TECHNICAL NOTE

# LANCE *Ultra* p300 Histone H3-Lysine Acetyltransferase Assay

*U*-TRF #37

LANCE® *Ultra*


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This LANCE *Ultra* immunodetection assay measures the acetylation of a biotinylated Histone H3 (1-21) peptide at lysine 9.

## Europium-anti-acetyl-Histone H3 Lysine 9 (H3K9ac) Antibody

- TRF0400-D: 10 µg, 1,562 assay points*
- TRF0400-M: 100 µg, 15,625 assay points*

**40 fmol/assay point*

## Peptidic Substrate Sequence:

ARTKQTAR**K**STGGKAPRKQLA-GG-K(BIOTIN)-NH2

## LANCE *Ultra* Assays

LANCE *Ultra* time-resolved fluorescence resonance energy transfer (TR-FRET) assays use a proprietary europium chelate donor dye, W1024 (Eu), together with U*Light*™, a small molecular weight acceptor dye with a red-shifted fluorescent emission.

In this technical note, we present the optimization of an epigenetic enzymatic assay using a biotinylated histone H3-derived peptide as substrate. The modified peptide is captured by the Eu-labeled antibody (Eu-Ab) and U*Light*-Streptavidin (SA) which bring the Eu donor and U*Light* acceptor dye molecules into close proximity. Upon irradiation at 320 or 340 nm, the energy from the Eu donor is transferred to the U*Light* acceptor dye which, in turn, generates light at 665 nm. The intensity of the light emission is proportional to the level of biotinylated substrate modification.

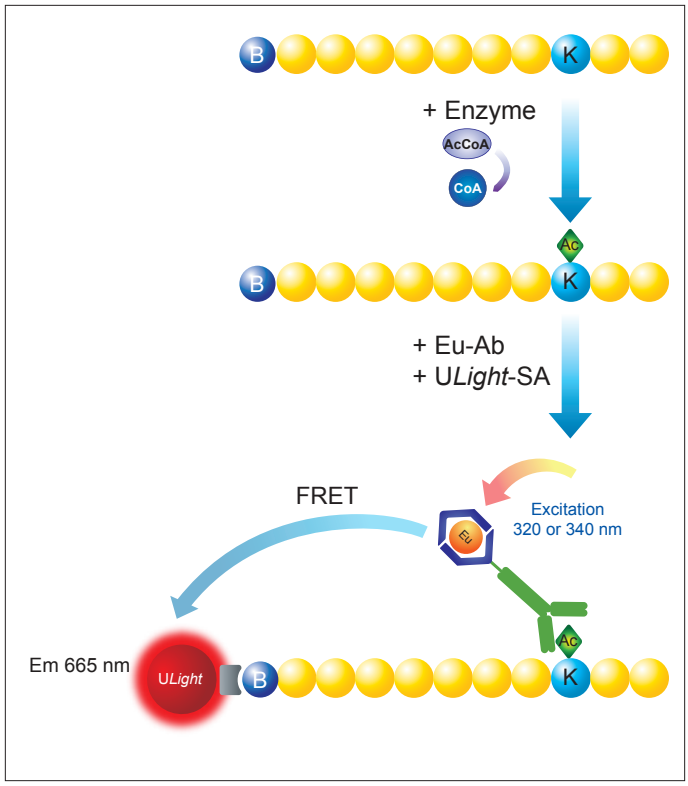


*Figure 1.* Schematic representation of the LANCE *Ultra* detection of a modified histone peptide

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# Development of a p300 Histone H3-Lysine Acetyltransferase Assay

## Reagents needed for the assay:

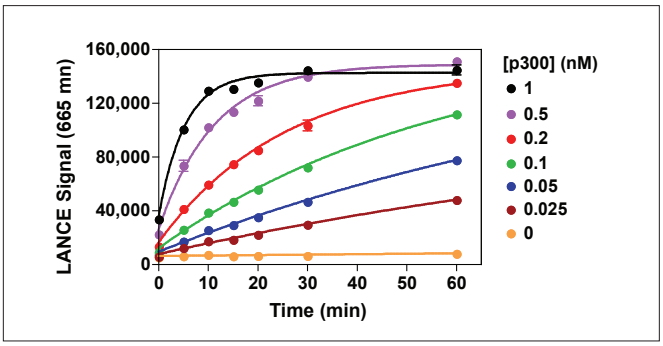
| | |
|---|---|
| Europium-anti-acetyl-Histone H3 Lysine 9 (H3K9ac) | PerkinElmer # TRF0400 |
| LANCE *Ultra* U*Light*-Streptavidin | PerkinElmer # TRF0102 |
| Histone H3 (1-21) peptide, biotinylated | AnaSpec # 61702 |
| LANCE Detection Buffer, 10X | PerkinElmer # CR97-100 |
| p300 (human), recombinant | Enzo # BML-SE451 |
| White opaque OptiPlate™-384 | PerkinElmer # 6007299 |
| TopSeal™-A films | PerkinElmer # 6005185 |
| Acetyl coenzyme A (acetyl CoA) | Sigma # A2056 |
| Anacardic Acid | Calbiochem # 172050 |
| Garcinol | Sigma # G5173 |
| Trichostatin A (TSA) | Sigma # T8552 |

Acetyl CoA is prepared at 56.9 mM in $H_2O$, aliquoted, and stored at -80 °C.
Assay Buffer: 50 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 1 mM DTT, 0.01% Tween-20, 0.01% BSA, 330 nM TSA

## Standard Protocol

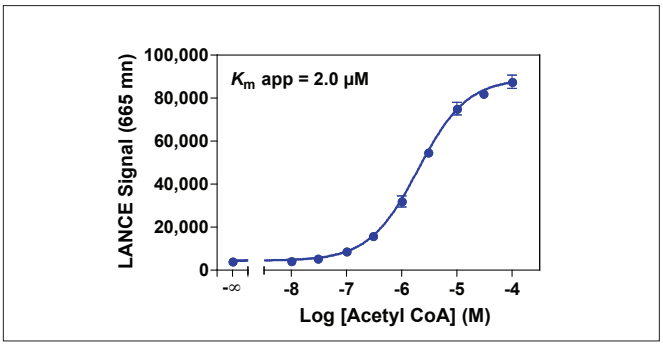
- Dilute p300 enzyme, acetyl CoA, inhibitors and biotinylated peptide substrate in Assay Buffer just before use.
- Add to the wells of a white Optiplate-384:
  - 5 µL of inhibitor (2X) or Assay Buffer
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of biotinylated Histone H3 (1-21) peptide/acetyl CoA mix (4X). For acetyl CoA titration, add acetyl CoA dilutions independently of substrate.
- Cover the plate with TopSeal-A film and incubate at room temperature (RT).
- Prepare Detection Mix by diluting the Eu-Ab to 8 nM and U*Light*-Streptavidin to 200 nM in 1X LANCE Detection Buffer (final concentrations of 2 nM and 50 nM, respectively, in 20 µL total assay volume).
- Prepare anacardic acid (4X) at 120 µM in 1X LANCE Detection buffer (final concentration of 30 µM in 20 µL total assay volume).
- Add 5 µL of anacardic acid to each well to stop the enzymatic reaction.
- Add 5 µL of Detection Mix.
- Cover with TopSeal-A film and incubate for 60 min at RT.
- Remove the TopSeal-A film and read signal with EnVision® Multilabel Reader in TR-FRET mode (excitation at 320 or 340 nm & emission at 665 nm).

## Experiment 1: Enzyme Titration and Time-Course



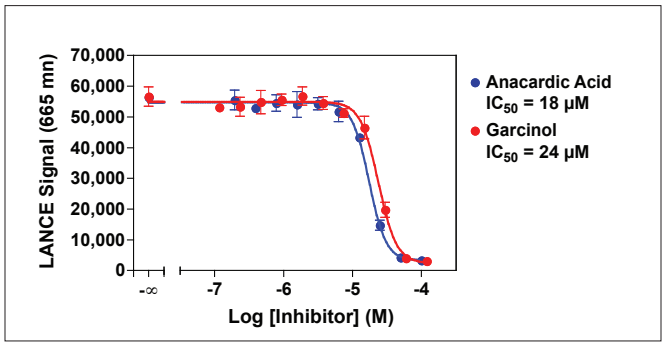

Enzymatic progress curves were performed by incubating p300 at concentrations ranging from 0.025 to 1 nM with 200 nM biotinylated H3 (1-21) peptide substrate and 25 µM acetyl CoA. Reactions were stopped by the addition of anacardic acid at the indicated times, followed by the Detection Mix. Signal was read after 60 min. A 30 min reaction time using 0.1 nM enzyme was selected for all subsequent experiments.

## Experiment 2: Acetyl CoA Titration



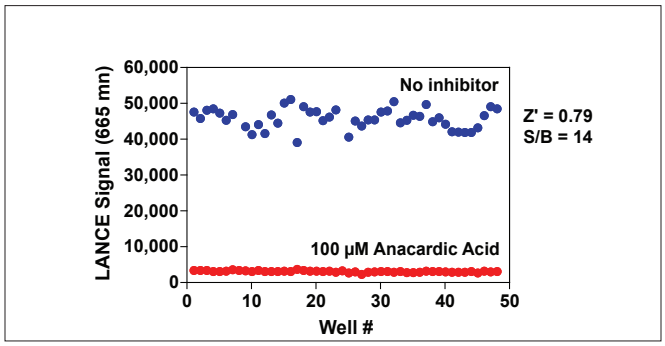

Serial dilutions of acetyl CoA ranging from 10 nM to 100 µM were added to 0.1 nM p300 and 200 nM biotinylated H3 (1-21) peptide substrate. A 5 µM acetyl CoA concentration was selected for subsequent experiments.

## Experiment 3: Enzyme Inhibition




Serial dilutions of anacardic acid ranging from 200 nM to 100 µM and garcinol ranging from 120 nM to 120 µM were pre-incubated for 10 min with 0.1 nM p300. Enzymatic reactions were initiated by the addition of 200 nM biotinylated H3 (1-21) peptide substrate plus 5 µM acetyl CoA. Enzymatic reactions contain 1% DMSO.

## Experiment 4: Z'-factor Determination




p300 (0.1 nM) was pre-incubated with or without 100 µM anacardic acid for 10 min. Enzymatic reactions were initiated by the addition of 200 nM biotinylated H3 (1-21) peptide substrate plus 5 µM acetyl CoA. Enzymatic reactions contain 1% DMSO.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