TECHNICAL NOTE

LANCE *Ultra* SIRT1 p53 Lysine 382 Deacetylase Assay

U-TRF #43

Authors

Mathieu Arcand
Julie Blouin
Mireille Caron
Claire Normand
Anne Labonté
Lucille Beaudet
Jaime Padrós

PerkinElmer, Inc.
Montreal, QC
Canada, H3J 1R4

LANCE® *Ultra*

This LANCE *Ultra* immunodetection assay measures the deacetylation of a biotinylated p53 (368-393) peptide acetylated at lysine 382.

Europium-anti-acetyl-p53 Lysine 382 (p53K382ac) Antibody

- TRF0409-D: 10 µg, 1,562 assay points*
- TRF0409-M: 100 µg, 15,625 assay points*

**40 fmol/assay point*

Peptidic Substrate Sequence:

Biotin-KGGHLKSKKGQSTSRHK**K(ac)**LMFKTEGPDSD-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 a signal decrease SIRT1 assay using as substrate a biotinylated p53-derived peptide acetylated at lysine 382. In the absence of enzyme or cofactor, the modified peptide is captured by the Eu-labeled antibody (Eu-Ab) and U*Light*-Streptavidin (U*Light*-SA), which brings 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 enzyme and cofactor are added to the reaction, the peptide substrate is deacetylated and the anti-p53K382ac 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 SIRT1 enzyme.

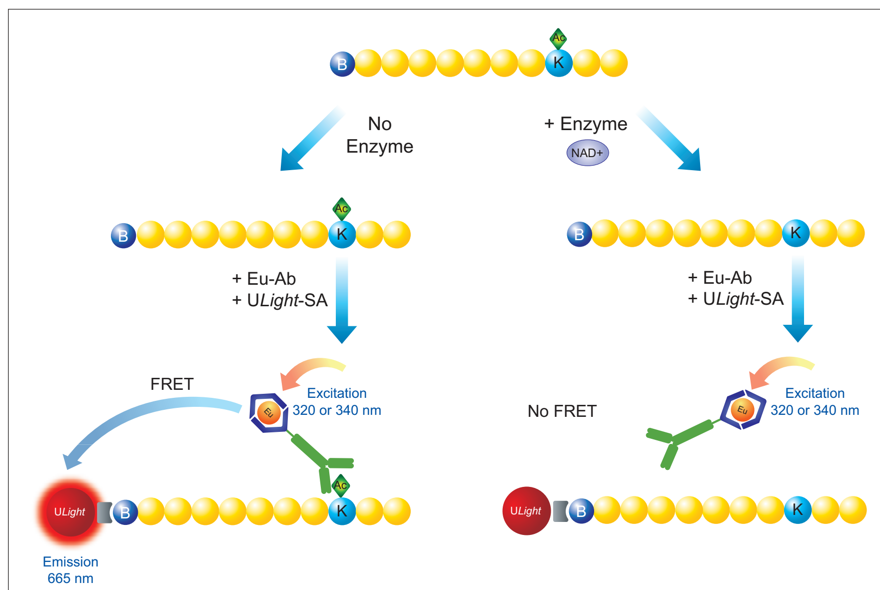

Figure 1. Schematic representation of the LANCE *Ultra* detection of a modified p53-derived peptide.

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Development of a SIRT1 p53 Lysine 382 Deacetylase Assay:

Reagents needed for the assay:

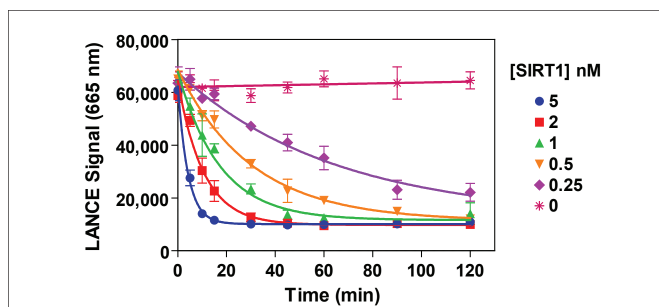
Reagent	Source
Europium-anti-acetyl-p53 Lysine 382 (p53K382ac) Antibody	PerkinElmer # TRF409
LANCE *Ultra* U*Light*-Streptavidin	PerkinElmer # TRF0102
p53 (368-393) acetyl-lysine 382 peptide (p53K382ac), biotinylated	AnaSpec # 64869
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
Sirtuin1 (human SIRT1), recombinant	BPS BioScience # 50012
EX-527	Tocris # 2780
SIRT1 Inhibitor III	Calbiochem # 566322
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

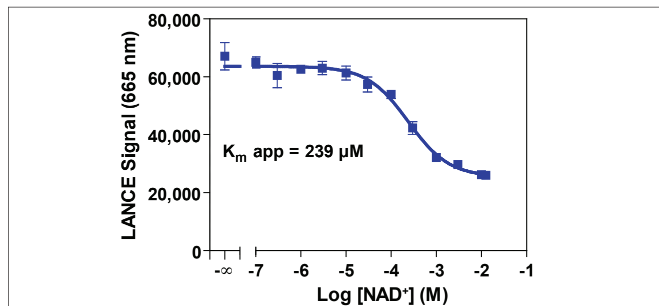
- Dilute SIRT1 enzyme, inhibitors, biotinylated p53K382ac peptide substrate and NAD^+ in Assay Buffer just before use.
- Add to the wells of a white Optiplate-384:
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of inhibitor (4X) or assay buffer
  - Incubate 5 min at RT
  - 2.5 µL of biotinylated p53K382ac peptide (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



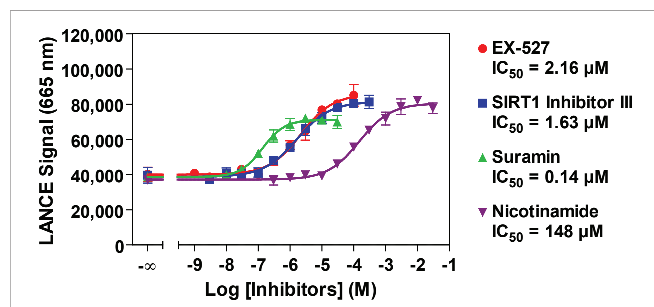
Enzymatic progress curves were performed by incubating SIRT1 at concentrations ranging from 0.25 to 5 nM with 3 nM biotinylated p53K382ac peptide substrate and 2 mM NAD^+. Reactions were stopped by the addition of EX-527 at indicated times. Detection Mix was then added and signal read after 60 min. A 0.5 nM enzyme concentration was selected for all subsequent experiments.

Experiment 2: NAD^+ Titration



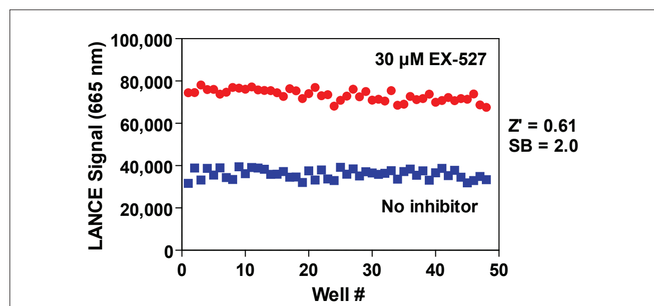
Serial dilutions of NAD^+ ranging from 100 nM to 12.5 mM were added to 0.5 nM SIRT1 and 3 nM biotinylated p53K382ac peptide substrate. A 200 µM NAD^+ concentration was selected for subsequent experiments.

Experiment 3: Enzyme Inhibition



Serial dilutions of inhibitors ranging from 1 nM to 100 µM (EX-527), 3 nM to 300 µM (SIRT1 Inhibitor III), 10 nM to 30 µM (suramin) and 300 nM to 30 mM (nicotinamide) were pre-incubated for 5 min with 0.5 nM of SIRT1. Enzymatic reactions were initiated by the addition of 3 nM biotinylated p53K382ac peptide substrate and 200 µM NAD^+. Enzymatic reactions contained 1% DMSO and proceeded for 60 min.

Experiment 4: Z'-factor Determination



SIRT1 (0.5 nM) was pre-incubated with or without 30 µM EX-527 for 5 min. Enzymatic reactions were initiated by the addition of 3 nM biotinylated p53K382ac peptide substrate and 200 µM NAD^+. Enzymatic reactions contained 1% DMSO and proceeded for 60 min.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

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