TECHNICAL NOTE

LANCE *Ultra* SIRT1 Histone H3-Lysine 4 Deacetylase Assay

U-TRF #44

LANCE® *Ultra*

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This LANCE *Ultra* immunodetection assay measures the deacetylation of a biotinylated Histone H3 (1-21) peptide acetylated at lysine 4.

Europium-anti-unmodified Histone H3 Lysine 4 (H3K4) Antibody

- TRF0404-D: 10 µg, 1,562 assay points*
- TRF0404-M: 100 µg, 15,625 assay points*

**40 fmol/assay point*

Peptidic Substrate Sequence:

ART**K(ac)**QTARKSTGGKAPRKQLA-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 an SIRT1 epigenetic assay using as substrate a biotinylated Histone H3-derived peptide acetylated at lysine 4. The deacetylated peptide product 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. The intensity of the light emission is proportional to the level of biotinylated substrate modification.

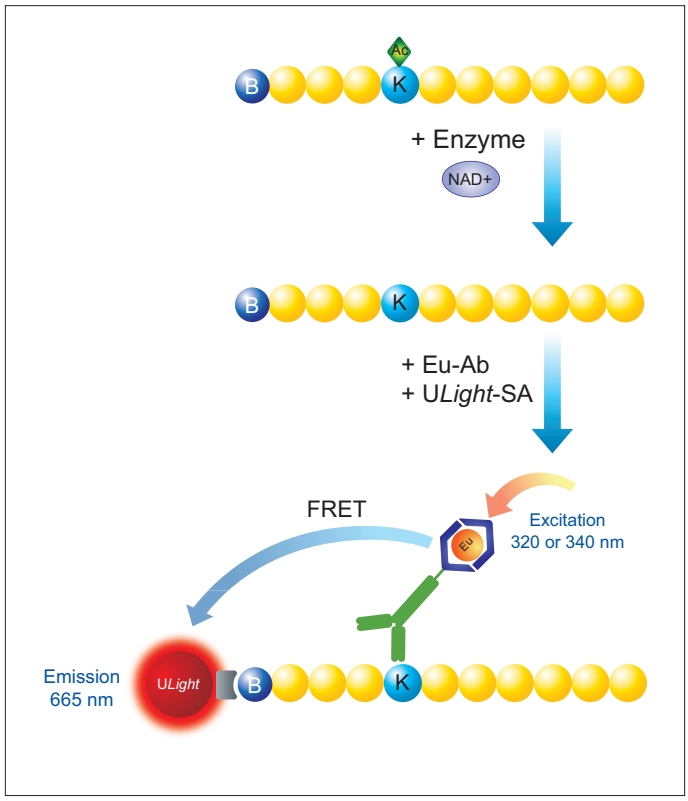

Figure 1. Schematic representation of the LANCE *Ultra* detection of a deacetylated histone peptide.

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Development of a SIRT1 Histone H3-Lysine 4 Deacetylase Assay:

Reagents needed for the assay:

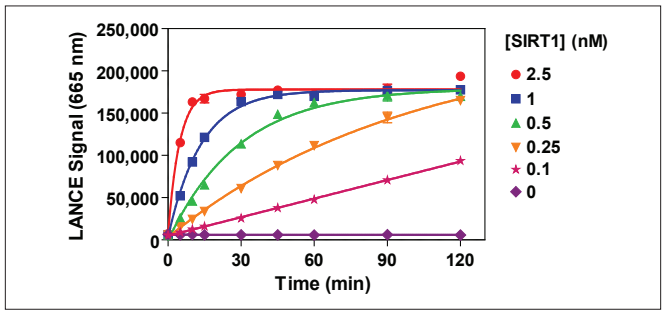
Europium-anti-unmodified Histone H3 Lysine 4 (H3K4) Antibody	PerkinElmer # TRF0404
LANCE *Ultra* U*Light*-Streptavidin	PerkinElmer # TRF0102
Histone H3 (1-21), H3K4ac peptide, biotinylated	AnaSpec # 65207
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
Sirtuin1 (human SIRT1), recombinant	BPS BioScience # 50012
EX-527	Tocris # 2780
Suramin	Calbiochem # 574625
Nicotinamide	Sigma # N3376
ß-Nicotinamide adenine dinucleotide hydrate (NAD^+)	Sigma # N1636
White opaque OptiPlate™-384	PerkinElmer # 6007299
TopSeal™-A films	PerkinElmer # 6005185

Assay Buffer: 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM DTT, 0.01% Tween-20 and 0.01% BSA.

Standard Protocol

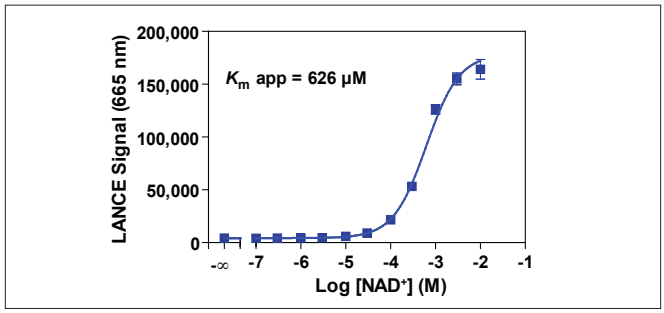
- Dilute SIRT1 enzyme, inhibitors, biotinylated Histone H3K4ac peptide substrate and NAD^+ in Assay Buffer just before use.
- Add to the wells of a white Optiplate-384:
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of inhibitor (4X) or assay buffer
  - Incubate 5 min at RT
  - 2.5 µL of biotinylated Histone H3K4ac peptide (4X)
  - 2.5 µL of NAD+ (4X)
- Cover the plate with TopSeal-A film and incubate at room temperature (RT).
- Prepare a 4X Stop Solution containing 400 µM of EX-527 in 1X LANCE Detection Buffer (final concentration of 100 µM EX-527 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 EX-527 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



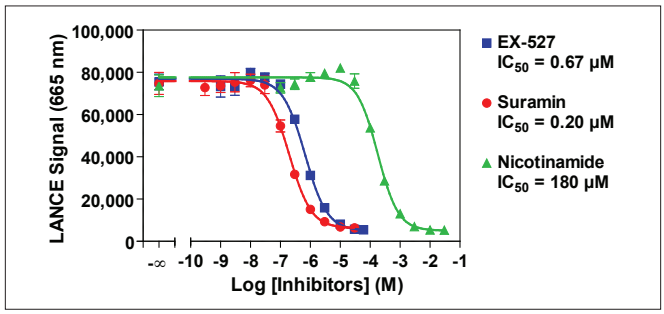
Enzymatic progress curves were performed by incubating SIRT1 at concentrations ranging from 0.1 to 2.5 nM with 300 nM biotinylated Histone H3K4ac peptide substrate and 1 mM NAD^+. Reactions were stopped by the addition of EX-527 at indicated times. Detection Mix was added and signal read after 60 min. A 30 min reaction time using 0.5 nM enzyme was selected for all subsequent experiments.

Experiment 2: NAD^+ Titration



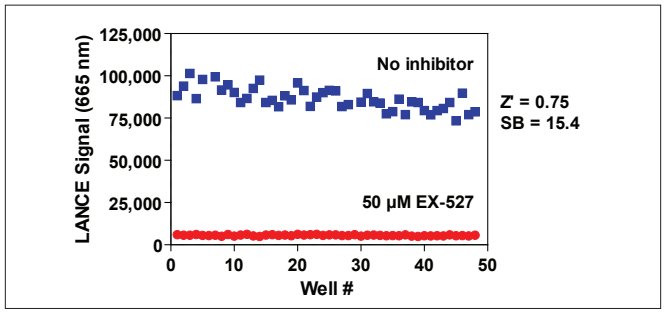
Serial dilutions of NAD^+ ranging from 100 nM to 10 mM were added to 0.5 nM SIRT1 and 300 nM biotinylated Histone H3K4ac peptide substrate. A 500 µM NAD^+ concentration was selected for subsequent experiments.

Experiment 3: Enzyme Inhibition



Serial dilutions of EX-527 ranging from 1 nM to 60 µM, serial dilutions of suramin ranging from 0.3 nM to 30 µM and serial dilutions of nicotinamide ranging from 100 nM to 30 mM were pre-incubated for 5 min with 0.5 nM of SIRT1. Enzymatic reactions were initiated by the addition of 300 nM biotinylated Histone H3K4ac peptide substrate plus 500 µM NAD^+. Enzymatic reactions contain 1% DMSO.

Experiment 4: Z'-factor Determination



SIRT1 (0.5 nM) was pre-incubated with or without 50 µM EX-527 for 5 min. Enzymatic reactions were initiated by the addition of 300 nM biotinylated Histone H3K4ac peptide substrate plus 500 µM NAD^+. Enzymatic reactions contain 1% DMSO.

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