TECHNICAL NOTE

LANCE *Ultra* G9a Histone H3-Lysine N-methyltransferase Assay

U-TRF #36

LANCE® *Ultra*

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This LANCE *Ultra* immunodetection assay measures the di-methylation of a biotinylated Histone H3 (1-21) peptide at lysine 9.

Europium-anti-methyl-Histone H3 Lysine 9 (H3K9me2) Antibody

- TRF0403-D: 10 µg, 1,562 assay points*
- TRF0403-M: 100 µg, 15,625 assay points*

**40 fmol/assay point*

Peptidic Substrate Sequence:

ARTKQTAR**K**STGGKAPRKQLA-GG-K(BIOTIN)-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 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 (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.

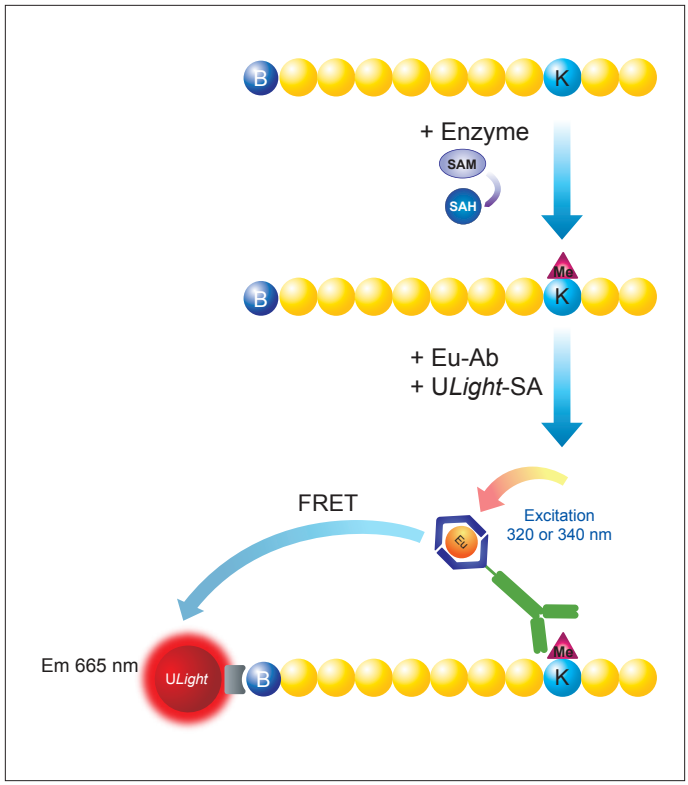

Figure 1. Schematic representation of the LANCE *Ultra* detection of a modified histone peptide.

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Development of a G9a Histone H3-Lysine N-methyltransferase Assay

Reagents needed for the assay:

Europium-anti-methyl-Histone H3 Lysine 9 (H3K9me2)	PerkinElmer # TRF0403
LANCE *Ultra* U*Light*-Streptavidin	PerkinElmer # TRF0102
Histone H3 (1-21) peptide, biotinylated	AnaSpec # 61702
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
G9a (human), recombinant	BPS Bioscience # 51001
White opaque OptiPlate™-384	PerkinElmer # 6007299
TopSeal™-A films	PerkinElmer # 6005185
S-(5'-Adenosyl)-L-methionine chloride (SAM)	Sigma # A7007
Sinefungin	Sigma # S8559
S-(5'-Adenosyl)-L-homocysteine (SAH)	Sigma # A9384
BIX 01294	Sigma # B9311

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.

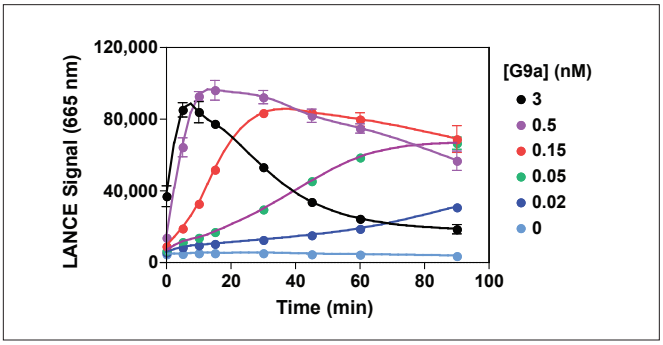
Assay Buffer: 50 mM Tris-HCl, pH 9.0, 50 mM NaCl, 1 mM DTT, 0.01% Tween-20

Standard Protocol

- Dilute G9a enzyme, SAM, inhibitors and biotinylated peptide substrate in Assay Buffer just before use.
- Add to the wells of a white Optiplate-384:
  - 5 µL of inhibitor (2X) or Assay Buffer
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of biotinylated Histone H3 (1-21) peptide/SAM mix (4X). For SAM titration, add SAM dilutions independently of substrate.
- Cover the plate with TopSeal-A film and incubate at room temperature (RT).
- Prepare Detection Mix by diluting the Eu-Ab to 4 nM, U*Light*-Streptavidin to 100 nM and poly-L-lysine* to 0.0002% in 1X LANCE Detection Buffer (final concentrations of 2 nM, 50 nM and 0.0001%, respectively, in 20 µL total assay volume).
- Add 10 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 EnVision® Multilabel Reader in TR-FRET mode (excitation at 320 or 340 nm & emission at 665 nm).

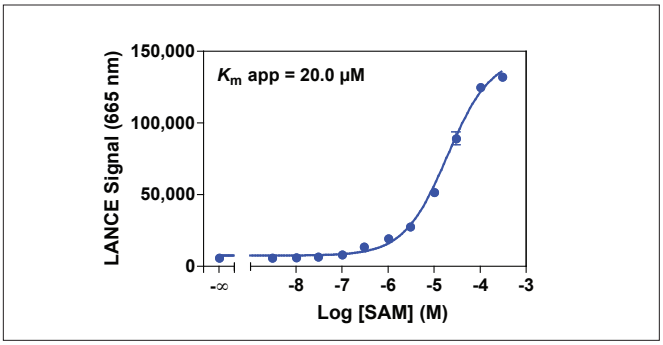
* *The poly-L-lysine (Sigma #P8920) present in the Detection Mix stops the enzymatic reaction.*

Experiment 1: Enzyme Titration and Time-Course



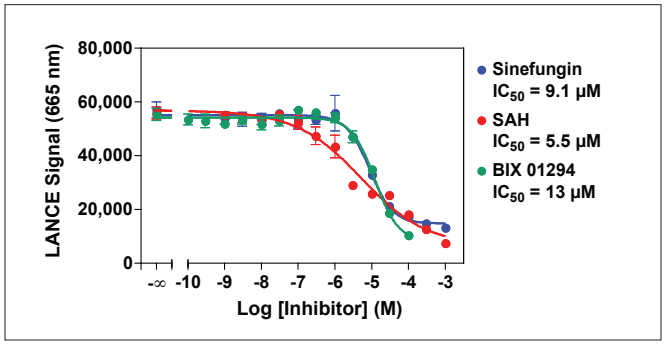
Enzymatic progress curves were performed by incubating G9a at concentrations ranging from 0.02 to 3 nM with 500 nM biotinylated H3 (1-21) peptide substrate and 300 µM SAM. Detection Mix was added to stop the reactions at the indicated times and signal was read after 60 min. A 30 min reaction time using 0.15 nM enzyme was selected for all subsequent experiments. Signal decrease observed at higher enzyme concentration or reaction time is due to the generation of peptides tri-methylated at lysine 9, which are not detected by the Eu-anti-methyl-Histone H3 Lysine 9 (H3K9me2) antibody.

Experiment 2: SAM Titration



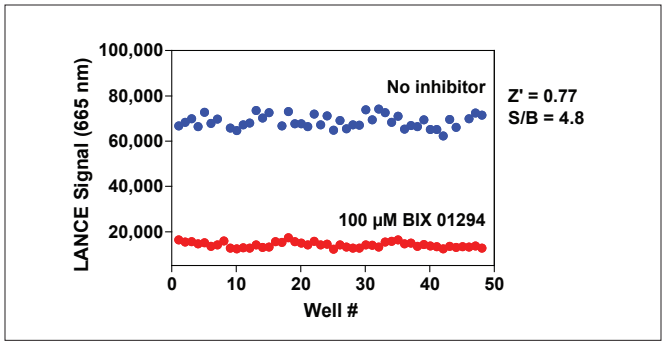
Serial dilutions of SAM ranging from 3 nM to 300 µM were added to 0.15 nM G9a and 500 nM biotinylated H3 (1-21) peptide substrate. A 20 µM SAM concentration was selected for subsequent experiments.

Experiment 3: Enzyme Inhibition



Serial dilutions of sinefungin and SAH ranging from 1 nM to 1 mM, and of BIX 01294 ranging from 100 pM to 100 µM were pre-incubated for 10 min with 0.15 nM G9a. Enzymatic reactions were initiated by the addition of 500 nM biotinylated H3 (1-21) peptide substrate plus 20 µM SAM. Enzymatic reactions contain 2% DMSO.

Experiment 4: Z'-factor Determination



G9a (0.15 nM) was pre-incubated with or without 100 µM BIX 01294 for 10 min. Enzymatic reactions were initiated by the addition of 500 nM biotinylated H3 (1-21) peptide substrate plus 20 µM SAM. Enzymatic reactions contain 2% DMSO.

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009388_01 Printed in USA Oct. 2010