TECHNICAL NOTE

LANCE *Ultra* JMJD3 Histone H3-Lysine 27 Demethylase Assay

U-TRF #42

LANCE® *Ultra*

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This LANCE *Ultra* immunodetection assay measures the demethylation of a biotinylated Histone H3 (21-44) peptide tri-methylated 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:

ATKAAR**K**(**me3**)SAPATGGVKKPHRYRP-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 anti-body (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.

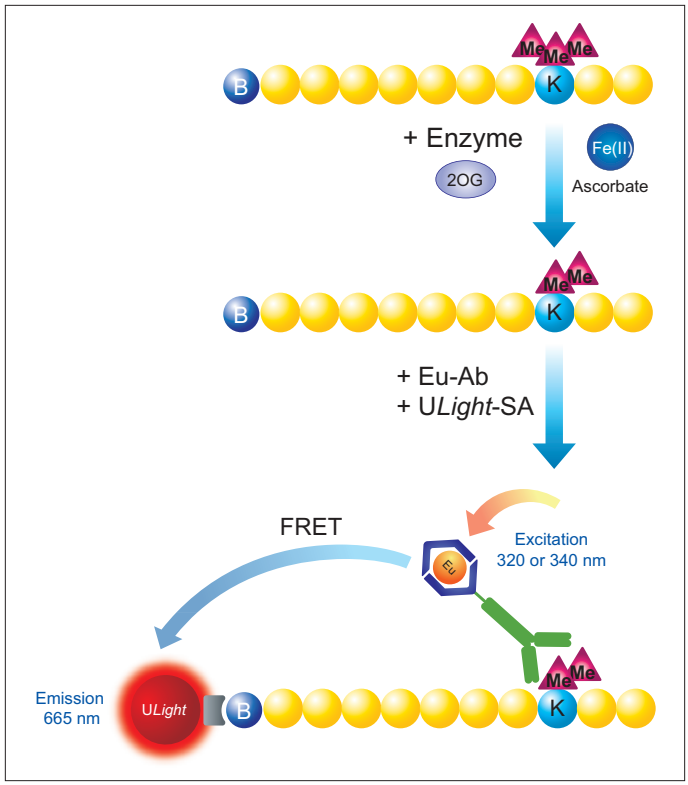

Figure 1. Schematic representation of the LANCE *Ultra* detection of a modified histone peptide.

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Development of a JMJD3 Histone H3-Lysine 27 Demethylase Assay:

Reagents needed for the assay:

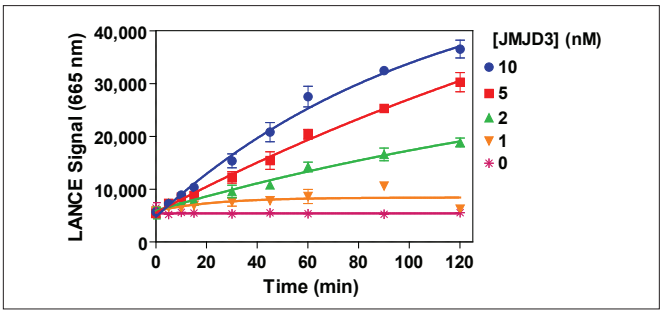
Europium-anti-di/mono-methyl-Histone H3 Lysine 27 (H3K27me2-1) Antibody	PerkinElmer # TRF0406
LANCE *Ultra* U*Light*-Streptavidin	PerkinElmer # TRF0102
Histone H3 (21-44), H3K27(me3) peptide, biotinylated	AnaSpec # 64367
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
JMJD3 (human), recombinant	BPS BioScience # 50115
White opaque OptiPlate™-384	PerkinElmer # 6007299
TopSeal™-A films	PerkinElmer # 6005185
α-Ketoglutaric acid potassium salt (2OG)	Sigma # K2000
(+) Sodium L-ascorbate	Sigma # 11140
Ammonium iron(II) sulfate hexahydrate (Fe(II))	Sigma # 215406
2,4-Pyridinedicarboxylic acid (2,4-PDCA)	Sigma # P63395
Ethylenediaminetetraacetic acid (EDTA)	Invitrogen #15575-038

Assay Buffer: 50 mM HEPES pH 7.5, 0.01% Tween-20 and 0.01% BSA

Standard Protocol

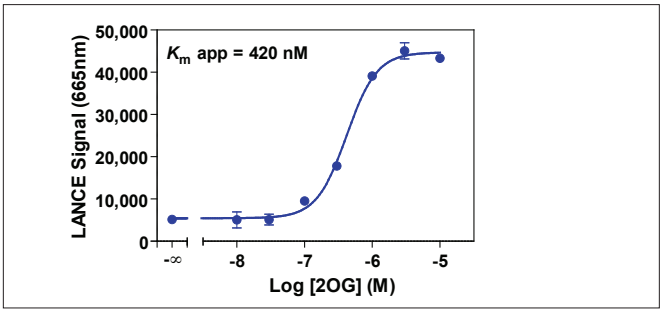
- Dilute JMJD3 enzyme, 2OG, Fe(II), ascorbate, 2,4-PDCA (inhibitor) and biotinylated peptide substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 5 µL of inhibitor (2X) or Assay Buffer
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of biotinylated Histone H3K27me3 peptide/2OG/Fe(II)/ascorbate mix (4X).
    For 2OG titration, add 2OG 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 4 mM EDTA in 1X LANCE Detection Buffer (final concentration of 1 mM in 20 µL total assay volume).
  - 5 µL of EDTA 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



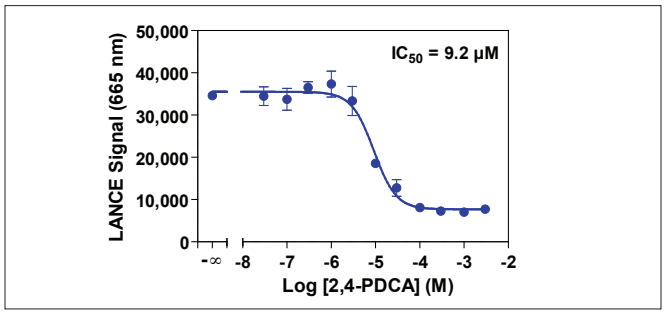
Enzymatic progress curves were performed by incubating JMJD3 at concentrations ranging from 1 to 10 nM with 200 nM biotinylated Histone H3K27me3 peptide substrate plus 50 µM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Reactions were stopped by the addition of EDTA at indicated times. The Detection Mix was then added and signal was read after 60 min. A 120 min reaction time using 5 nM enzyme was selected for all subsequent experiments.

Experiment 2: 2OG Titration



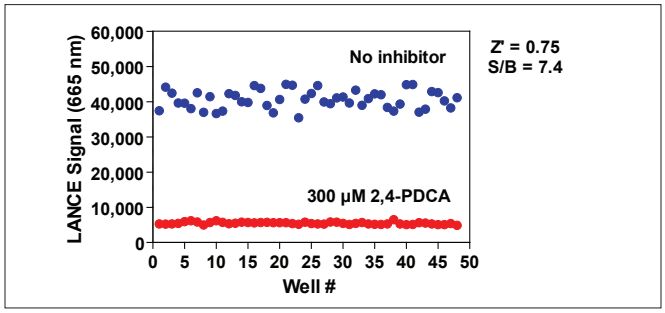
Serial dilutions of 2OG ranging from 10 nM to 10 µM were added to 5 nM JMJD3 and 200 nM biotinylated Histone H3K27me3 peptide substrate plus 5 µM Fe(II) and 100 µM ascorbate. A 500 nM 2OG concentration was selected for subsequent experiments.

Experiment 3: Enzyme Inhibition



Serial dilutions of 2,4-PDCA ranging from 30 nM to 3 mM were pre-incubated for 15 min with 5 nM JMJD3. Enzymatic reactions were initiated by the addition of 200 nM biotinylated Histone H3K27me3 peptide substrate plus 500 nM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Enzymatic reactions contain 1% DMSO.

Experiment 4: Z'-factor Determination



JMJD3 (5 nM) was incubated with or without 300 µM 2,4-PDCA. Enzymatic reactions were initiated by the addition of 200 nM biotinylated Histone H3K27me3 peptide substrate plus 500 nM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Enzymatic reactions contain 0.05% BSA and 1% DMSO.

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