High-throughput homogeneous epigenetics assays using HTRF® technology and the SpectraMax® Paradigm® microplate detection platform

Cathy Olsen¹, Nicolas Pierre², Arturo Gonzalez-Moya², Suzanne Graham², François Degorce²
¹Molecular Devices, LLC, 1311 Orleans Drive, Sunnyvale, CA 94089
²Cisbio US, 135 South Road, Bedford, MA 01730

Overview

Histone proteins are dynamic proteins that form the scaffold for DNA packaging and also undergo a variety of post-translational modifications that help to determine which areas of the genome are active or repressed. Histone methylation is a post-translational modification that occurs on arginine and lysine residues and that represents one of the most studied post-translational modifications involved in epigenetic regulation. On histone H3, several lysines are potential targets for methyltransferases and demethylases, enzymes that convert these residues to mono-, di-, or trimethylated marks. Over the past decade, these modifications have been shown to have a prominent role in a broad variety of diseases, and the related enzymes and chromatin-interactive proteins are now considered as important druggable therapeutic targets in the drug discovery field. Cisbio Bioassays has developed assays that are suitable for high throughput screening of inhibitors of these disease-relevant enzymes.

HTRF® Technology

HTRF® technology uses a light- and energy-transfer mechanism to deliver accurate and robust results. It involves the use of a cryptate-labeled ligand and a fluorophore labeled with europium cryptate, which can be combined to form a pair of reagents able to detect a specific target of interest, in the context of a homogeneous detection scheme. A look-up table is generated that allows the determination of each concentration and comparison of results.

Results: K_m determination

Figure 3. SAM titration. This experiment allowed the determination of K_m for SAM. The K_m value was determined using 0.2 mM G9a, and a range of substrate concentrations were tested. The K_m for SAM at 40 mM substrate was determined to be 7.2 µM, which is in agreement with published values.

Figure 4. Substrate titration at different SAM concentrations. K_m values ranging from 80 to 100 nM SAM were calculated.

Figure 5. FAO titration with LSD1 and different concentrations of substrate. A K_m value of 0.013 µM was calculated for all but the highest substrate concentration.

Figure 6. G9a (left) and LSD1 (right) inhibition curves. In this experiment, an IC_50 value of 2.3 µM was calculated for SAM, in close agreement with published values. An IC_50 value of 7.8 µM was obtained for transgenic yeast, similar to published values.

Conclusions

- The assay is shown to be highly sensitive, accurate, and robust, with no requirement for transfer or separation steps.
- HTRF® G9a histone methyltransferase and LSD1 demethylase assays are suitable for high throughput screening of inhibitors of G9a.
- HTRF® epigenetic toolboxes generate reagents that allow the design of highly specific, custom histone modification assays.

SpectraMax Paradigm Microplate Reader

- Sensitive, high-performance HTS system for HTRF® epigenetic assays
- Modular system with HTRF®-certified detection and pre-configured SoftMax® Pro software protocols for rapid data analysis
- Dual emission design for accurate results with shorter read times

Additional HTRF-certified readers from Molecular Devices

- SpectraMax i3 Multi-Mode Platform
  - Option to add cellular imaging capability with MicroMax™ Imaging Cytometer
- SpectraMax M5e Multi-Mode Microplate Reader
- FlexStation® 3 Multi-Mode Microplate Reader

References

- Additional references can be found in the supplementary material provided with the manuscript.