TECHNICAL NOTE

AlphaLISA #12

AlphaLISA JMJD2A Histone H3-Lysine 9 Demethylase Assay

AlphaLISA®

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

Anti-methyl-Histone H3 Lysine 9 (H3K9me2) AlphaLISA® Acceptor Beads

- AL117C: 250 µg, 500 assay points*
- AL117M: 5 mg, 10,000 assay points*
- AL117R: 25 mg, 50,000 assay points*

**0.5 µg/assay point*

Peptidic Substrate Sequence:

ARTKQTAR-**K(me3)**-STGGKAPRKQLA-GG-K(Biotin)

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 an JMJD2A enzymatic assay using a biotinylated histone H3K9me3 peptide as substrate. Detection of the modified product was performed by the addition of Streptavidin (SA) Alpha Donor beads and AlphaLISA Acceptor beads conjugated to an antibody (Ab) directed against the di-methylated 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 light emission is proportional to the level of biotinylated substrate modification.

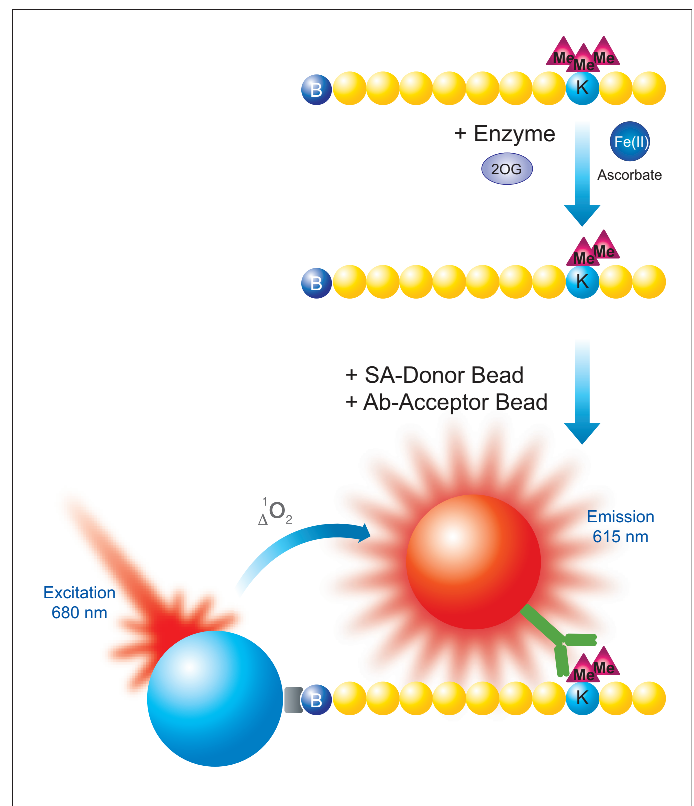

Figure 1. Schematic representation of the AlphaLISA detection of a modified histone peptide.

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Development of a JMJD2A Histone H3-Lysine 9 Demethylase Assay

Reagents needed for the assay:

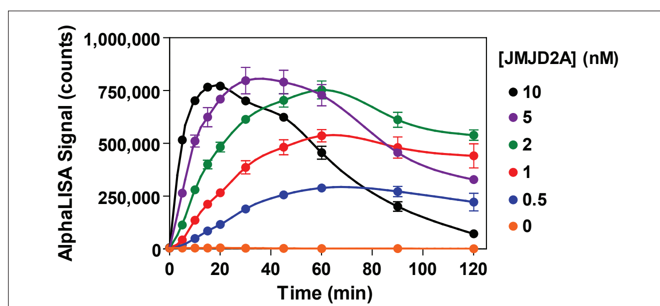
Anti-methyl-Histone H3 Lysine 9 (H3K9me2) AlphaLISA Acceptor beads	PerkinElmer # AL117
Alpha Streptavidin Donor beads	PerkinElmer # 6760002
Histone H3 (1 - 21) lysine 9 tri-methylated peptide, biotinylated (H3K9me3)	AnaSpec # 64360
AlphaLISA 5X Epigenetics Buffer 1 Kit	PerkinElmer # AL008
JMJD2A (human), recombinant	BPS BioScience # 50103
White opaque OptiPlate™-384	PerkinElmer # 6007299
TopSeal™-A films	PerkinElmer # 6005185
⊠-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

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, 0.1% BSA

Standard Protocol

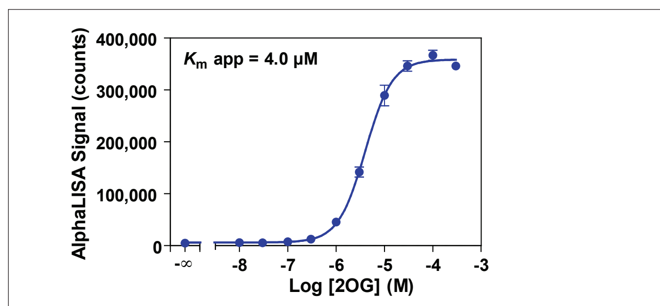
- Dilute JMJD2A enzyme, 2OG, Fe(II), ascorbate, 2,4-PDCA inhibitor and biotinylated peptide substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 2.5 µL of inhibitor (4X) or Assay Buffer
  - 5 µL of enzyme (2X)
  - 2.5 µL of biotinylated H3K9me3 peptide/2OG/Fe(II)/ascorbate 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 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 the signal in Alpha mode with an EnVision® or EnSpire® reader.

Experiment 1: Enzyme Titration and Time-Course



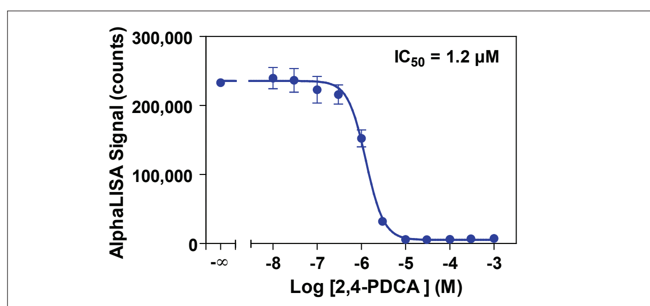
Enzymatic progress curves were performed by incubating JMJD2A at concentrations ranging from 0.5 to 10 nM with 100 nM biotinylated H3K9me3 peptide substrate plus 50 µM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Acceptor beads were added at the indicated times. Donor beads were added 60 min later and signal was read after 30 min. A 30 min reaction time using 1 nM enzyme was selected for all subsequent experiments. Signal decrease observed at higher enzyme concentration or reaction time is due to the generation of either mono-methylated lysine 9 or unmethylated peptides, which are not detected by the anti-methyl-Histone H3 Lysine 9 (H3K9me2) AlphaLISA Acceptor beads.

Experiment 2: 2OG Titration



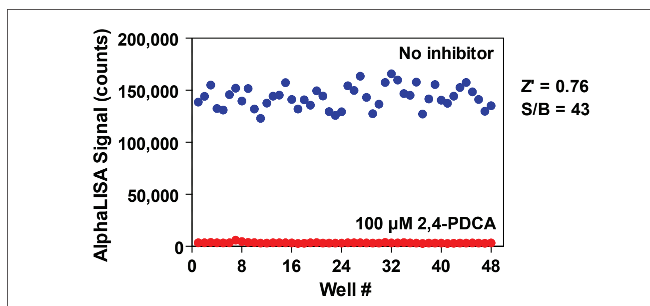
Serial dilutions of 2OG ranging from 10 nM to 300 µM were added to 1 nM JMJD2A and 100 nM biotinylated H3K9me3 peptide substrate plus 5 µM Fe(II) and 100 µM ascorbate. A 5 µM 2OG concentration was selected for subsequent experiments.

Experiment 3: Enzyme Inhibition



Serial dilutions of 2,4-PDCA ranging from 10 nM to 1 mM were pre-incubated for 10 min with 1 nM JMJD2A. Enzymatic reactions were initiated by the addition of 100 nM biotinylated H3K9me3 peptide substrate plus 5 µM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Enzymatic reactions contain 2% DMSO.

Experiment 4: Z′-factor Determination



JMJD2A (1 nM) was pre-incubated with or without 100 µM 2,4-PDCA for 10 min. Enzymatic reactions were initiated by the addition of 100 nM biotinylated H3K9me3 peptide substrate plus 5 µM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Enzymatic reactions contain 2% DMSO.

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