Quantitation of Sirtuin Activity with a One-Step Luminescent Assay
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1. Abstract/Introduction
The Sirtuin family of histone/protein deacetylating enzymes has gained considerable interest both for their recognized importance in processes such as gene silencing and expression and in cellular metabolism. We have developed a convenient and sensitive screening methodology to detect and measure both inhibitors and activators of sirtuin activity. Here we describe a one-step, homogeneous luminescent assay for the detection of activity for multiple sirtuin isotypes. The reagent formulation contains a pro-luminescent sirtuin peptide substrate, NAD+, ATP, thermostable luciferase, and a developer enzyme that cleaves deacetylated lysine residues. The addition of an active sirtuin enzyme source leads to a robust luminescent signal within minutes with “glow-type” characteristics lasting for several hours.

2. Assay Concept
Promega’s novel pro-luminescent substrate contains an acetylated lysine attached to aminoluciferin, along with a peptide sequence derived from p53. Upon deacetylation of the lysine by a sirtuin, the deacetylated peptide becomes a substrate for the developer reagent present in the reaction mixture. This yields free aminoluciferin which reacts with luciferase to generate a luminescent signal. This signal is stable and quantifiable. This assay requires only the addition of the reagent mixture and your sirtuin of interest.

3. Reagent Preparation and Work Flow

1. Step 1. Create SIRT-Glo™ Reagent by adding buffer and developer reagent to lyo cake
2. Step 2. Add Reagent to rSirtuin enzyme source
3. Step 3. Incubate 15-60 min at RT, then measure luminescence (stable signal for hours)

4. Linear Response Comparison
SIRT-Glo™ shows a linear response over a wide rSirt2 concentration range. In addition, the signal to noise ratio observed is greater than 100-fold better than a commercially available fluorescent assay. Comparable results were obtained with rSirt1 and rSirt3.

5. Scalability (Z’ in HTS format)
SIRT-Glo™ produces large signal windows suitable for high density HTS plate formats. The figure above demonstrates Z’ factor “fitness” in a 384 well plate using rSirt3 with or without 10mM nicotinamide.

6. Control Assay Chemistry
Inhibition of Developer enzyme or luciferase rather than a Sirtuin could cause a false positive. Our non-acetylated control substrate is shown. When assayed under the same conditions as the SIRT-Glo™ substrate a decrease in light indicates a false positive. Although the number of false positives in this assay is usually low, preliminary experiments have shown that inclusion of a detergent such as Tergitol MP9 and lengthening the read-time when running a large HTS screen can reduce the number.

7. Control Assay Utility
Both Cambinol and Salermide are confirmed as specific inhibitors of rSirt1 and rSirt2 through inhibition of SIRT-Glo™ but not the control assay. A known luciferase inhibitor shows strong inhibition of both SIRT-Glo™ and the control assay.

8. Orthogonal Counter-Confirmation Testing
SIRT-Glo™ provides an alternative to fluorescence methods for verifying sirtuin augmentation. The data above show no augmentation of luminescent signal with increasing concentrations of a compound previously shown to produce an increase in signal in a fluorescent assay.

9. Conclusions
The SIRT-Glo™ Deacetylase Activity Assay provides:
• A homogeneous, one-step reagent
• Maximal signal (sensitivity) in 15-20 minutes
• A steady signal that allows measurement over a range of time
• Broad linearity response (2-3 logs)
• Scalability into high density formats
• Comparable IC50s to published values and a control substrate for detecting false positives

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