HDAC-Glo™ 2 Assay

INSTRUCTIONS FOR USE OF PRODUCT G9590.

Biochemical Assay

1. Prepare HDAC-Glo™ 2 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 SAHA control at 100X of the final assay concentration in 100% compound. Be sure to include a no-compound (DMSO-only) control.
   b. Combine the 100X compound serial dilutions with HDAC-Glo™ 2 Assay Buffer to make a final 2X compound/2% DMSO concentration (i.e., 5µl of 100X compound + 245µl of HDAC-Glo™ 2 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 2 enzyme source to 2X final desired enzyme concentration in HDAC-Glo™ 2 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 2 enzyme titration may be necessary before inhibitor titrations are made to determine the optimal concentration of HDAC 2 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™ 2 Final Detection Reagent (Step 2 of Figure 1).
6. Add 100µl of HDAC-Glo™ 2 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.

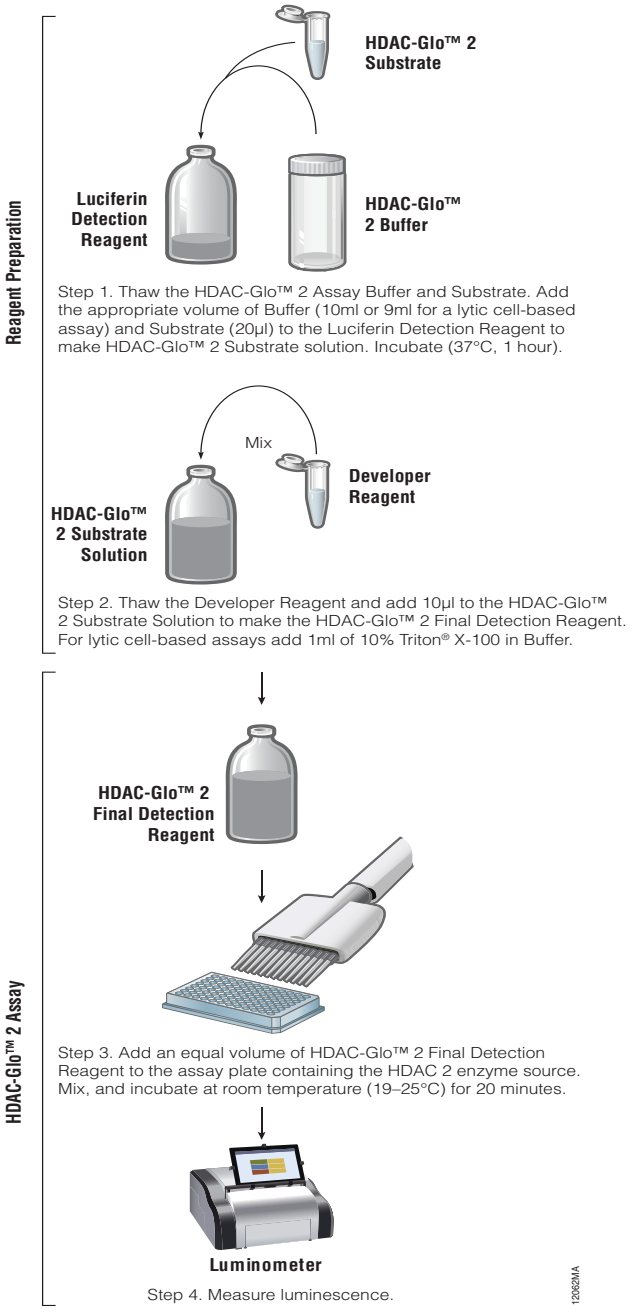

Figure 1. HDAC-Glo™ 2 Assay Protocol. See Section 3 of TM406 for a detailed description of reagent preparation.

HDAC-Glo™ 2 Assay

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Cell-Based Assay

1. Seed 50µl of attachment-dependent cells into a white 96-well plate at a density of about 10,000 cells/well (about 5,000 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™ 2 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 SAHA 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 dilution series 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.
6. Incubate cell/inhibitor mixes for at least 30 minutes at room temperature.
7. Prepare HDAC-Glo™ 2 Final Detection Reagent (Step 2 of Figure 1).
8. Add 100µl of HDAC-Glo™ 2 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 (19–25°C) to achieve enzyme steady state, then measure luminescence.

For detailed protocol information see Technical Manual #TM406, available online at: **www.promega.com/protocols**

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