TECHNICAL NOTE

LANCE *Ultra* JMJD1A Histone H3-Lysine 9 Demethylase Assay

U-TRF #48

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LANCE® *Ultra*

This LANCE *Ultra* immunodetection assay measures the demethylation of a biotinylated histone H3 (1-21) peptide mono-methylated 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(me1)**-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 JMJD1A enzymatic assay using as substrate a biotinylated histone H3-derived peptide mono-methylated at lysine 9. The demethylated 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 demethylation activity of the JMJD1A enzyme.

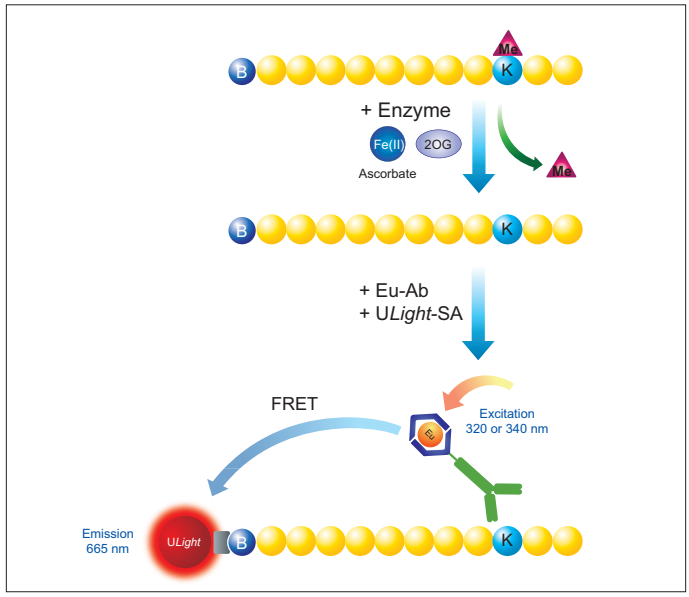

Figure 1. Schematic representation of the LANCE *Ultra* detection of a demethylated histone peptide (B: biotin group; K: lysine residue; Me: methyl group).

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Development of a JMJD1A Histone H3-Lysine 9 Demethylase 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 mono-methylated peptide, biotinylated (H3K9me1)	AnaSpec # 64358
LANCE Detection Buffer, 10X	PerkinElmer # CR97-100
JMJD1A (human), recombinant	BPS Bioscience # 50130
White opaque OptiPlate™-384	PerkinElmer # 6007290
TopSeal™-A film	PerkinElmer # 6050195
α-Ketoglutaric acid potassium salt (2OG)	Sigma # K2000
(+)-Sodium L-ascorbate	Sigma # 11140
Ammonium iron(II) sulfate hexahydrate (Fe(II))	Sigma # 215406
Ethylenediaminetetraacetic acid (EDTA)	Gibco # 15575
N-Oxalylglycine	Sigma # O9390
2,4-Pyridinedicarboxylic acid (2,4-PDCA)	Sigma # P63395
Dimethyloxalylglycine (DMOG)	Sigma # D3695

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.

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, 0.1% BSA, 1X Halt™ protease inhibitor cocktail (Thermo Scientific # 87786)

Standard Protocol

- Dilute JMJD1A enzyme, 2OG, Fe(II), ascorbate, inhibitors and biotinylated histone H3K9me1 peptide substrate in Assay Buffer just before use.

  Note the JMJD1A preparation used throughout this technical note was found to have limited stability in terms of enzymatic activity once thawed; it is advised to prepare single-use aliquots that are kept at -80 °C.

- Add to the wells of a white OptiPlate-384:

  – 2.5 µL of inhibitor (4X) or Assay Buffer

  – 5 µL of enzyme (2X)

  – Incubate for 5 min at room temperature (RT).

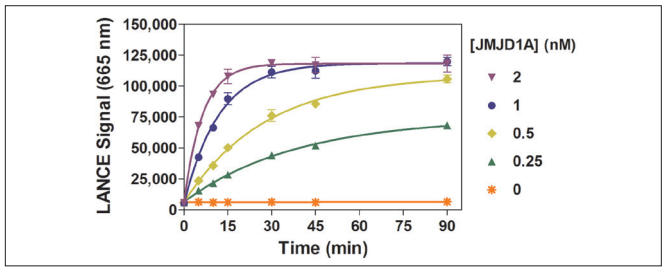
  – 2.5 µL of biotinylated H3K9me1 peptide/2OG/Fe(II)/ascorbate mix (4X)

  For 2OG titration, add 2OG dilutions independently of substrate.

- Cover the plate with TopSeal-A film and incubate at 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).

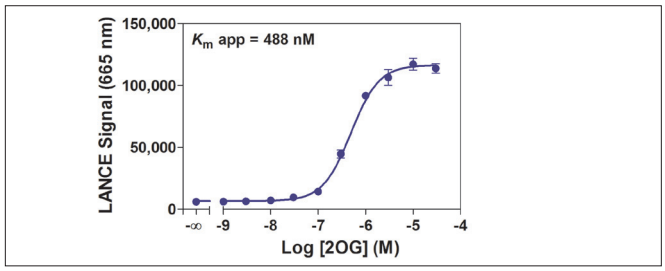
Results

Experiment 1: Enzyme Titration and Time-Course



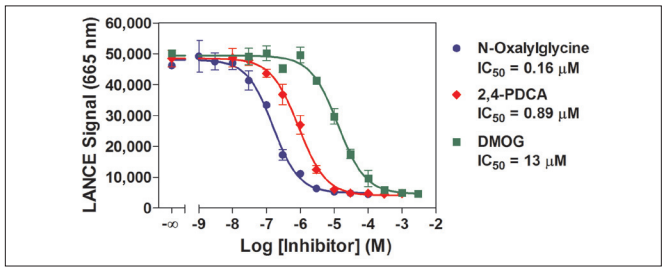
Enzymatic progress curves were performed by incubating JMJD1A at concentrations ranging from 0.25 to 2 nM with 50 nM biotinylated H3K9me1 peptide substrate plus 50 µM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Detection Mix containing EDTA was added to stop the reactions at the indicated times and signal was read after 60 min. A 15 min reaction time using 1 nM enzyme was selected for all subsequent experiments.

Experiment 2: 2OG Titration



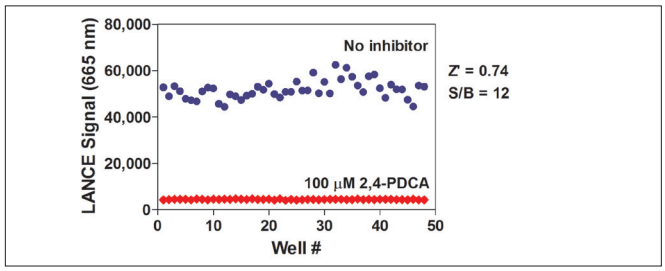
Serial dilutions of 2OG ranging from 1 nM to 30 µM were added to 1 nM JMJD1A and 50 nM biotinylated H3K9me1 peptide substrate plus 5 µM Fe(II) and 100 µM ascorbate. A 1 µM 2OG concentration was selected for subsequent experiments.

Experiment 3: Enzyme Inhibition



Serial dilutions of N-Oxalylglycine, 2,4-PDCA and DMOG ranging from 1 nM to 100 µM, 10 nM to 1 mM, and 30 nM to 3 mM, respectively, were pre-incubated for 5 min with 1 nM JMJD1A. Enzymatic reactions were initiated by the addition of 50 nM biotinylated H3K9me1 peptide substrate plus 1 µM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Enzymatic reactions contain 1% DMSO. **Note** the JMJD1A preparation used throughout this technical note was found to be negatively affected by the presence of increasing concentrations of DMSO (data not shown).

Experiment 4: Z'-factor Determination



JMJD1A (1 nM) was pre-incubated with or without 100 µM 2,4-PDCA for 5 min. Enzymatic reactions were initiated by the addition of 50 nM biotinylated H3K9me1 peptide substrate plus 1 µM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Enzymatic reactions contain 1% DMSO.

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