Universal Homogenous Bioluminescent Assay to monitor the Effect of Modulators on Various Classes of Methyltransferases in vitro

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1. Abstract

Methylation/demethylation of DNA and Proteins play major roles in modulation of the epigenome and has been implicated in a wide variety of human diseases. Recent biochemical and biological data suggest that the enzymatic activities of several of these enzymes have pathogenic roles in cancer, inflammation, and neurodegenerative diseases. Thus, pharmacological modulation of these enzymes by small molecules will be beneficial in developing novel therapeutics for multiple unmet medical needs. Towards this goal of searching for activators/inhibitors of these enzymes for the development of next generation of drugs, screening assays for these modulators are urgently needed. To address these unmet needs, we have developed a novel assay that monitors the activity of these enzymes and their modulation by small molecules. The assay is bioluminescent based, HTS formatted and highly sensitive. The assay is universal since it is based on the principle of bioluminescence, and capable of detecting changes in activity of a broad range of methyltransferases such as DNA, protein, and small molecules methyltransferases. In addition, the assay has been validated for all classes of protein methyltransferases (Lysine and Arginine), and with different types of substrates (small peptide, large proteins, or even nucleosomes). This enables determining the specificity of these enzymes and their substrate requirements. The assay has high signal to background and low C.V. (Z’ value > 0.7) and has been validated using various plate densities such as 96-, 384-, and 1536-well plates. A strong feature of this assay is its utility with broad range of substrates with no limitations on the use of high concentrations of substrates or the composition of the substrates (short vs. long peptide), thus making it a very versatile tool of kinetic data and determining the mechanism of action of various modulators of methyltransferases of interest.

2. Universal Methyltransferase MT-Glo™ Scheme

Flexibility

- Multiple Substrates: Histone derived peptides, Histone 3, Histone 4, or Nucleosomes
- Any Substrate: Histone derived peptides, Histone 3, Histone 4, or Nucleosomes
- Any Buffer: Any buffer can be used in MT-Glo™ assay
- Any Strain: Can be used in MT-Glo™ assay

3. Assay Performance:

MT-Glo™ Assay

- High sensitivity:
  - Sensitivity: Detects Low SAH (< 20nM)
  - Broad range of concentrations of SAM can be used in MT-Glo™
  - High S/B: >50 using 384LV-well format

- Robust:
  - Z’ > 0.8 (at 1µM of SAH using 384LV-well format)

- Universal Assay:
  - Any Substrate: Histone derived peptides, Histone 3, Histone 4, or Nucleosomes
  - Any Buffer: Any buffer can be used in MT-Glo™ assay

- Fast performance: in less than 40min after MT reaction using MT-Glo™

4. SAM and SAH Standard Curve in MT-Glo™

5. Effect of Temperature & Buffer on The Activity of MT Using MT-Glo™

6. Methylation Transferase Activity of EHMT2-9a & SET7/9 Using MT-Glo™

7. Methylation Transferase Activity of EZH1 & EZH2 Using MT-Glo™

8. Methylation Transferase Activity of DNMT1 & DNMT3a Using MT-Glo™

9. Substrate Effect on Lysine & Arginine Methyltransferases (PRMT5 & DOT1L) Using MT-Glo™

10. Z’ Value: MT-Glo™ (Using SAH as Readout)

11. LOPAC Screening Using MT-Glo™ Assay

12. Summary

- Sensitivity: Detects Low SAH (< 20nM)
- Broad range of concentrations of SAM can be used in MT-Glo™
- High S/B: >50 using 384LV-well format
- Minimal interference from fluorescent compounds
- Linear (S/B ratio)
- “Low Signal” Ctrl.
- Any Substrate: Histone derived peptides, Histone 3, Histone 4, or Nucleosomes
- Any Buffer: Any buffer can be used in MT-Glo™ assay
- Fast performance: in less than 40min after MT reaction using MT-Glo™

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