Development of High-Throughput Assays to Study Histone H3K4 Methyltransferases & H3K9 Methyl- and Acetyltransferases


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Abstract

Post-translational modifications of histone proteins play an important part in a wide array of cellular processes including regulation of gene transcription, DNA repair, cell cycle, and metabolism control. For instance, transcriptional activation is associated with acetylation at the promoter region of histone H3 and H4, and methylation on K4. On the other hand, gene repression has been linked to H3K9, H3K27, and H3K79 methylation. In this regard, the activity of several histone modifying enzymes (e.g., acetylases and methyltransferases, deacetylases, demethylases) have been linked to diseases such as cancer and neurological disorders. Thus, the development of simple and reliable assays for these enzymes could facilitate the identification of new modulatory compounds, eventually leading to the development of clinically relevant drugs.

In this work, we describe the development of enzymatic assays to perform high throughput screening of H3K9 acetyltransferases, H3K4 methyltransferases, and H3K9 and H3K36 methyltransferases, using homogeneous proximity assays based on two non-radioactive technologies: LANCE® Ultra time-resolved fluorescence energy transfer and AlphaLISA® bead-based chemiluminescent assays. To this end, we used as substrate a biotinylated peptide derived from the N-terminus of histone H3 (residues 1-21) to assay acetyltransferase p300 and methyltransferases G9a and SET7/9. In all cases, we found conditions suitable for screening: enzyme concentrations between 0.05 to 5 nM and substrate concentrations between 50 and 500 nM. Acetyl-CoA or S-adenosylmethionine (SAM) concentrations were in the 0.1-20 µM range, enabling sensitive screenings of compounds competing with these cofactors. Enzyme assays could be carried out in less than 4 hours, with an order of potency for known inhibitors (sinefungin, SAH, SAM) in good correlation with published literature.

The results presented herein demonstrate how to optimally assay p300, G9a, and SET7/9 activities in vitro using a biotinylated H3-derived peptide in non-radioactive, homogeneous assay formats. This could enable simple and fast screening of compound libraries, facilitating the discovery of novel modulators of histone modifying enzymes.

2. Assay Principle

AlphaLISA® Assays

The antibody-conjugated Acceptor bead binds specifically to the modified histone H3 peptide, while the Luminostat Streptavidin binds to the biotin group attached to it, allowing TR-FRET to occur.

LANCE Ultra Assays

The Europium-labeled antibody specifically recognizes the modified histone H3 peptide, while the Luminostat Streptavidin binds to the biotin group attached to it, allowing AlphaLISA signal.

3. AlphaLISA Assays

p300 acetyltransferase

G9a methyltransferase

SET7/9 methyltransferase

4. LANCE Ultra Assays

p300 acetyltransferase

G9a methyltransferase

SET7/9 methyltransferase

5. Materials

Common materials (PKI cat#)

- AlphaLISA 96-Well plates (E6201)
- Biotin-H3 peptide (P7650)
- Tween 20 (83402)
- 5X Tris Buffer (E1620)
- Acetyl-CoA (Sigma, P8789)

- LANCE® H3K9me3 (HD520)
- LANCE® H3K4me2 (HD522)
- LANCE® H3K4me3 (HD523)
- LANCE® H3K9ac (HD521)
- Antibody (Sigma, P8789)

- Anti-H3K4me1-2 antibody (Sigma, P8789)
- Anti-H3K9ac antibody (Sigma, P8789)
- Anti-H3K9me1 antibody (Sigma, P8789)
- Anti-H3K9me2 antibody (Sigma, P8789)
- Anti-H3K9me3 antibody (Sigma, P8789)
- AlphaLISA Antibody (Sigma, P8789)

- AlphaLISA H3K9me3 protocol
- AlphaLISA H3K4me2 protocol
- AlphaLISA H3K4me3 protocol
- AlphaLISA H3K9ac protocol

- AlphaLISA H3K9me2 protocol
- AlphaLISA H3K9me3 protocol

- AlphaLISA H3K9ac protocol

6. Methods

AlphaLISA Detection Assay

LANCE Ultra Detection Assay

Standard protocol for enzymatic reactions

1. 3 µl of corresponding assay buffer (2.5X solution)
2. 0.5 µl of substrate ([H3]acetyl or [H3]methylated peptide substrate) (mM)
3. 0.1 µl of biotinylated antibody (100 µg/ml)
4. 0.1 µl of enzyme in 50 µl of 5X enzyme buffer

Notes

- p300 enzymatic assays were stopped by the addition of acetic acid (50 µL 30%) in the presence of AlphaLISA and LANCEUltra antibodies, respectively. For AlphaLISA assays, an additional step was included to wash away the non-specifically bound biotinylated peptide (3X with 50 µL PBS, 3X with 50 µL acetate buffer). The plate was air-dried before the addition of the biotin-antibody [H3]acetyl/H3 [H3]methylated Ultra antibodies.

- AlphaLISA enzymatic reactions were performed by the addition of the Acceptor bead diluted in AlphaLISA 1X Enzyme Buffer 1. AlphaLISA LANCE Ultra enzymatic reactions were stopped by adding the Luminostat Streptavidin-H3 (1X) diluted in LANCE Ultra Labelling Buffer supplemented with 0.002% Tween-20 (final 0.001% in 20 µL).

- LANCE Ultra enzymatic reactions (AlphaLISA and LANCEUltra) were found to be stopped by the addition of the detection reagents diluted in the corresponding detection buffer.

7. Summary

- Non-radio, homogeneous immunoassays for monitoring histone H3 modifications in vitro were developed for AlphaLISA and LANCEUltra platforms.

- All assays employ an H3-derived biotin-peptide substrate, paired with immobilization-specific, antibody conjugates.

- H3K9 acetylation, and H3K4 and H3K9 methylation assays were successfully optimized in all-one-well format, and then validated for high-throughput screening on HTS, demonstrated with 2-factor ranges varying from 0.7 to 0.5 (not shown).

- A comprehensive description of these assays and their optimization is available on our website at www.perkinelmer.com/immunoassays.