**Setting-up HTRF technology for G9a & DNMT1 High-Throughput Screening**

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**Introduction**

- Epigenetic modifications play a crucial role in human disease. Epigenetic phenomena have gained increased attention in the field of cancer research, with many studies indicating that they are significantly involved in tumor establishment and progression.

- Histone methyltransferases (HMTs) are a large group of enzymes that specifically methylate protein lysine and arginine residues, especially in histones, using S-adenosyl-methionine (SAM) as the methyl donor. DNA methylation is also an important epigenetic mark in eukaryotes, and aberrant patterns of this modification is involved in numerous diseases such as cancers. Therefore, there is a need for identifying new small inhibitors of DNA methyltransferases (DNMTs).

- However, in general, HMTs have no widely accepted high-throughput screening (HTS) assay format, and reference inhibitors are not available for many of the enzymes. Moreover, despite the development of numerous in vitro DNMT assays, there is a lack of reliable tests suitable for high-throughput screening, which can also give insights into inhibitor mechanisms of action.

- We describe the setting up of a G9a enzyme activity assay using HTRF® technology in a HTS 384-well plate format which can also give insights into inhibitor mechanisms of action.

- A homogeneous HTRF assay for DNMT1 enzyme activity was also optimized in a HTS 384-well plate format which could be used for identifying new inhibitors fulfilling the requirements of robustness and reproducibility.

**G9a & DNMT1 Optimization**

**Mode of action studies**

**Biochemical assay to measure G9a enzyme activity**

![G9a enzyme activity](image)

**DNMT1 Activity**

**G9a & DNMT1 Optimization**

**Conclusions**

- The key methyltransferases enzymes activities G9 and DNMT1 have been optimized in a 384-well plate format able to be used with screening purposes.

- The assays permitted to find and characterize reversible inhibitor compounds with a high potency and selectivity on these targets, confirmed by direct measurements of cellular epigenetic marks.

- The assays could be used to explore mechanisms of action such as processivity and compound profiling on different steps of G9a activity, and substrate competition analysis in both epigenetic enzymes.