TECHNICAL NOTE

LANCE *Ultra* HDAC1 Histone H3-Lysine 27 Deacetylase Assay

U-TRF #39

LANCE® *Ultra*

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This LANCE *Ultra* immunodetection assay measures the deacetylation of a biotinylated Histone H3 (21-44) peptide acetylated at lysine 27.

Europium-anti-acetyl Histone H3 Lysine 27 (H3K27ac) Antibody

- TRF0405-D: 10 µg, 1,562 assay points*
- TRF0405-M: 100 µg, 15,625 assay points*

**40 fmol/assay point*

Peptidic Substrate Sequence:

ATKAAR**K(ac)**SAPATGGVKKPHRYRP-GG-K(Biotin)-OH

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 a signal decrease HDAC1 assay using as substrate a biotinylated Histone H3-derived peptide acetylated at lysine 27. In the absence of enzyme, the modified peptide is captured by the Eu-labeled antibody (Eu-Ab) and U*Light*-Streptavidin (U*Light*-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 (left panel). When the enzyme is added to the reaction, the peptide substrate is deacetylated and the anti-H3K27ac Eu-Ab does not recognize the biotinylated peptide anymore, causing a decrease in signal (right panel). This signal decrease is proportional to the deacetylation activity of the HDAC1 enzyme.

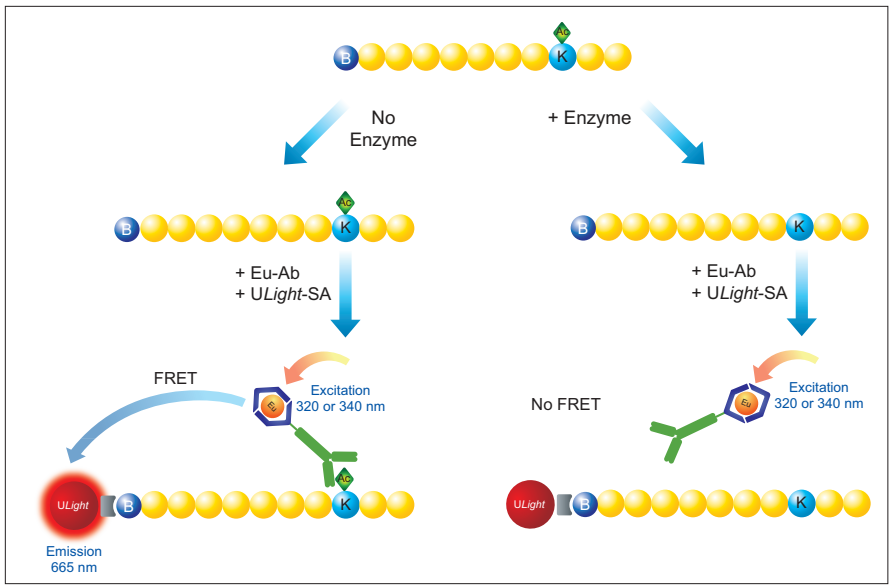

Figure 1. Schematic representation of the LANCE *Ultra* detection of a modified histone peptide.

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Development of a HDAC1 Histone H3-Lysine 27 Deacetylase Assay:

Reagents needed for the assay:

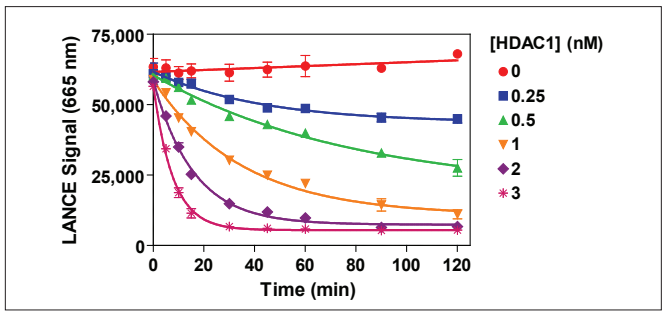
Europium-anti-acetyl-Histone H3 lysine 27 (H3K27ac) Antibody	PerkinElmer # TRF0405
LANCE *Ultra* U*Light*-Streptavidin	PerkinElmer # TRF0102
Histone H3 (21-44), H3K27ac peptide, biotinylated	AnaSpec # 64846
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
HDAC1 (human), recombinant	Cayman Chemical # 10009231
Trichostatin A	Sigma # T8552
SAHA	Cayman Chemical # 10009929
White opaque OptiPlate™-384	PerkinElmer # 6007299
TopSeal™-A films	PerkinElmer # 6005185

Assay Buffer: 50 mM Tris-HCl pH 8.0, 0.1 mM EDTA, 1 mM DTT, 0.01% Tween-20 and 0.01% BSA.

Standard Protocol

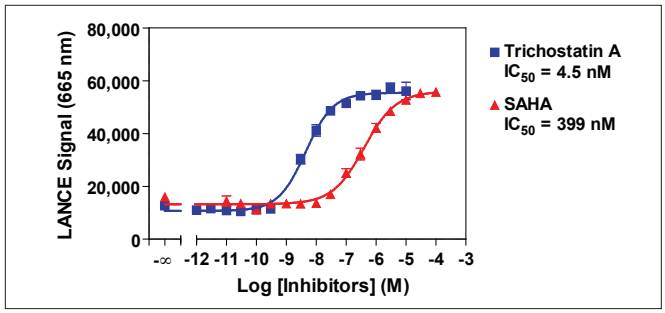
- Dilute HDAC1 enzyme, inhibitors and biotinylated Histone H3K27ac peptide substrate in Assay Buffer just before use.
- Add to the wells of a white Optiplate-384:
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of inhibitor (4X) or assay buffer
  - Incubate 5 min at RT
  - 5 µL of biotinylated Histone H3K27ac peptide (2X)
- Cover the plate with TopSeal-A film and incubate at room temperature (RT).
- Prepare a 4X Stop Solution containing 400 nM of Trichostatin A in 1X LANCE Detection Buffer (final concentration of 100 nM Trichostatin A in 20 µL total assay volume).
- Prepare a 4X 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).
  - 5 µL of Trichostatin A Stop Solution and incubate 5 min at RT
  - 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 the EnVision® Multilabel Reader in TR-FRET mode (excitation at 320 or 340 nm & emission at 665 nm).

Experiment 1: Enzyme Titration and Time-Course



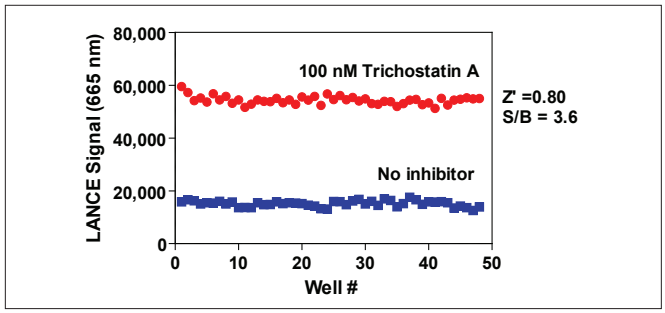
Enzymatic progress curves were performed by incubating HDAC1 at concentrations ranging from 0.25 to 3 nM with 3 nM biotinylated Histone H3K27ac peptide substrate. Reactions were stopped by the addition of Trichostatin A at indicated times. The Detection Mix was then added and signal read after 60 min. A 45 min reaction time using 1 nM enzyme was selected for all subsequent experiments.

Experiment 2: Enzyme Inhibition



Serial dilutions of Trichostatin A ranging from 1 pM to 10 µM and serial dilutions of SAHA ranging from 10 pM to 100 µM were pre-incubated for 5 min with 1 nM of HDAC1. Enzymatic reactions were initiated by the addition of 3 nM biotinylated Histone H3K27ac peptide substrate. Enzymatic reactions contain 1% DMSO.

Experiment 3: Z'-factor Determination



HDAC1 (1 nM) was pre-incubated with or without 100 nM Trichostatin A for 5 min. Enzymatic reactions were initiated by the addition of 3 nM biotinylated Histone H3K27ac peptide substrate. Enzymatic reactions contain 1% DMSO.

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