A Bioluminescent HDAC Assay for Cell-Based Drug Discovery

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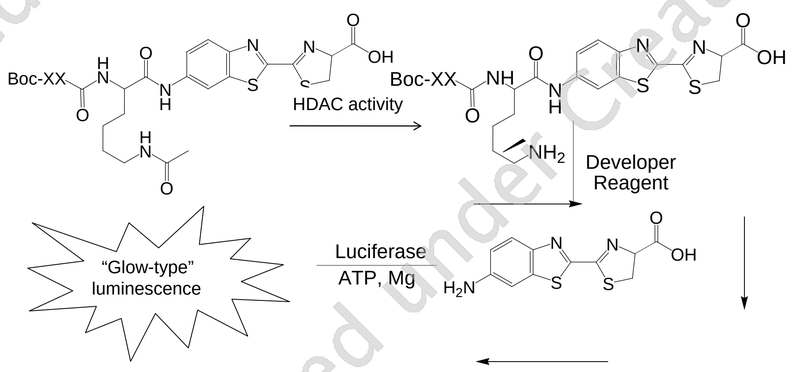
1. Abstract

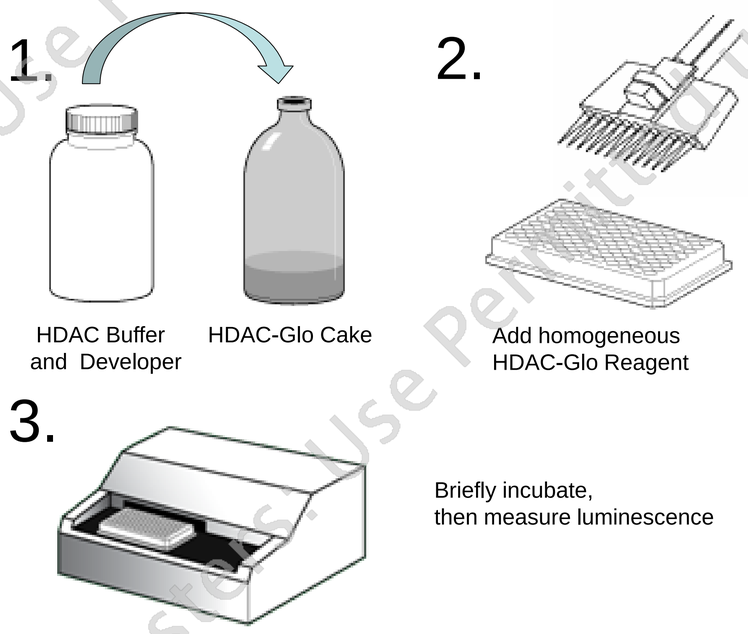
Cell-based models continue to be an important tool in the drug discovery and development toolbox because they provide a more biologically relevant context to study the complex changes occurring after targeted intervention. Histone deacetylase (HDAC) enzymes are an emerging group of epigenetic modifiers that are of particular interest for targeted intervention because they are well-validated and chemically tractable. We have developed a bioluminescent, single addition, homogeneous deacetylase assay that is sufficiently sensitive and robust for cell-based screening or characterization efforts in either non-lytic or lytic formats. Here we describe our efforts to define the utility of the assay using structurally diverse HDAC inhibitors with both attachment-dependent and suspension cell lines. Furthermore, HDAC inhibition will be correlated with cellular fate by various multiplexed viability and cytotoxicity measures in the same well.

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.

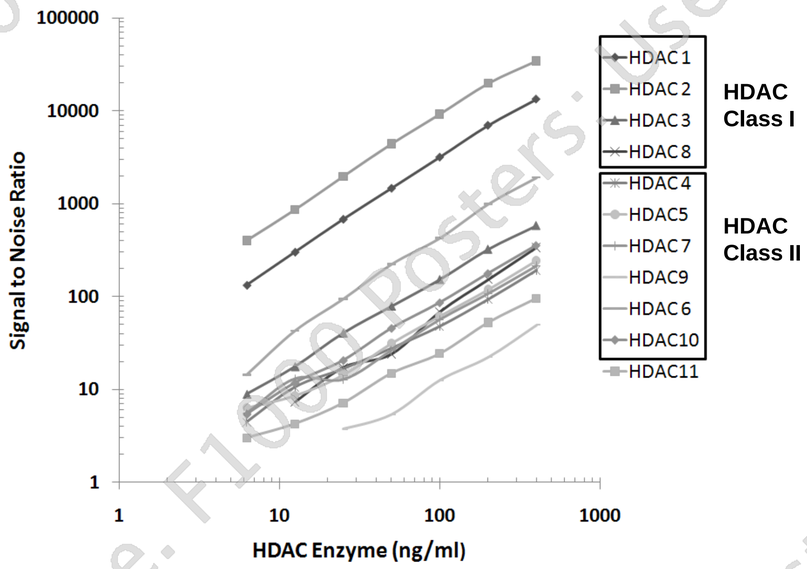


3. Simplified, Single Addition Protocol



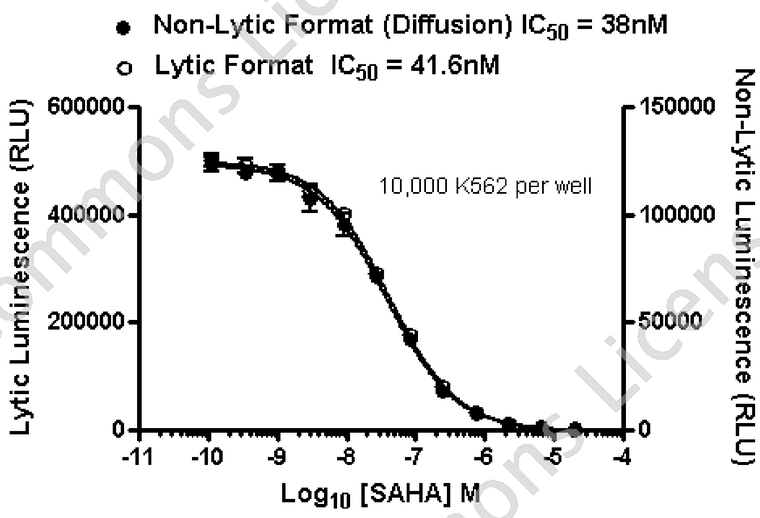
Step 1. Create HDAC-Glo™ Class I/II Reagent by adding buffer and developer enzyme to lyophilized luciferase reaction components
Step 2. Add Reagent to HDAC enzyme source (cells, nuclear extract or recombinant) with or w/o inhibitor
Step 3. Incubate 15-45 min at RT, then measure stable luminescent signal

4. Broad Isoenzyme Utility



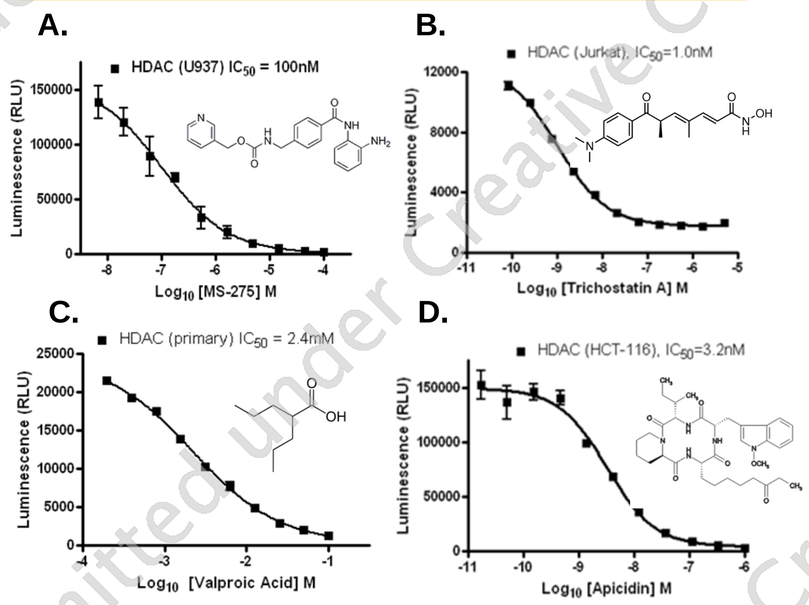
The assay sensitively measures Class I/II lysine deacetylase activities in living or lysed cells. Here, recombinant isoenzymes were serially diluted and activities measured to demonstrate assay utility. Note: Class I enzymes are ubiquitously expressed, whereas particular Class II expression is tissue specific. Therefore, any cell-based signals represent blended and/or averaged isoenzyme activities.

5. Substrate Permeability



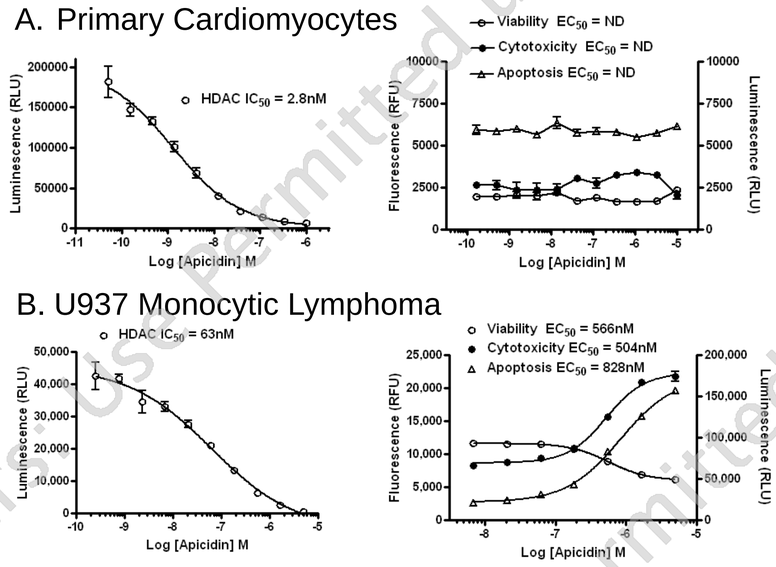
The pro-luminogenic lysine deacetylase substrate is cell permeable, which enables measurement of HDAC activity in either non-lytic or lytic formats. Cell permeability also enables real-time measurement of HDAC inhibition and allows other analyses to be conducted on the cell sample (e.g. Western blot, etc.)

6. Different Cells and HDACi Scaffolds



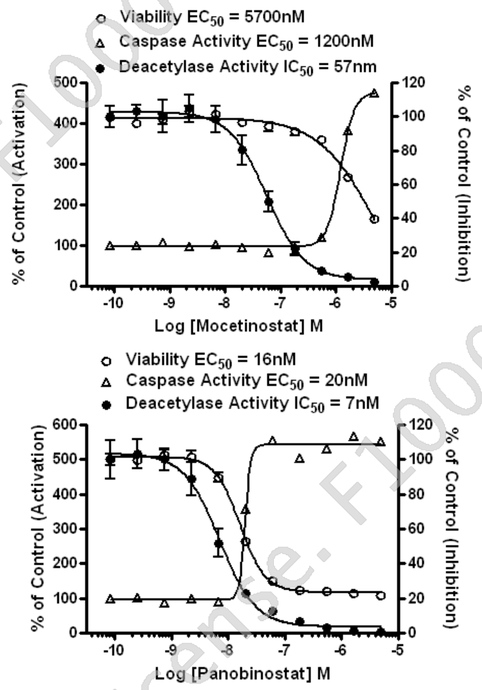
HDAC inhibition profiles were determined in both primary and cancer cell lines that were either suspension or attachment dependent. Inhibitors from the A) Benzamides, B) Hydroxamates, C) Short-chain fatty acids, or D) Cyclic Peptides classes were tested. The resulting data were in good agreement with published IC_{50} values.

7. On- vs. Off-Target HDACi Effects



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 formats because it delivers:

- An easy to use, homogeneous, one-step reagent
- 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 with 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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