TECHNICAL NOTE

LANCE *Ultra* HDAC1 Histone H3-Lysine 9 Deacetylase Assay

U-TRF #47

Authors

Nancy Gauthier
Marie-Claude Loiselle
Marjolaine Roy
Liliana Pedro
Lucille Beaudet
Roberto Rodriguez-Suarez

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

LANCE® *Ultra*

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

Europium-anti-unmodified Histone H3 Lysine 9/Lysine 27 (H3K9/K27) Antibody

- TRF0411-D: 10 µg, 1,562 assay points*
- TRF0411-M: 100 µg, 15,625 assay points*

**40 fmol/assay point*

Peptidic Substrate Sequence:

ARTKQTAR-**K(ac)**-STGGKAPRKQLA-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 HDAC1 enzymatic assay using as substrate a biotinylated histone H3-derived peptide acetylated at lysine 9. 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 deacetylation activity of the HDAC1 enzyme.

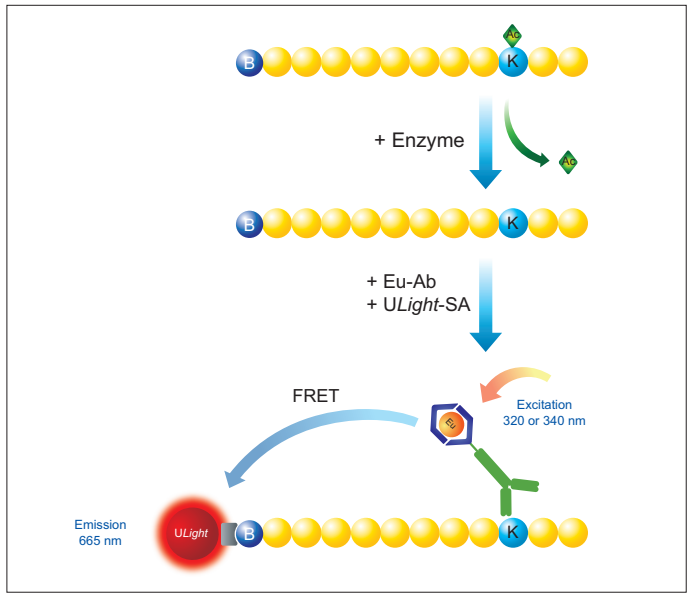


Figure 1. Schematic representation of the LANCE *Ultra* detection of a deacetylated histone peptide (B: biotin group; K: lysine residue; Ac: acetyl group).

PerkinElmer®
For the Better

Development of a HDAC1 Histone H3-Lysine 9 Deacetylase Assay

Reagents needed for the assay:

Europium-anti-unmodified Histone H3 Lysine 9/ Lysine 27 (H3K9/K27) Antibody	PerkinElmer # TRF0411
LANCE *Ultra* U*Light*-Streptavidin	PerkinElmer # TRF0102
Histone H3 (1-21) lysine 9 acetylated peptide, biotinylated (H3K9ac)	AnaSpec # 64361
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
HDAC1 (human), recombinant	Cayman # 10009231
White opaque OptiPlate™-384	PerkinElmer # 6007290
TopSeal™-A film	PerkinElmer # 6050195
Trichostatin A	Sigma # T8552
SAHA	Cayman # 10009929

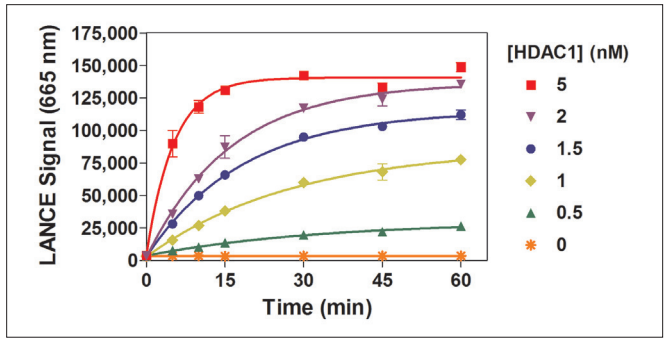
Assay Buffer: 50 mM Tris-HCl pH 8.0, 1 mM DTT, 0.01% Tween-20

Standard Protocol

- Dilute HDAC1 enzyme, inhibitors and biotinylated histone H3K9ac peptide substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 2.5 µL of inhibitor (4X) or Assay Buffer
  - 2.5 µL of enzyme (4X)
  - Incubate for 10 min at room temperature (RT).
  - 5 µL of biotinylated H3K9ac peptide (2X)
- Cover the plate with TopSeal-A film and incubate at 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).
- Add 5 µL of Trichostatin A Stop Solution (4X).
- Add 5 µL of Detection Mix (4X).
- 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).

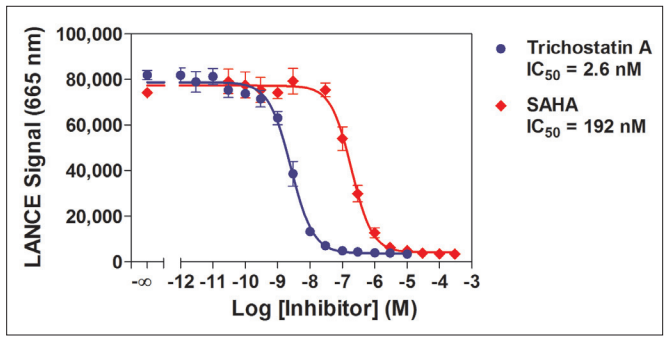
Results

Experiment 1: Enzyme Titration and Time-Course



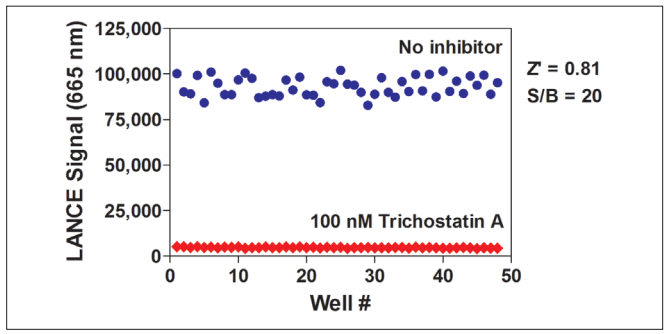
Enzymatic progress curves were performed by incubating HDAC1 at concentrations ranging from 0.5 to 5 nM with 50 nM biotinylated H3K9ac peptide substrate. Reactions were stopped by the addition of Trichostatin A at indicated times, followed by the Detection Mix. Signal was read after 60 min. A 30 min reaction time using 1.5 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 SAHA ranging from 30 pM to 300 µM were pre-incubated for 10 min with 1.5 nM HDAC1. Enzymatic reactions were initiated by the addition of 50 nM biotinylated H3K9ac peptide substrate. Enzymatic reactions contain 2% DMSO.

Experiment 3: Z'-factor Determination



HDAC1 (1.5 nM) was pre-incubated with or without 100 nM Trichostatin A for 10 min. Enzymatic reactions were initiated by the addition of 50 nM biotinylated H3K9ac peptide substrate. Enzymatic reactions contain 2% DMSO.

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

PerkinElmer®

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

010042_01 Printed in USA Dec. 2011