TECHNICAL NOTE

LANCE *Ultra* JMJD2C Histone H3-Lysine 9 Demethylase Assay

U-TRF #45

LANCE® *Ultra*

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This LANCE *Ultra* immunodetection assay measures the demethylation of a biotinylated Histone H3 (1-21) peptide tri-methylated 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(me3)**-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 a JMJD2C epigenetic assay using as substrate a biotinylated histone H3-derived peptide tri-methylated at lysine 9. The modified peptide 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 level of biotinylated substrate modification.

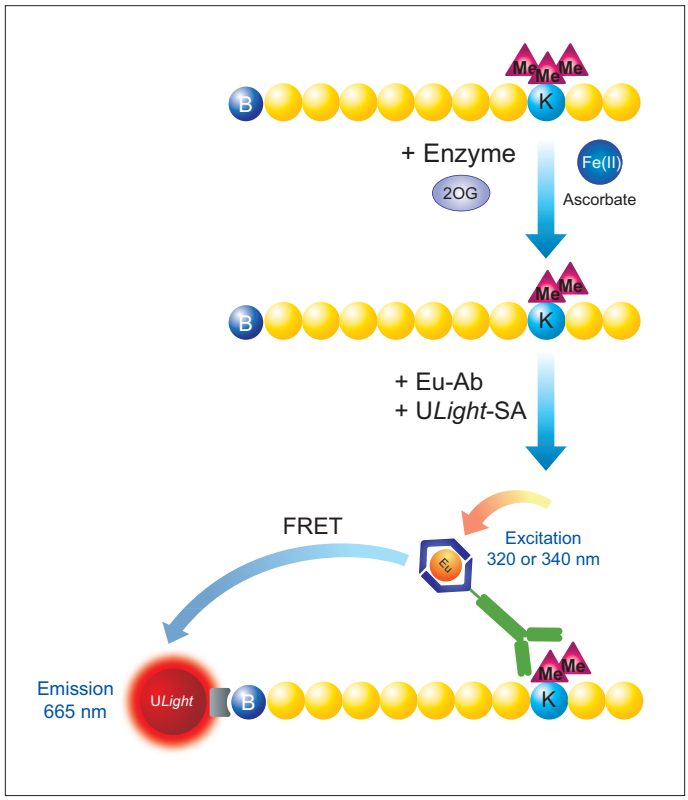

Figure 1. Schematic representation of the LANCE *Ultra* detection of a modified histone peptide.

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Development of a JMJD2C Histone H3-Lysine 9 Demethylase Assay

Reagents needed for the assay:

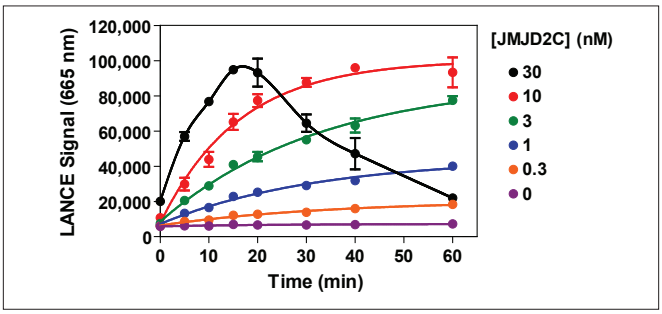
Europium-anti-methyl-Histone H3 Lysine 9 (H3K9me2)	PerkinElmer # TRF0403
LANCE *Ultra* U*Light*-Streptavidin	PerkinElmer # TRF0102
Histone H3 (1 - 21) lysine 9 tri-methylated peptide, biotinylated (H3K9me3)	AnaSpec # 64360
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
JMJD2C (human), recombinant	BPS BioScience # 50105
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)	Gibco # 15575038

2OG is prepared at 100 mM in H_2O, aliquoted and stored at -80°C.
Ascorbate is prepared at 1 M in H_2O, aliquoted and stored at -80°C up to 2 weeks.
Fe(II) is prepared at 500 mM in H_2O, aliquoted and stored at -80°C.
Assay Buffer: 50 mM Hepes pH 7.5, 0.01% Tween-20, 0.01 % BSA

Standard Protocol

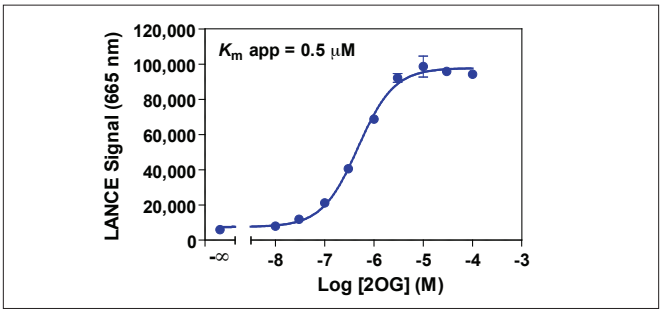
- Dilute JMJD2C enzyme, 2OG, Fe(II), ascorbate, 2,4-PDCA inhibitor and biotinylated peptide substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 2.5 µL of inhibitor (4X) or Assay Buffer
  - 5 µL of enzyme (2X)
  - 2.5 µL of biotinylated H3K9me3 peptide/2OG/Fe(II)/ascorbate 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 Detection Mix by diluting the Eu-Ab to 4 nM, U*Light*-Streptavidin to 100 nM and EDTA to 2 mM in 1X LANCE Detection Buffer (final concentrations of 2 nM, 50 nM and 1 mM, respectively, in 20 µL total assay volume).
- Add 10 µL of Detection Mix.
- Cover with TopSeal-A film and incubate 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



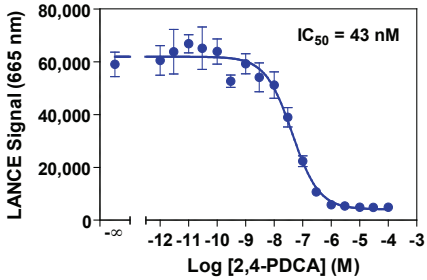
Enzymatic progress curves were performed by incubating JMJD2C at concentrations ranging from 0.3 to 30 nM with 300 nM biotinylated H3K9me3 peptide substrate plus 50 µM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Detection Mix containing EDTA was added to stop the reactions at the indicated times and signal was read after 60 min. A 30 min reaction time using 10 nM enzyme was selected for all subsequent experiments. Signal decrease observed at higher enzyme concentration is due to the generation of either mono-methylated lysine 9 or unmethylated peptides, which are not detected by the Eu-anti-methyl-Histone H3 Lysine 9 (H3K9me2) antibody.

Experiment 2: 2OG Titration



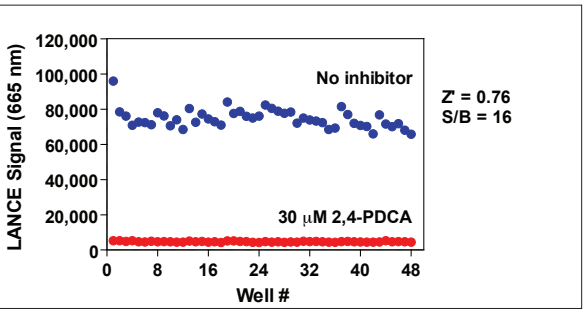
Serial dilutions of 2OG ranging from 10 nM to 100 µM were added to 10 nM JMJD2C and 300 nM biotinylated H3K9me3 peptide substrate plus 5 µM Fe(II) and 100 µM ascorbate. A 1 µM 2OG concentration was selected for subsequent experiments.

Experiment 3: Enzyme Inhibition



Serial dilutions of 2,4-PDCA ranging from 1 pM to 100 µM were pre-incubated for 10 min with 10 nM JMJD2C. Enzymatic reactions were initiated by the addition of 300 nM biotinylated H3K9me3 peptide substrate plus 1 µM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Enzymatic reactions contain 2% DMSO.

Experiment 4: Z'-factor Determination



JMJD2C (10 nM) was pre-incubated with or without 30 µM 2,4-PDCA for 10 min. Enzymatic reactions were initiated by the addition of 300 nM biotinylated H3K9me3 peptide substrate plus 1 µM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Enzymatic reactions in the presence of inhibitor contain 2% DMSO.

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