IIa Final Detection Reagent (see Step 2 of Figure 3 or Section 3). Incubate for 15 minutes at room temperature.
6. Add 100µl of HDAC-Glo™ Class IIa Final Detection Reagent to each well (10µl for a 384-well plate).
7. Mix briefly using an orbital shaker at 500–700rpm to ensure homogeneity.
8. Incubate for 20 minutes at room temperature to achieve enzyme steady state, then measure luminescence.

Table 1. Recommended Plate Layout (96-well plate format). Shaded (upper) portion of Table 1 is an example of an unknown compound dilution series (dilution factor is shown). Nonshaded (lower) portion of the table is an example of the control inhibitor (TMP269 = 1X nM).

Initial	1:3	1:9	1:27	1:81	1:243	1:729	1:2,187	1:6,561	1:19,683	1:59,049	0
Initial	1:3	1:9	1:27	1:81	1:243	1:729	1:2,187	1:6,561	1:19,683	1:59,049	0
Initial	1:3	1:9	1:27	1:81	1:243	1:729	1:2,187	1:6,561	1:19,683	1:59,049	0
Initial	1:3	1:9	1:27	1:81	1:243	1:729	1:2,187	1:6,561	1:19,683	1:59,049	0
10,000	3,333.3	1,111.1	370.4	123.5	41.2	13.7	4.6	1.5	0.5	0.2	0
10,000	3,333.3	1,111.1	370.4	123.5	41.2	13.7	4.6	1.5	0.5	0.2	0
10,000	3,333.3	1,111.1	370.4	123.5	41.2	13.7	4.6	1.5	0.5	0.2	0
10,000	3,333.3	1,111.1	370.4	123.5	41.2	13.7	4.6	1.5	0.5	0.2	0

4.B. HDAC Class IIa Inhibitor Potency Determination using Cells

See Figure 4, Panel B, for sample IC_{50} data from a cell-based assay.

1. Seed 50µl of attachment-dependent cells into a white 96-well plate at a density of about 20,000 cells/well (about 10,000 cells/well in 10µl for a 384-well plate). Allow cells to attach by incubation at 37°C.

   Note: Suspension cells can be added directly to inhibitor dilutions in Step 4 at a desired number of cells/well (50µl per well for a 96-well plate; 10µl per well for a 384-well plate). Best assay performance is achieved in serum-free medium.

   Note: A cell titration may be needed before inhibitor titrations are made to determine the optimal number of cells to use per well in either a lytic or nonlytic format.

2. Follow Step 1 of Figure 3 (see Section 3 for detailed reagent preparation information). During the 1-hour incubation, prepare a compound dilution series in a parallel plate as follows:

   a. Make threefold serial dilutions of the unknown compound or TMP269 control at 100X concentration in 100% DMSO. Be sure to include a no-compound (DMSO-only) control.

   b. Transfer the 100X compound dilution series to serum-free culture medium to obtain a final 2X/2% DMSO concentration (i.e., 5µl of 100X compound + 245µl of serum-free culture medium). This is the master intermediate dilution series of compound.

3. Remove culture medium from attachment-dependent cells by gentle aspiration and replace with 50µl of serum-free medium for a 96-well plate format (10µl for a 384-well format).
4. Transfer 50µl of each dilution from the 2X master intermediate dilution series (prepared in Step 2.b) to the white 96-well assay plate (10µl for a 384-well plate). Replicates should come from the same master intermediate dilution series, and DMSO percentage should be equal across the inhibitor titration.
5. Mix briefly using an orbital shaker at 500–700rpm to ensure homogeneity.
6. Incubate cell/inhibitor mixes for at least 30 minutes at room temperature.

4.B. HDAC Class IIa Inhibitor Potency Determination using Cells (continued)

7. Prepare HDAC-Glo™ Class IIa Final Detection Reagent (see Step 2 of Figure 3 or Section 3). Incubate for 15 minutes at room temperature.

   Note: The HDAC-Glo Class IIa Substrate is moderately cell permeant, making nonlytic cell formats possible. However, a greater signal window (3–4X) can be achieved in a lytic assay format by adding Triton® X-100 to the Reagent for a final concentration of 1%. If performing the assay in nonlytic mode, it will be necessary to increase the number of cells per assay well to increase the signal window.

8. Add 100µl of HDAC-Glo™ Class IIa Final Detection Reagent to each well (20µl for a 384-well plate).

9. Mix briefly using an orbital shaker at 500–700rpm to ensure homogeneity.

10. Incubate for 20 minutes at room temperature (19–25°C) to achieve enzyme steady state, then measure luminescence.

5. General Considerations

5.A. Background Luminescence and Inherent Serum Activity

Animal serum used to supplement tissue culture medium may contain detectable levels of the deacetylase activity. Because the assay results can be collected within less than 1 hour, we recommend that you use serum-free medium during the assay. If serum is used, a cell-free medium control should be used as a background control. For longer incubation periods with compounds/cells, when serum-supplemented media is essential, the assay reagent may take a longer period of time to reach a steady state luminescent signal, especially in a nonlytic format.

5.B. Temperature

The enzymes measured in this assay are influenced by temperature. For best results, incubate at a constant controlled temperature to ensure uniformity across the plate. After compound addition and a brief mix, pre-incubate/equilibrate to room temperature (19–25°C) prior to adding the Final Detection Reagent.

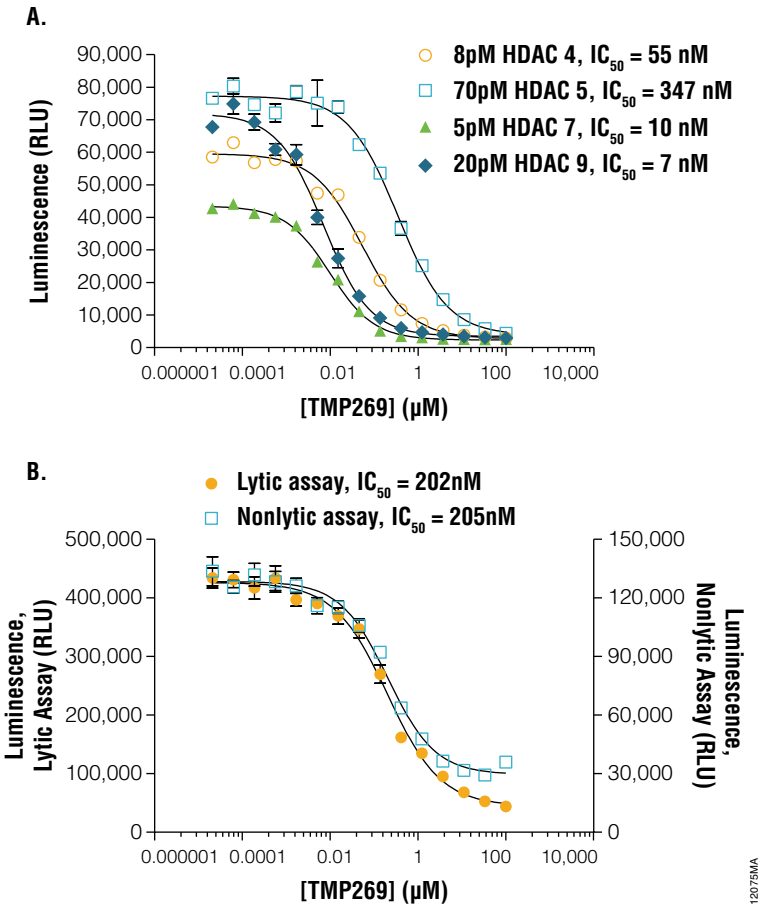

Figure 4. Sample potency data for the HDAC-Glo™ Class IIa Assay. Panel A, biochemical format. Assay was performed using recombinant HDAC 4, 5, 7 and 9 as the enzyme source (1X = 8, 70, 5 and 20pM, respectively) in a 384-well plate format (Corning Cat.# 3673) at a final assay volume of 20µl. **Panel B, cell-based format**. Assay was performed using K562 cells as the HDAC IIa enzyme source (2,500 cells/well in lytic format; 10,000 cells/well in a nonlytic format) in a 384-well plate (Corning Cat.# 3570) at a final assay volume of 40µl. Final concentration of HDAC-Glo™ Class IIa Substrate per well for all experiments was 35µM. Data was plotted and IC_{50} determined using GraphPad Prism® software.

5.C. Incubation Time and Signal Stability

Enzymatic steady state is typically achieved after approximately 20 minutes at room temperature. We recommend that luminescence be measured after this signal plateau is achieved (20 minutes at room temperature or within 30 minutes thereafter). Signal will gradually decay as a function of time with a half-life of about 60–90 minutes after steady state is achieved. If the assay plate is read before enzymatic steady state, variation may be seen in replicates from one end of the assay plate to the other end depending on the plate reader pattern. This is especially important in a 384-well plate, where it can take up to 5 minutes to read a single plate. We recommend performing an enzyme or cell titration before running additional experiments in order to determine the optimal concentration of enzyme or number of cells/well to use. After performing an enzyme or cell titration, use a concentration of enzyme or number of cells/well in the lower end of the linear portion of the assay that still gives an acceptable signal:background ratio. If too much enzyme is added or too many cells/well are added you can oversaturate the assay, and once steady state is achieved, the signal will decay faster as a function of time. If the amount of enzyme or number of cells/well is in the lower end of the linear portion of the assay once steady state is achieved, the signal is more "stable" and will decay slower as a function of time. Figure 5 illustrates this principle. Another factor that can affect signal stability is the stability of the recombinant HDAC enzyme itself. We have found certain HDAC isoforms to be more stable than other isoforms. We have also found that enzyme stability can vary from vendor to vendor with the same HDAC isoform. If running biochemical assays, it may be useful to evaluate samples from multiple vendors for a given HDAC isoform.

5.D. Assay Controls

Medium or Buffer Background Control: Set up at least triplicate wells without cells or enzyme to serve as the negative control to determine background luminescence.

Untreated Cells or Uninhibited Enzyme Control: The maximal signal control is established by the addition of vehicle only (used to deliver the test compound to test wells). In most cases, this consists of a buffer system or medium and solvent (DMSO, methanol, etc.) diluted to the same concentration as found in the treatment. Set up at least triplicate wells with untreated cells or uninhibited enzyme. Add the same solvent used to deliver the test compounds to the vehicle control wells.

Optional Inhibitor Control: Set up a dilution series using a known HDAC class IIa inhibitor as a control for known inhibition of HDAC class IIa activity from cells or recombinant enzyme source.

5.E. High-Throughput Screening (HTS) Considerations

When conducting a compound screen in a high-throughput format, it is often beneficial to add a small amount of detergent (such as Triton® X-100) to the final detection reagent whether the assay is run in a biochemical or cell-based format. Although statistically rare, the detergent can reduce the possibility of "false hits"—or compounds that may inhibit luciferase activity. The HDAC-Glo™ Class IIa Assay is robust and resilient to assay interferences. However, a small false-inhibition rate is possible through interference with 1) the Developer Reagent; or 2) the luciferase detection component. We have developed the non-acetylated HDAC-Glo™ I/II Control Substrate, which can be used with the HDAC-Glo™ Class IIa Assay to confirm HDAC inhibition in secondary screens. The non-acetylated HDAC-Glo™ I/II Control Substrate (Cat.# G6550) can be purchased separately. Assays with the HDAC-Glo™ I/II Control Substrate do not require deacetylation to produce luminescence and therefore reveal non-HDAC interferences.

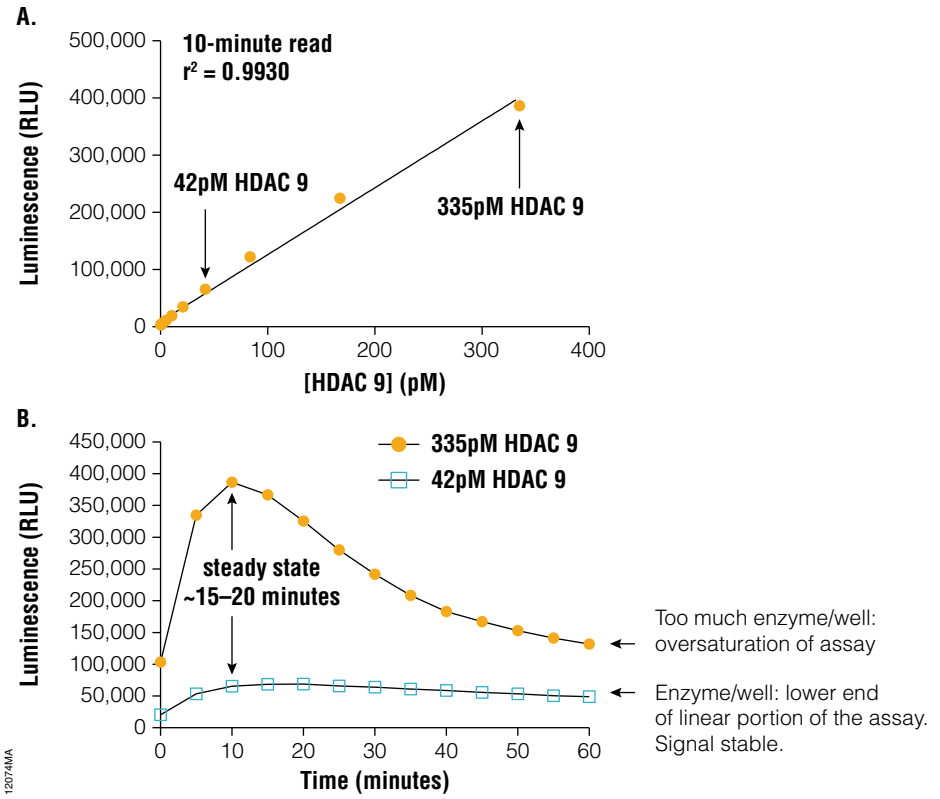

Figure 5. Example of linear range data for the HDAC-Glo™ Class IIa Assay and signal stability in a biochemical format. Panel A shows the linear range of the HDAC-Glo™ Class IIa Assay. **Panel B** shows that if the amount of enzyme or number of cells/well is at the lower end of the linear portion of the assay, acceptable signal:background ratios can be achieved once steady state is reached. That signal is stable with a half-life of about 60–90 minutes after steady state is reached.

5.F. IC_{50} Value Considerations in Cell-Based Assay Format

Sometimes differences in IC_{50} values can be observed when performing a cell-based assay in a lytic format versus a nonlytic format. There are many possible explanations for this including (but not limited to): 1) lack of compound cell permeability; or 2) the lytic assay conditions adversely affecting certain compound/enzyme interactions. Therefore, when performing IC_{50} value determinations in a cell-based assay, we recommend performing the assay in both a lytic and nonlytic format.

6. Supplemental Data

Table 2. HDAC-Glo™ Class IIa Substrate K_m determination. An 11-point, twofold serial titration of HDAC-Glo™ Class IIa Substrate was performed in HDAC-Glo™ Class IIa Assay Buffer (point 12 was a no-substrate control). After the titration series samples were incubated at 37°C for 1 hour, a proportional volume of Developer Reagent was added to each titration series sample and incubated at room temperature for 15 minutes. For each titration series sample, 100µl was added to wells of a white 96-well plate (Corning Costar® Cat.# 3917). To this, 100µl of two separate HDAC class IIa enzyme concentrations (n = 3 replicates each) and no-enzyme controls (n = 2 replicates) were added to start the reaction. Luminescence was measured after 10 minutes at room temperature. The background luminescence for each substrate concentration (determined from the no-enzyme controls) was subtracted from each substrate concentration data point for the two separate HDAC class IIa enzyme tests. Data was plotted and K_m was determined using GraphPad Prism® software.

Enzyme	HDAC-Glo™ Class IIa Substrate K_m (µM)	10mM Substrate/10ml Rehydrated Luciferin Detection Reagent
HDAC 4	13	26µl (2X = 26µM)
HDAC 5	61	122µl (2X = 122µM)
HDAC 7	32	64µl (2X = 64µM)
HDAC 9	33	66µl (2X = 66µM)

Note: Individual, purified class IIa enzymes can be assayed at their respective K_m by creating a 2X substrate solution. We have empirically determined that mixed pools of class IIa activity found in lysates or cells are best represented and measured using a K_m of 35µM (70µl of substrate per 10ml of rehydrated Luciferin Detection Reagent).

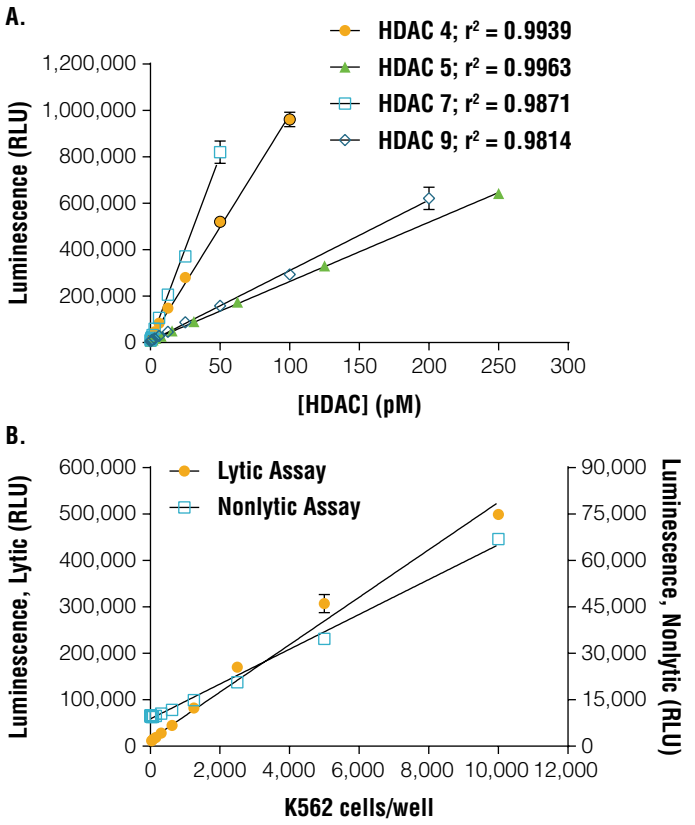

Figure 6. Examples of linear range data for the HDAC-Glo™ Class IIa Assay. Panel A, biochemical format. Recombinant HDAC IIa enzymes were diluted to the appropriate starting concentration in HDAC-Glo™ Class IIa Assay Buffer, then serially diluted twofold (15 points, plus no-enzyme control) in 100µl volumes in a 96-well plate. From this master enzyme titration series, 10µl replicates (n = 4) were transferred to a 384-well white assay plate (Corning Cat.# 3673). An equal volume (10µl) of HDAC-Glo™ Class IIa Final Detection Reagent was added to all wells and luminescence was measured after 20 minutes at room temperature. **Panel B, cell-based format (lytic and nonlytic).** K562 cells were diluted to the appropriate number of cells/ml in serum-free media, then serially diluted twofold (15 points, plus no-enzyme control) in 100µl volumes in a 96-well plate. From this master cell titration series (both lytic and nonlytic assay titrations start at 10,000 cells/well), 20µl replicates (n = 4) were transferred to a 384-well white assay plate (Corning Cat.# 3570). An equal volume (20µl) of HDAC-Glo™ Class IIa Final Detection Reagent (with 1% Triton® X-100 for lytic assay) was added to all wells and luminescence was measured after 15 minutes at room temperature. For both assays, the final concentration of HDAC-Glo™ Class IIa Substrate was 35µM. The data for both figures was plotted and linearity determined using GraphPad Prism® software.

6. Supplemental Data (continued)

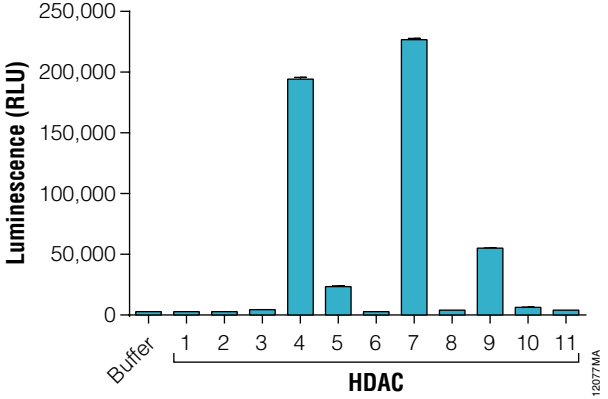

Figure 7. Recombinant HDAC isoenzyme selectivity panel using the HDAC-Glo™ Class IIa Assay. Each recombinant HDAC enzyme was diluted to equal molar equivalents (25pM) based on concentration, molecular weight and % purity in HDAC-Glo™ Class IIa Assay Buffer. For each enzyme (n = 8 replicates), 100µl volumes were transferred to a white 96-well assay plate (Corning Costar® Cat.# 3917). An equal volume (100µl) of HDAC-Glo™ Class IIa Final Detection Reagent was added to all wells and luminescence was measured after 15 minutes at room temperature. For all test conditions, the final concentration of HDAC-Glo™ Class IIa Substrate was 35µM.

6. Supplemental Data (continued)

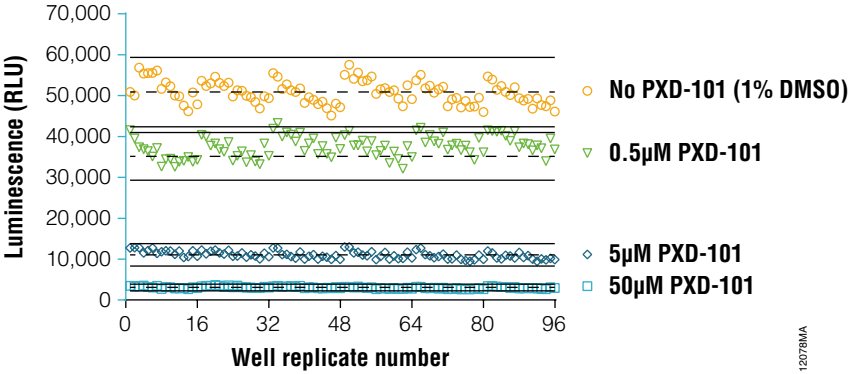

Figure 8. Z´ analysis in 384-well plate format using the HDAC-Glo™ Class IIa Assay. Four separate pools of 2X PXD-101/2% DMSO were prepared in HDAC-Glo™ Class IIa Assay Buffer and 5µl for each test condition was added to 96 replicates in a white 384-well assay plate (Corning Cat.# 3673). To this, 5µl of 2X HDAC 7 in HDAC-Glo™ Class IIa Buffer was added to all wells. The inhibitor/enzyme mix was incubated at room temperature for 30 minutes. An equal volume (10µl) of HDAC-Glo™ Class IIa Final Detection Reagent was added to all wells (20µl final assay volume). Luminescence was measured after 15 minutes at room temperature. For all test conditions, the final concentration of HDAC-Glo™ Class IIa Substrate was 35µM. The final concentration of HDAC 7/well was 5pM. The data was plotted using GraphPad Prism® software. The dotted lines for each set of 96 replicates represent the mean and the solid lines indicate ±3 standard deviations.

Compound Test Condition (n = 96)	Signal:Background	% CV	Z´
no PXD-101 (1% DMSO)	N.A.	5.6	N.A.
0.5µM PXD-101	1.4	5.5	0.10
5µM PXD-101	4.6	8.3	0.72
50µM PXD-101	16.7	8.7	0.81

6. Supplemental Data (continued)

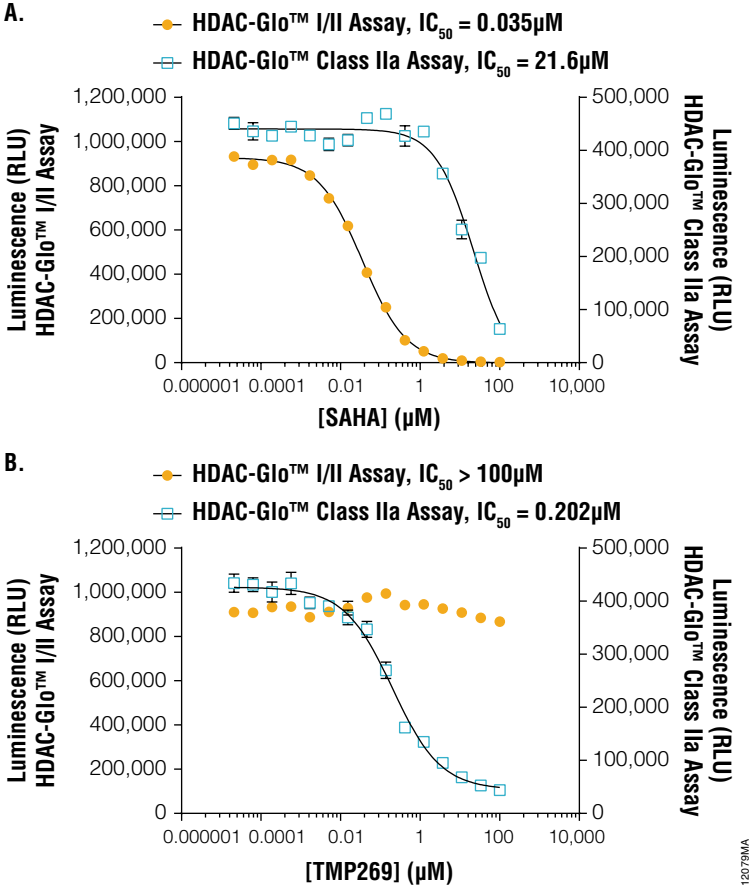

Figure 9. Sample inhibitor potency data using K562 cells and the HDAC-Glo™ I/II or the HDAC-Glo™ Class IIa Assay with inhibitors SAHA (Panel A) or TMP269 (Panel B). A 15-point threefold serial dilution of inhibitor was performed at 100X in 100% DMSO in a 96-well plate. A 5µl aliquot of this 100X/100% DMSO titration series was added to 245µl of media (without serum) to make a 2X/2% DMSO master intermediate titration series of SAHA or TMP269 in a 96-well plate. From this master intermediate titration series, 10µl of replicates were transferred to the white 384-well assay plate (Corning Cat.# 3570). A 10µl addition of K562 cells (250,000 cells/ml in media without serum) was added to all wells for a final 2,500 K562 cells/well. The cell/inhibitor mixes were pre-incubated for 45 minutes at room temperature (19–25°C). An equal volume (20µl) of HDAC-Glo™ I/II Final Detection Reagent or HDAC-Glo™ Class IIa Final Detection Reagent (both with 1% Triton® X-100 for the lytic assay) was added to the appropriate wells (40µl final assay volume) and luminescence was measured after 15 minutes at room temperature. For all test conditions, the final concentration of HDAC-Glo™ I/II Substrate and HDAC-Glo™ Class IIa Substrate was 50µM and 35µM, respectively. Data was plotted using GraphPad Prism® software.

7. Related Products

Product	Size	Cat.#
HDAC-Glo™ I/II Assay	10ml	G6420
	5 × 10ml	G6421
	100ml	G6422
HDAC-Glo™ I/II Screening System	10ml	G6430
	5 × 10ml	G6431
SIRT-Glo™ Assay	10ml	G6450
	5 × 10ml	G6451
	100ml	G6452
SIRT-Glo™ Screening System	10ml	G6470
	5 × 10ml	G6471
CellTox™ Green Cytotoxicity Assay	10ml	G8741
	50ml	G8742
	100ml	G8743
CellTiter-Fluor™ Cell Viability Assay	10ml	G6080
	5 × 10ml	G6081
	2 × 50ml	G6082

Available Separately

Product	Size	Cat.#
HDAC-Glo™ I/II Control Substrate	10µl	G6550
SIRT-Glo™ Control Substrate	35µl	G6460
HeLa Nuclear Extract	10µl	G6570
Trichostatin A	10µl	G6560
Nicotinamide	30µl	G6540

8. Summary of Changes

The following changes were made to the 5/15 revision of this document:

1. Storage Conditions and Additional Storage Considerations were updated.

[a]U.S. Pat. Nos. 6,602,677, 7,241,584 and 8,030,017, European Pat. No. 1131441, Japanese Pat. Nos. 4537573 and 4520084 and other patents pending.

[b]U.S. Pat. No. 8, 632,992 and other patents pending.

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