A Multiplexed, Bioluminescent HDAC Assay for Determining Target-Specific, Anti-Cancer Potency

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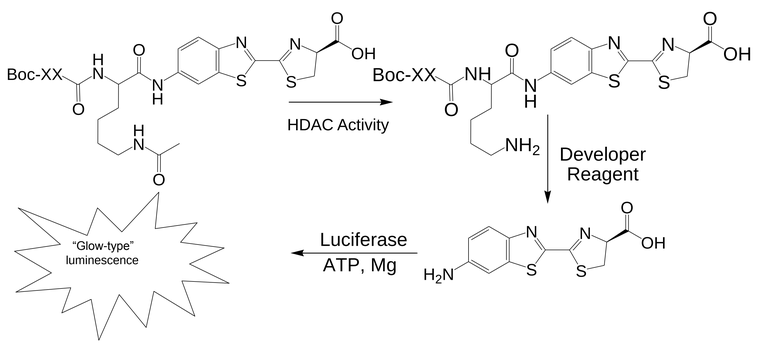
1. Abstract

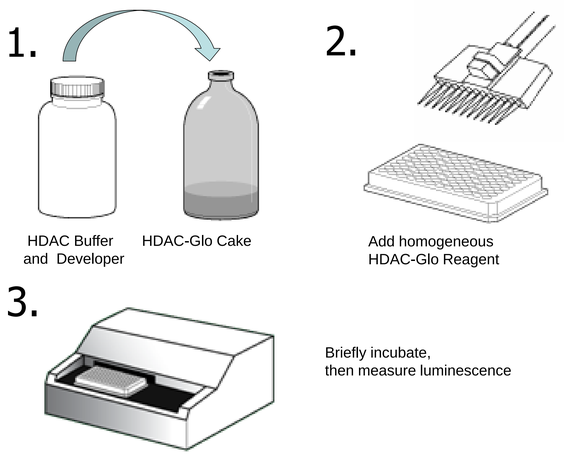
Histone deacetylase (HDAC) class I and II enzymes are zinc dependent protein deacetylases. They play many roles in normal gene regulation events of development and homeostasis, but their dysregulation has been linked to a variety of solid tumors and hematological malignancies. We have recently developed a one-step, homogeneous, luminescent assay for activity detection from multiple HDAC Class I and II isoforms, thereby providing a useful tool for cell culture, enriched or recombinant sources of lysine deacetylases. This luciferase-containing, "glow-type" assay reagent is cell-permeable and can directly measure HDAC activity in living attachment-dependent and suspension cells. Furthermore, this luminescent HDAC assay can be paired in same well multiplex formats with fluorescent assays which measure biomarkers associated with changes in viability and cytotoxicity. Here we correlate HDAC inhibition with cellular fate, using representative inhibitors from the hydroxamate, short-chain fatty acid, benzamide and cyclic peptide classes. We will also demonstrate relative on-target anti-cancer efficacies and off-target safety using transformed, primary or iPS-derived cell lineages.

2. Assay Concept

Promega's novel luminogenic substrate contains an acetylated lysine peptide sequence derived from Histone 4 and conjugated to aminoluciferin. Like all peptidyl aminoluciferins, this compound *is not* a substrate for firefly luciferase. In the assay, HDAC Class I/II enzymes deacetylate the lysine residue, allowing the developer enzyme to recognize and cleave the peptide from the aminoluciferin. The released aminoluciferin *can then* act as a substrate for luciferase. The luminescent signal generated is stable and quantifiable using standard luminometry in plate-based formats.

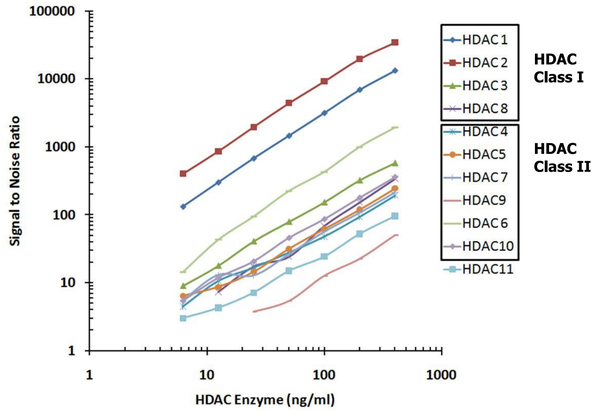


3. Simplified, Single Addition Protocol



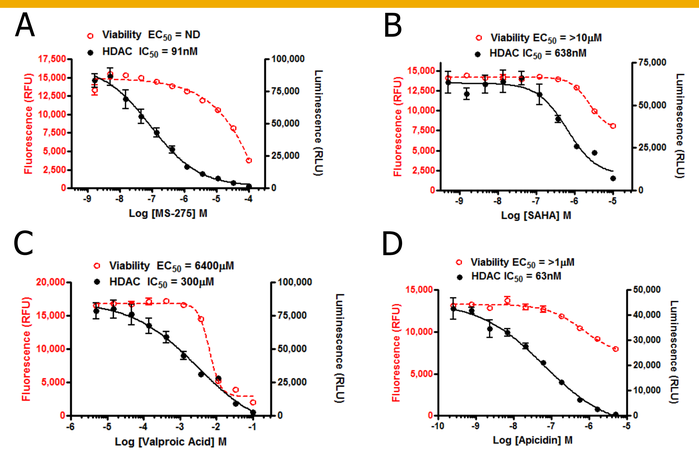
Step 1: Create HDAC-Glo™ Class I/II Reagent by adding buffer and developer enzyme to lyophilized luciferase reaction components
Step 2: Add Reagent to HDAC enzyme source (cells, nuclear extract or recombinant) with or w/o inhibitor
Step 3: Incubate 15-45 min at RT, then measure stable luminescent signal

4. Broad Isoenzyme Utility



The assay sensitively measures both Class I and Class II lysine deacetylase activities using recombinant enzyme sources. Here, recombinant isoenzymes were serially diluted and activities measured to demonstrate assay utility.

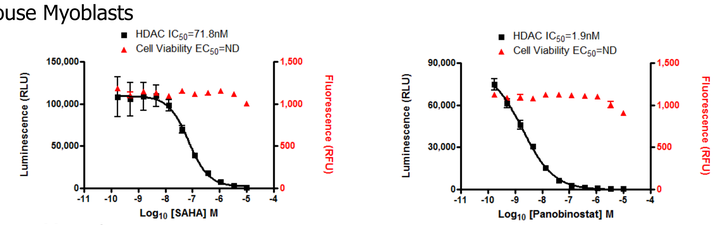
5. Multiplexed Applications Using HDACi Scaffolds



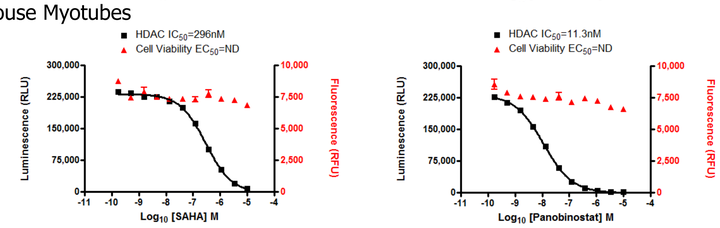
HDAC inhibition and cell viability profiles were monitored in U937 cancer cell lines. HDAC inhibition was determined with HDAC-Glo™ and cell viability was measured with CellTiter-Fluor™. HDAC inhibitors (HDACi) from the A) Benzamides, B) Hydroxamates, C) Short-chain fatty acids, or D) Cyclic peptides classes were tested. The resulting data were consistent with published IC_{50} values.

6. HDACi Potency in Pre- and Post-Differentiated Cells

A. Mouse Myoblasts



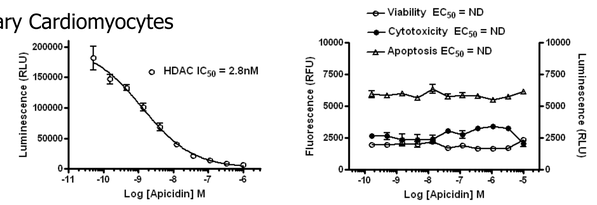
B. Mouse Myotubes



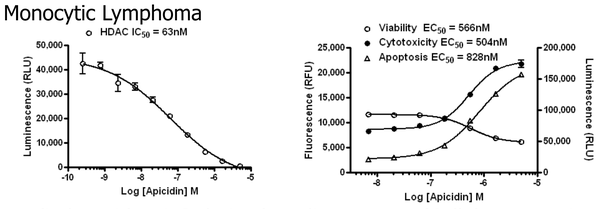
SAHA or Panobinostat was applied to either mouse myoblasts or myotubes for 1hr. HDAC inhibition was determined with HDAC-Glo™ and cell viability was measured with CellTiter-Fluor™. Increased HDAC inhibitor potency was observed in myoblasts, suggesting activation of alternate HDAC isoenyzmes upon differentiation.

7. On- vs. Off-Target HDACi Effects

A. Primary Cardiomyocytes

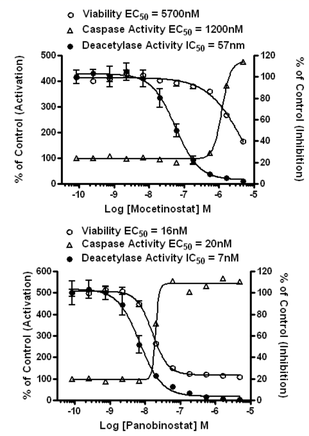


B. U937 Monocytic Lymphoma



Apicidin was applied to either iPS-derived cardiomyocytes (Cellular Dynamics International) or U937 cells for 24hrs. HDAC inhibition was determined using HDAC-Glo™ whereas cell viability, cytotoxicity and caspase activation were measured using ApoTox-Glo™. Potent HDAC inhibition was observed in both cell types, but only cancer cells underwent apoptosis as a consequence of HDAC inhibition.

8. HDACi IC_{50} vs. Cytotoxic Potency



Mocetinostat (MGCD0103) is a potent and semi-selective (HDAC 1 and 2) inhibitor.

Panobinostat (LBH589) is a potent and non-selective HDACi

HDACi were applied to K562 cells for 48hrs. Both compounds demonstrate strong inhibition of HDAC activity but show markedly different cytotoxicity profiles. This result demonstrates the relevance of HDAC isozyme selectivity on antitumor activity.

9. Conclusions

The HDAC-Glo™ Deacetylase Activity Assay offers significant utility in cell-based, recombinant and extract formats because it delivers:

- An easy to use, homogeneous, one-step reagent
- Robust signal with maximal sensitivity achieved in 15-45 minutes
- Broad linearity responses (2-3 logs) using HDAC Class I and II enzymes
- Lytic or Non-lytic formats useful in primary or cancer cell lines and suspension or attachment dependent cultures
- Comparable IC_{50} to published values using well-studied HDAC inhibitors
- The possibility of linking HDACi to phenotypic outcomes via same well multiplexing.

Questions or comments?

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