The dynamic equilibrium of lysine acetylation of histones in vivo is governed by the opposing activity of acetyltransferases and deacetylases. Certain disease processes also alter this equilibrium, the acetylating/deacetylating events and the corresponding enzymes have become important therapeutic targets in recent years. Specific histone modification inhibitors can also help biologists in studying the complex role of histone post-translational modifications. There are no tools available for high-throughput screening of potential histone acetyltransferase/deacetylase inhibitors. The objective was to develop a high-throughput assay to quantify the level of acetylation upon treatment with histone modification inhibitors. Acetylation of histone H3 lysine (Lys) 9 was chosen as a model system. Initial experiments with purified histone and AlphaLISA immunoassay technology demonstrated that the assay was not stable and interacted non-specifically with the antibody and/or bead reagents. The assay involved two steps: 1) acetylation of the Lys 9 model using reconstituted histone H3, followed by 2) reaction with 5-Fluorescein acetyl CoA that was optimized so that the Histone was exposed to low salt conditions.  

**Summary**  

Histone H3 acetylation was monitored using a fluorescence assay. The enzyme reaction was stopped at 10, 30, and 60 min and the resulting fluorescence intensity was measured.  

**Materials and Methods**  

Materials:  

Acetyltransferase Activity Kits and anti-histone H3 Acetyl Lys9 were from Assay Designs. Anti-Histone H2 (C-terminal) anti-acetyl-histone H2 peptide, pCAF, and Histones extracted from HeLa cells were from Millipore. Human Histone H2 (C-term) Acetyl CoA, Histone H3 (C-term), Histone H4 (C-term) Acetyl CoA, and recombinant Acetyltransferase were from Pierce Chemicals. Anti-Acetyl transferase antibody was from Millipore. Histone H3 from HeLa Cells. A highly sensitive, specific, and automated assay was developed. The signal was measured using Histone H3 from HeLa Cells and the signal was detected using the Envision Multilabel Reader. 

AlphaLISA Assay Principle  

The biotinylated anti-analyte antibody binds to the Streptavidin-coated donor beads while another anti-analyte antibody is immobilized on the AlphaLISA Acceptor beads. In the presence of the antibody, the beads coated with a reporter molecule. The excitation of the donor beads provides the release of singlet oxygen molecules that triggers a cascade of energy transfer to the Acceptor beads resulting in a sharp peak of light emission at 615 nm.

**Optimized in vitro Assay**

A very high maximum signal and a good signal to background (S/B) ratio were obtained at the 30 min concentration. At 0.3 M NaI, histone was a significant difference between the control and acetylated histone signal.

**Results**

AlphaLISA Assay Principle  

The reaction of pCAF and histone H3 were optimized using the Assay Designs acetyl transferase kit, which measures the release of CoA. Different buffers, as well as enzyme and substrate concentrations, were tested. A time course of the optimized reaction is illustrated.

**Optimization of Detection Buffer**

Above are illustrated the competition curves before and after buffer optimization. The binding of the anti-acetyl antibody to the acetylated histone peptide was competed by both acetylated or unmodified histone (histone H3Ac), indicating the requirement of non-specific interactions occurring. The addition of 0.5 M NaI to the detection buffer was critical to decrease the non-specific interactions, so that only acetylated histone competed the binding (high NaI).

**Detecting Ac-H3 from HeLa Cells**

Histone hyperacetylation could be measured in inhibitor-treated HeLa cells. HeLa cells were treated with either sodium butyrate or trichostatin A (TSA). In the sodium butyrate experiment, the histones were extracted from a nucleus-free preparation and used to assay the formation of acetylated histone H3. NanoLC-MS/MS analysis of the peptide mixture was carried out using the nanoLC system to generate a mass spectrometric signature for the tryptic peptide of histone acetylation. The extracts from control or treated cells were then assayed using the optimized immunoassay.