Development of High-Throughput Assays to Study Methyllases, Demethylases and Deacetylases Targeting Histone H3K4, H3K27 and H3K36 Residues

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Abstract
Several assay methods have been developed for quantifying the activity of histone demethylases (JMD2A and JMJD3), histone methyltransferases (HMTs), and histone demethylases (HDMs). These include radiolabeled assays, enzyme-linked immunosorbent assays (ELISAs), and spectrophotometric assays. Assays differ in their substrate, reaction conditions (e.g., S-adenosylhomocysteine, formyltetrahydrofolate, and histone peptides), and detection formats (e.g., radioactive, fluorescent, and enzyme-coupled assays). Generation of false positives and/or negatives may occur.

In this study, we describe the development and optimization of homogeneous antibody-based assays for measuring the catalytic activity of a series of commercial inhibitors and enzymatic and demethylase inhibitors, including a series of histone demethylase (HDMs) and H3K27me3 demethylase (H3K27, JMJD1A, JMJD3, and JMJD3A) inhibitors. Two different non-radiative, no-wash technologies were used for detection of the enzymatic reaction products, the LANCE Ultra (LANCE H3K27) and AlphaLISA (LANCE H3K27) assay and time-resolved Förster energy transfer (LANCE Ultra) assay.

Results demonstrated that all assays were sensitive, rapid and robust (Z" factors ≤ 0.65), requiring only nanomolar concentrations of enzyme and peptide. Furthermore, profiling of known inhibitors for each enzyme of interest showed the expected potency with either technology. These assays will therefore be ideal for the identification of selective small molecule inhibitors. The approach described here is broadly applicable for measuring the catalytic activity of other histone-modifying enzymes by combining the appropriate biotinylated histone-derived peptides and mark-selective antibodies.

Materials
Enzymes and biotinylated peptide substrates. Recombinant enzymes L3D1, SIRT1, JMJD2A, JMJD3 and the EZH2/EMT/SUZ12/RNAi4/NSPY2 protein complexes were from Bio-Techne Europe. Antibodies were obtained from Cayman Chemical. Biotinylated peptides were from AlphaLISA.

Reagents and inhibitors. S-(S-adenosyl-L-methionine chloride (SAM), S-adenosyl-L-homocysteine (SAH), S-(S-adenosyl-L-homocysteine chloride (SAHC)), S-adenosyl-L-homocysteine hydrochloride (SAH-HCl), S-adenosyl-L-homocysteine sulfate (SAH-SO4)), acetylated and unmodified histone H3, histone H3 (S10), histone H3 peptide (H3K27me3), histone H3 peptide (H3K27me2), histone H3 peptide (H3K36me2), histone H3 peptide (H3K4ac), histone H3 peptide (H3K4K), and histone H3 peptide (H3K4m). Sinefungin, formic acid, and formamide were obtained from Merck Canada. Turpentine was obtained from Turpentine Canada.

Histone deacetylase (HDAC) assays. The AlphaLISA HDAC Assay (PerkinElmer) was used. This uses recombinant HDACs and AlphaLISA Acceptor beads coated with a biotinylated histone H3 peptide substrate.

Histone methyltransferase (HMT) assays. The EZH2/EMT/SUZ12/RNAi4/NSPY2 protein complex is used. Biotinylated peptides are used as substrates. These are detected by AlphaLISA (PerkinElmer). A comprehensive description of these assays and their optimization is available on our website at perkinelmer.com/epigenetics.