TECHNICAL NOTE

AlphaLISA #33

AlphaLISA NSD2 Histone H3 Lysine-N-methyltransferase Assay

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AlphaLISA® Technology

This AlphaLISA immunodetection assay measures in recombinant nucleosomes the di-methylation of histone H3 at lysine 36 using anti-H3K36me2 Acceptor Beads for Full-length Histone H3 and Nucleosomes and a biotinylated anti-Histone H3 antibody.

AlphaLISA Anti-Histone H3K36me2 Acceptor Beads for Full-length Histone H3 and Nucleosomes

- AL152C: 250 µg, 500 assay points*
- AL152M: 5 mg, 10,000 assay points*
- AL152R: 25 mg, 50,000 assay points*

**0.5 µg/assay point*

AlphaLISA Biotinylated anti-Histone H3 Antibody

- AL118C: 2 µg, 500 assay points*
- AL118M: 40 µg, 10,000 assay points*
- AL118R: 200 µg, 50,000 assay points*

**4 ng/assay point*

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 NSD2 enzymatic assay using recombinant nucleosomes as substrate. Detection of histone H3 di-methylated at lysine 36 is achieved through the recognition of the epigenetic mark by AlphaLISA anti-H3K36me2 FL and Acceptor beads for Full-length Histone H3 and Nucleosomes combined with a biotinylated anti-histone H3 antibody which is then captured by Streptavidin (SA) Alpha Donor beads. 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 methylation activity of the NSD2 enzyme.

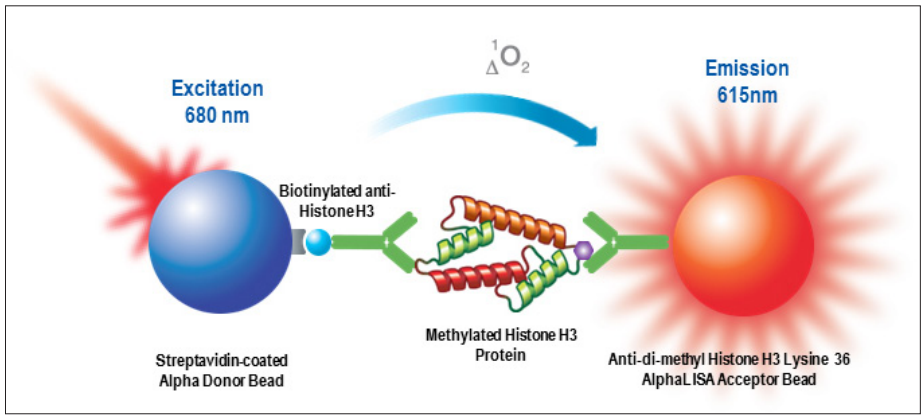

Figure 1. Schematic representation of the AlphaLISA detection of full-length histone H3 di-methylated at lysine 36.

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Development of a NSD2 Histone H3-Lysine N-methyltransferase Assay

Reagents needed for this assay:

AlphaLISA Anti-di-methyl-Histone H3 Lysine 36 (H3K36me2) Acceptor Beads for Full-length Histone H3 and Nucleosomes	PerkinElmer # AL152
AlphaLISA Biotinylated anti-Histone H3 Antibody	PerkinElmer # AL118
Alpha Streptavidin Donor beads	PerkinElmer # 6760002
EpiMark Nucleosome Assembly Kit	New England Biolabs # E5350S
Recombinant NSD2, human	BPS Bioscience # 51026
White opaque OptiPlate™-384	PerkinElmer # 6007290
TopSeal™-A film	PerkinElmer # 6050195
S-(5′-Adenosyl)-L-methionine chloride (SAM)	Sigma # A7007
Poly-L-lysine	Sigma # P1399
Chaetocin	Sigma # C9492
BIX-01338	Sigma # B5313
Sinfungin	Sigma # S8559

Recombinant nucleosomes were prepared as per the dilution method provided in the New England Biolabs' kit instructions.

SAM is prepared at 30 mM in 5 mM H_2SO_4/10% ethanol (v/v) in H_2O, aliquoted and stored at -80 °C.

Assay Buffer: 50 mM Tris-HCl, pH 8.5, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 0.01% Tween-20

High Salt Buffer: 50 mM Tris-HCl pH 7.4, 0.1% Tween-20, 1 M NaCl, 0.3% poly-L-lysine

Detection Buffer: 50 mM Tris-HCl pH 7.4, 0.1% Tween-20, 300 mM NaCl, 0.001% poly-L-lysine

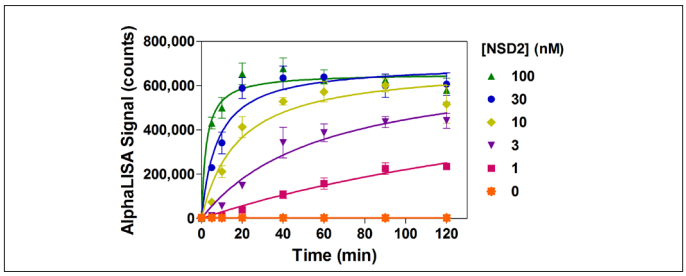
Standard Protocol

- Dilute NSD2 enzyme, SAM, inhibitors and recombinant nucleosomes 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)
  - Incubate for 10 min at 37 °C.
  - 2.5 µL of recombinant nucleosome/SAM mix (4X)

  For SAM titration, add SAM dilutions independently of substrate.
- Cover the plate with TopSeal-A film and incubate at 37 °C.
- Add 5 µL of High Salt Buffer. *Addition of High Salt Buffer stops the NSD2 enzymatic reaction.*
- Cover the plate with TopSeal-A film and incubate 15 min at room temperature (RT).
- Prepare a 5X mix of anti-Histone H3K36me2 Acceptor beads and biotinylated anti-H3 antibody at 100 µg/mL and 5 nM, respectively, in Detection Buffer. Final concentrations are 20 µg/mL and 1 nM, respectively, in 25 µL total assay volume.
- Add 5 µL of 5X Acceptor beads/biotinylated antibody mix.
- Cover with TopSeal-A film and incubate 60 min at RT.
- Prepare 5X Streptavidin Donor beads at 100 µg/mL in Detection Buffer in subdued light (final concentration of 20 µg/mL in 25 µL total assay volume).
- Add 5 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® Multilabel Plate Reader or EnSpire® Multimode Plate Reader.

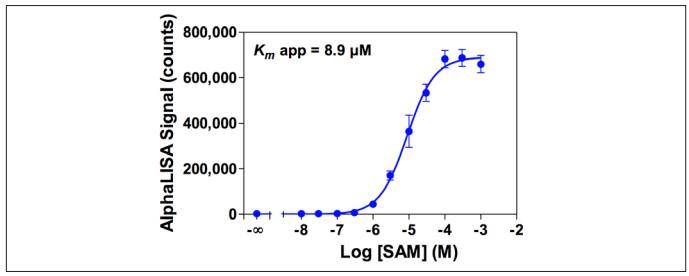
Results

Experiment 1: Enzyme Titration and Time Course



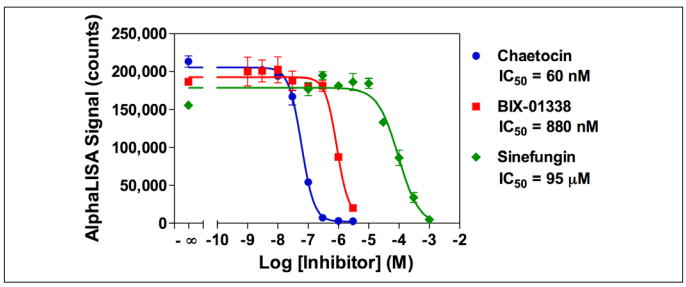
Enzymatic progress curves were performed by incubating NSD2 at concentrations ranging from 1 to 100 nM with 1.5 µg/mL recombinant nucleosome substrate and 100 µM SAM. High salt buffer was added to stop the reactions at the indicated times. After 15 min, a mixture of Acceptor beads and biotinylated anti-H3 antibody was added and product detection was carried out for 60 min. Donor beads were finally added and signal was read after 30 min. A 20 min reaction time using 10 nM enzyme was selected for all subsequent experiments.

Experiment 2: SAM Titration



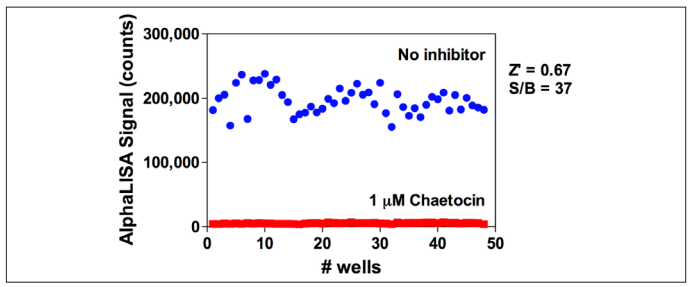
Serial dilutions of SAM ranging from 10 nM to 1 mM were added to 10 nM NSD2 and 1.5 µg/mL recombinant nucleosome substrate. A 10 µM SAM concentration was selected for subsequent experiments.

Experiment 3: Enzyme Inhibition



Serial dilutions of chaeotocin (3 nM to 3 µM), BIX 01338 (1 nM to 3 µM) and sinefungin (300 nM to 1 mM) were pre-incubated for 10 min (37 °C) with 10 nM NSD2. Enzymatic reactions were initiated by the addition of 1.5 µg/mL recombinant nucleosome substrate and 10 µM SAM. Enzymatic reactions contain 1% DMSO.

Experiment 4: Z′-factor Determination



NSD2 (10 nM) was pre-incubated with or without 1 µM chaetocin for 10 min (37 °C). Enzymatic reactions were initiated by the addition of 1.5 µg/mL recombinant nucleosome substrate and 10 µM SAM. Enzymatic reactions contain 1% DMSO.

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010913_01 Jan. 2013