TECHNICAL NOTE

AlphaLISA JMJD1A Histone H3-Lysine 9 Demethylase Assay

AlphaLISA #18

AlphaLISA®

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This AlphaLISA immunodetection assay measures the demethylation of a biotinylated histone H3 (1-21) peptide mono-methylated at lysine 9.

Anti-unmodified-Histone H3 Lysine 9/Lysine 27 (H3K9/K27) AlphaLISA® Acceptor Beads

- AL138C: 250 µg, 500 assay points*
- AL138M: 5 mg, 10,000 assay points*
- AL138R: 25 mg, 50,000 assay points*

**0.5 µg/assay point*

Peptidic Substrate Sequence:

ARTKQTAR-**K(me1)**-STGGKAPRKQLA-GG-K(BIOTIN)-OH

AlphaLISA Assays

AlphaLISA technology is a powerful and versatile platform that offers highly sensitive, no-wash immunoassays using Alpha Donor and AlphaLISA Acceptor beads. In this technical note, we present the optimization of a JMJD1A enzymatic assay using a biotinylated histone H3K9me1 peptide as substrate. Detection of the demethylated product was performed by the addition of Streptavidin (SA) Alpha Donor beads and AlphaLISA Acceptor beads conjugated to an antibody (Ab) directed against the unmodified H3K9 residue. Upon laser irradiation of the beads-target complexes at 680 nm, short-lived singlet oxygen molecules produced by the Donor beads can reach the Acceptor beads in proximity to generate an amplified chemiluminescent signal at 615 nm. The intensity of the light emission is proportional to the demethylation activity of the JMJD1A enzyme.

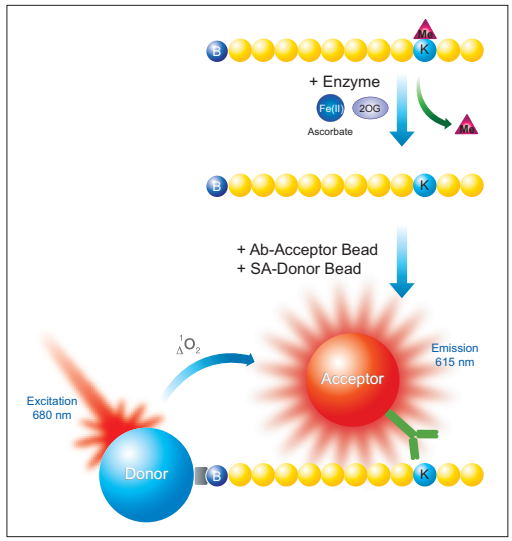

Figure 1. Schematic representation of the AlphaLISA detection of a demethylated histone peptide (B: biotin group; K: lysine residue; Me: methyl group).

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Development of a JMJD1A Histone H3-Lysine 9 Demethylase Assay

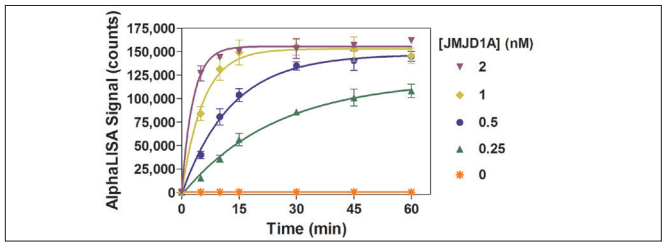
Reagents needed for the assay:

Anti-unmodified-Histone H3 Lysine 9/Lysine 27 (H3K9/K27) AlphaLISA® Acceptor beads	PerkinElmer # AL138
Alpha Streptavidin Donor beads	PerkinElmer # 6760002
Histone H3 (1-21) lysine 9 mono-methylated peptide, biotinylated (H3K9me1)	AnaSpec # 64358
AlphaLISA 5X Epigenetics Buffer 1 Kit	PerkinElmer # AL008
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
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)

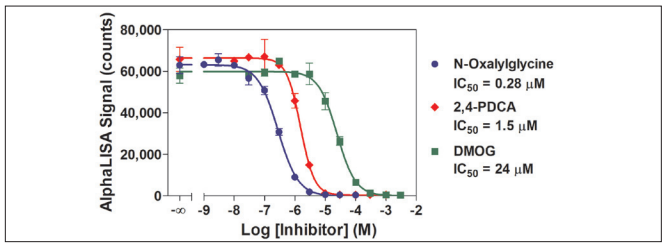
Results

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. Acceptor beads were added at the indicated times. Donor beads were added 60 min later and signal was read after 30 min. A reaction time of 15 min using 0.5 nM enzyme was selected for all subsequent experiments.

Experiment 3: Enzyme Inhibition



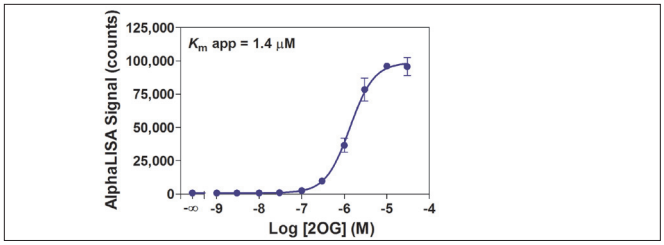
Serial dilutions of N-Oxalylglycine, 2,4-PDCA and DMOG ranging from 1 nM to 100 µM, 10 nM to 1 mM, and 30 nM to 3 mM, respectively, were pre-incubated for 5 min with 0.5 nM JMJD1A. Enzymatic reactions were initiated by the addition of 50 nM biotinylated H3K9me1 peptide substrate plus 3 µM 2OG, 5 µM Fe(II) 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).

Standard Protocol

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

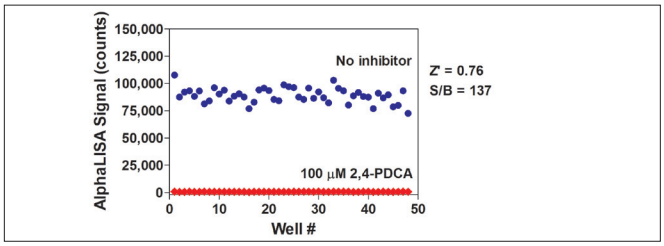
  Note the JMJD1A preparation used throughout this technical note was found to have limited stability in terms of enzymatic activity once thawed; it is advised to prepare single-use aliquots that are kept at -80 °C.
- Add to the wells of a white OptiPlate-384:
  – 2.5 µL of inhibitor (4X) or Assay Buffer
  – 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 1X Epigenetics Buffer 1 as recommended in the buffer technical data sheet.
- Prepare Acceptor beads at 100 µg/mL in 1X Epigenetics Buffer 1 (final concentration of 20 µg/mL in 25 µL total assay volume).
- Add 5 µL of Acceptor beads. *Addition of Acceptor beads prepared in Epigenetics Buffer 1 stops the enzymatic reaction.*
- Cover with TopSeal-A film and incubate 60 min at RT.
- Prepare Streptavidin Donor beads at 50 µg/mL in 1X Epigenetics Buffer 1 in subdued light (final concentration of 20 µg/mL in 25 µL total assay volume).
- Add 10 µL of Donor beads in subdued light.
- Cover with TopSeal-A film and incubate in subdued light for 30 min at RT.
- Read signal in Alpha mode with the EnVision® or EnSpire® readers.

Experiment 2: 2OG Titration



Serial dilutions of 2OG ranging from 1 nM to 30 µM were added to 0.5 nM JMJD1A and 50 nM biotinylated H3K9me1 peptide substrate plus 5 µM Fe(II) and 100 µM ascorbate. A 3 µM 2OG concentration was selected for subsequent experiments.

Experiment 4: Z'-factor Determination



JMJD1A (0.5 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 3 µM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Enzymatic reactions contain 1% DMSO.

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