HDAC-Glo™ Class IIa Assay

INSTRUCTIONS FOR USE OF PRODUCT G9560.

Biochemical Assay

1. Prepare HDAC-Glo™ Class IIa Substrate Solution as instructed in Figure 1, Step 1. During the 1-hour incubation (at 37°C), prepare a compound dilution series in a parallel plate as follows:
   a. Prepare threefold serial dilutions of 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. Combine the 100X compound serial dilutions with HDAC-Glo™ Class IIa Assay Buffer to make a final 2X compound/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 (Step 1.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 compound titration.
2. Dilute HDAC Class IIa enzyme source to 2X final desired enzyme concentration in HDAC-Glo™ Class IIa Assay Buffer, and dispense 50µl into inhibitor dilutions and no-compound controls in 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 Class IIa enzyme titration may be necessary before inhibitor titrations are made to determine the optimal concentration of HDAC Class IIa enzyme to use per well.
3. Mix briefly using an orbital shaker at 500–700rpm.
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 1). 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.
8. Incubate for 20 minutes at room temperature to achieve enzyme steady state, then measure luminescence.

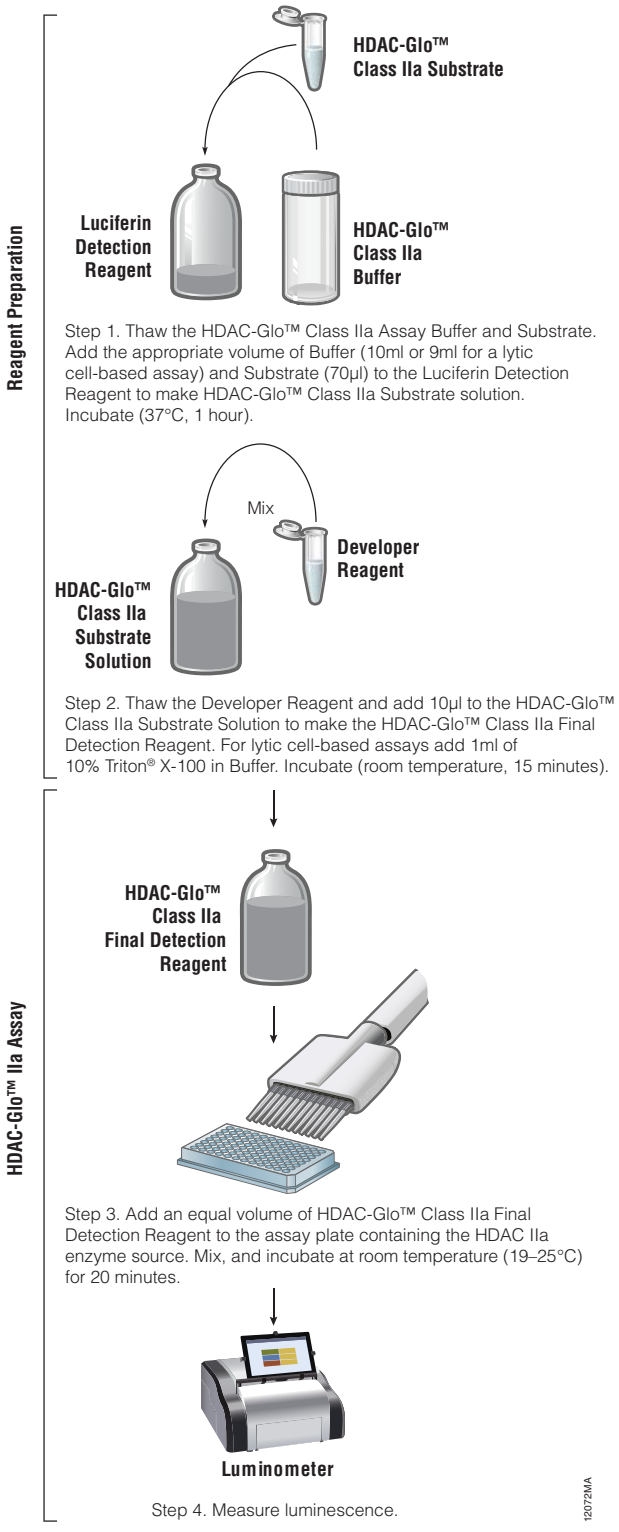

Figure 1. HDAC-Glo™ Class IIa Assay Protocol. See Section 3 of TM407 for a detailed description of reagent preparation.

HDAC-Glo™ Class IIa Assay

INSTRUCTIONS FOR USE OF PRODUCT G9560.

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 the desired number of cells/well (50µl per well for a 96-well plate; 10µl per well for a 384-well plate).
2. Prepare HDAC-Glo™ Class IIa Substrate Solution as instructed in Figure 1, Step 1. During the 1-hour incubation (at 37°C), prepare a compound dilution series in a parallel plate as follows:
   a. Prepare a threefold serial dilutions of the unknown compound or TMP269 control at 100X of the final concentration in 100% DMSO. Be sure to include a no-compound (DMSO-only) control.
   b. Transfer the 100X compound serial dilutions to serum-free culture medium to obtain a final 2X compound/2% DMSO concentration (i.e., 5µl of 100X compound + 245µl of serum-free culture medium). This is the master intermediate dilution series.
3. Remove culture medium from attachment-dependent cells by aspiration and replace it 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 compound dilution series (prepared in Step 2.b) to the white 96-well assay plate (10µl for a 384-well plate).
5. Mix briefly using an orbital shaker at 500–700rpm. Incubate for 15 minutes at room temperature.
6. Incubate cell/inhibitor mixes for at least 30 minutes at room temperature.
7. Prepare HDAC-Glo™ Class IIa Final Detection Reagent (Step 2 of Figure 1).
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.
10. Incubate for 20 minutes at room temperature to achieve enzyme steady state, then measure luminescence.

For detailed protocol information see Technical Manual #TM407, available online at: **www.promega.com/protocols**

Ordering and Technical Information
www.promega.com • Phone 608-274-4330
Toll free in USA 800-356-9526 • Fax 608-277-2601

Printed in USA 1/14. Part# 9FB152