TECHNICAL NOTE

# LANCE *Ultra* EZH2 Histone H3-Lysine 27 N-methyltransferase Assay

*U*-TRF #40


## Authors

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

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


**LANCE® *Ultra***

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

## Europium-anti-di/mono-methyl-Histone H3 Lysine 27 (H3K27me2-1) Antibody

- TRF0406-D: 10 µg, 1,562 assay points*
- TRF0406-M: 100 µg, 15,625 assay points*

**40 fmol/assay point*

## Peptidic Substrate Sequence:

ATKAARKSAPATGGVKKPHRYRP-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 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 (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. The intensity of the light emission is proportional to the level of biotinylated substrate modification.

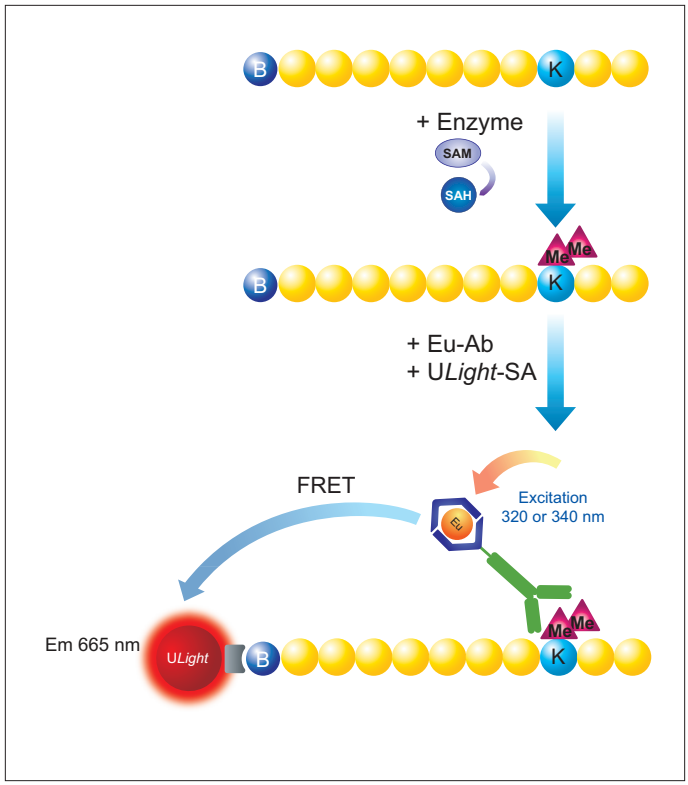



*Figure 1.* Schematic representation of the LANCE *Ultra* detection of a modified histone peptide.

PerkinElmer®
*For the Better*

# Development of an EZH2 Histone H3-Lysine 27 N-methyltransferase Assay:

## Reagents needed for the assay:

| | |
|---|---|
| Europium-anti-di/mono-methyl-Histone H3 Lysine 27 (H3K27me2-1) Antibody | PerkinElmer # TRF0406 |
| LANCE *Ultra* U*Light*-Streptavidin | PerkinElmer # TRF0102 |
| Histone H3 (21-44) peptide, biotinylated | AnaSpec # 64440 |
| LANCE Detection Buffer, 10X | PerkinElmer # CR97-100 |
| EZH2/EED/SUZ12/RbAp48/AEBP2 Complex | BPS BioScience # 51004 |
| White opaque OptiPlate™-384 | PerkinElmer # 6007299 |
| TopSeal™-A films | PerkinElmer # 6005185 |
| Sinefungin | Sigma # S8559 |
| S-(5'-Adenosyl)-L-methionine chloride (SAM) | Sigma # A7007 |
| Poly-L-lysine solution – 0.1% (w/v) | Sigma # P8920 |

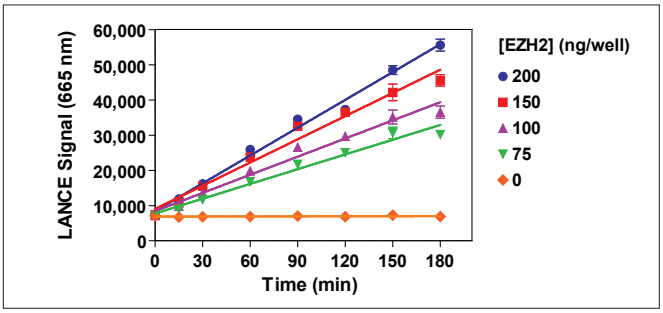
SAM is prepared at 30 mM in 5 mM $H_2SO_4$/10% ethanol (v/v) in $H_2O$, aliquoted and stored at -80 °C.

Assay Buffer: 50 mM Tris-HCl pH 9.0, 50 mM NaCl, 1 mM DTT, 0.01% Tween-20 and 0.01% BSA.

## Standard Protocol

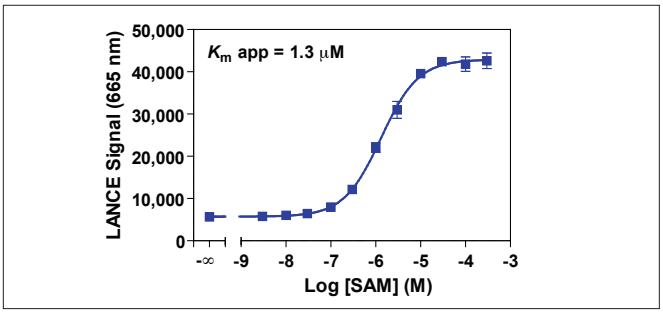
- Dilute EZH2 enzyme complex, SAM, sinefungin (inhibitor) and biotinylated peptide substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of inhibitor (4X) or Assay Buffer
  - 5 µL of biotinylated Histone H3 (21-44) peptide/SAM Mix (2X). *For SAM titration, add SAM dilutions independently of substrate*.
- Cover the plate with TopSeal-A film and incubate at room temperature (RT).
- 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).
- Prepare a 4X Stop Solution containing 0.0004% of poly-L-lysine in 1X LANCE Detection Buffer (final concentration of 0.0001% in 20 µL total assay volume).
  - 5 µL of poly-L-lysine 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



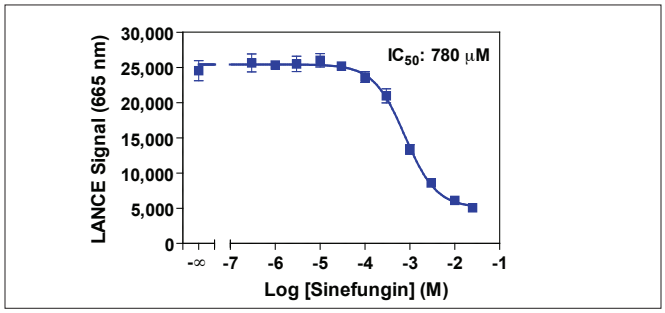

Enzymatic progress curves were performed by incubating EZH2 complex at concentrations ranging from 75 to 200 ng/well with 500 nM biotinylated Histone H3 (21-44) peptide substrate and 30 µM SAM. Reactions were stopped by the addition of poly-L-lysine at indicated times. The Detection Mix was then added and signal was read after 60 min. A 180 min reaction time using 150 ng/well enzyme was selected for all subsequent experiments.

## Experiment 2: SAM Titration



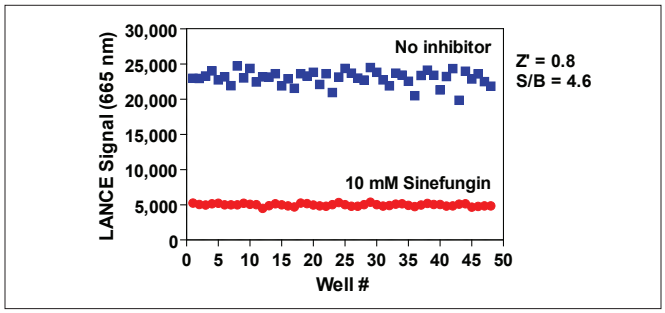

Serial dilutions of SAM ranging from 3 nM to 300 µM were added to 150 ng/well EZH2 complex and 500 nM biotinylated Histone H3 (21-44) peptide substrate. A 3 µM SAM concentration was selected for subsequent experiments.

## Experiment 3: Enzyme Inhibition




Serial dilutions of sinefungin ranging from 300 nM to 30 mM were pre-incubated for 10 min with 150 ng/well EZH2 complex. Enzymatic reactions were initiated by the addition of 500 nM biotinylated Histone H3 (21-44) peptide substrate plus 3 µM SAM. Enzymatic reactions contain 1% DMSO.

## Experiment 4: Z'-factor Determination




EZH2 complex (150 ng/well) was pre-incubated with or without 10 mM sinefungin for 10 min. Enzymatic reactions were initiated by the addition of 500 nM biotinylated Histone H3 (21-44) peptide substrate plus 3 µM SAM. Enzymatic reactions contain 1% 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**



009570_01 Printed in USA Mar. 2011