TECHNICAL NOTE

AlphaLISA #25

AlphaLISA DOT1L Histone H3 Lysine-N-methyltransferase Assay

AlphaLISA® Technology

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This AlphaLISA immunodetection assay measures the methylation at lysine 79 of nucleosomal Histone H3 using anti-histone H3 (C-ter) Acceptor Beads and a biotinylated anti-dimethyl-histone H3 lysine 79 (H3K79me2) antibody.

AlphaLISA Anti-Histone H3 (C-ter) Acceptor Beads

- AL147C: 250 µg; 500 assay points*
- AL147M: 5 mg, 10,000 assay points*
- AL147R: 25 mg, 50,000 assay points*

**0.5 µg/assay point*

AlphaLISA Biotinylated anti-dimethyl-Histone H3 Lysine 79 (H3K79me2) Antibody

- AL148C: 2 µg; 500 assay points*
- AL148M: 40 µg, 10,000 assay points*
- AL148R: 200 µg, 50,000 assay points*

**4 ng/assay point*

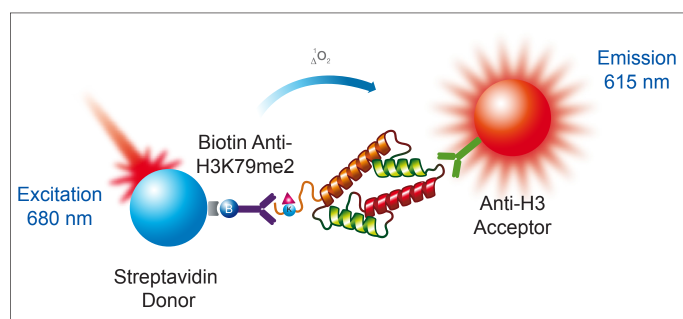

Figure 1. Schematic representation of the AlphaLISA detection of a dimethylated full-length histone H3 (B: biotin group; K: modified lysine residue).

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 DOT1L enzymatic assay using oligonucleosomes as substrate. Detection of the histone H3 lysine 79 dimethylated product is achieved through the recognition of the epigenetic mark of interest by a biotinylated anti-H3K79me2 antibody captured by Streptavidin (SA) Alpha Donor beads, combined with AlphaLISA Acceptor beads conjugated to an anti-histone H3 antibody directed against the carboxy-terminal (C-ter) sequence of human histone H3. 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 DOT1L enzyme.

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Development of a DOT1L Histone H3 Lysine-N-methyltransferase Assay

Reagents needed for the assay:

Reagent	Source
Anti-Histone H3 (C-ter) AlphaLISA® Acceptor Beads	PerkinElmer # AL147
AlphaLISA® Biotinylated anti-dimethyl-Histone H3 Lysine 79 (H3K79me2) Antibody	PerkinElmer # AL148
Alpha Streptavidin Donor beads	PerkinElmer # 6760002
Purified oligonucleosomes (HeLa cells)	Reaction Biology # HMT-35-130
DOT1L (human), recombinant	Reaction Biology # HMT-11-101
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
BIX-01338	Sigma # B5313
5′-Deoxy-5′-(methylthio)adenosine (MTA)	Sigma # D5011
S-(5'-Adenosyl)-L-homocysteine (SAH)	Sigma # A9384
Sinefungin	Sigma # S8559

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 8.0, 150 mM NaCl, 3 mM $MgCl_2$, 0.1% BSA

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

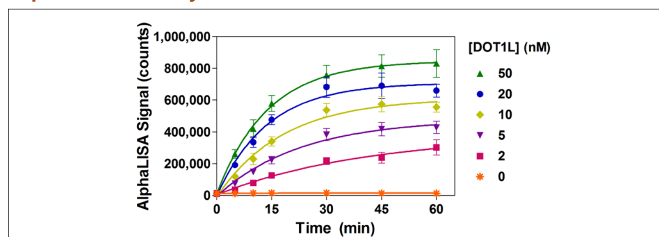
Detection Buffer 1X: 25 mM HEPES pH 7.4, 0.01% Tween-20, 0.005% Proclin-300

Standard Protocol

- Dilute DOT1L enzyme, SAM, inhibitors and oligonucleosomes 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 room temperature (RT).
  - 2.5 µL of oligonucleosomes/SAM mix (4X)

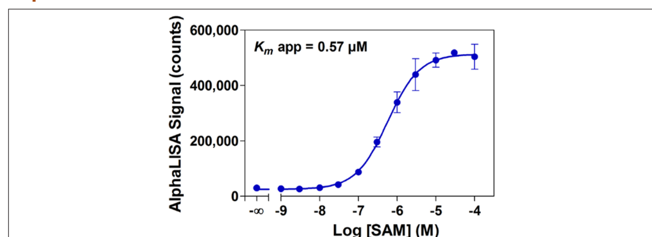
    For SAM titration, add SAM dilutions independently of substrate.
- Cover the plate with TopSeal-A film and incubate at RT.
- Add 5 µL of High Salt Buffer. *Addition of High Salt Buffer stops DOT1L enzymatic reaction.*
- Cover the plate with TopSeal-A film and incubate 15 min at RT.
- Prepare a 5X mix of anti-Histone H3 Acceptor beads and biotinylated anti-H3K79me2 antibody at 100 µg/mL and 5 nM, respectively, in 1X Detection Buffer. Final concentrations are respectively 20 µg/mL and 1 nM 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 1X 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® or EnSpire® readers.

Experiment 1: Enzyme Titration and Time-Course



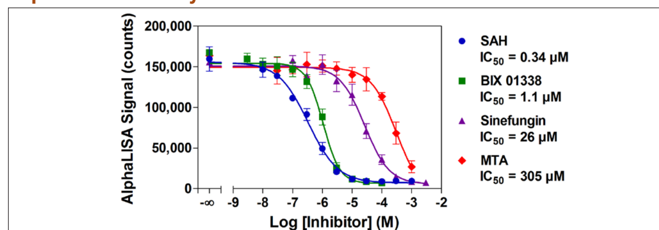
Enzymatic progress curves were performed by incubating DOT1L at concentrations ranging from 2 to 50 nM with 0.25 ng oligonucleosomes plus 100 µM SAM. High Salt Buffer was added to stop the reactions at the indicated times. After 15 min, a mixture of Acceptor beads and biotinylated anth-H3K79me2 antibody was added and product detection was carried out for 60 min. Donor beads were finally added and signal was read after 30 min. A 15 min reaction time using 20 nM enzyme was selected for all subsequent experiments.

Experiment 2: SAM Titration



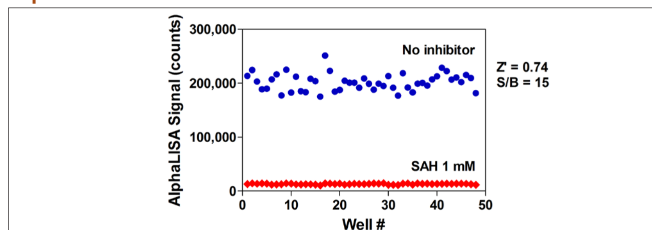
Serial dilutions of SAM ranging from 1 nM to 100 µM were added to 20 nM DOT1L and 0.25 ng oligonucleosomes. A 2 µM SAM concentration was selected for subsequent experiments.

Experiment 3: Enzyme Inhibition



Serial dilutions of SAH, BIX 01338, sinefungin and MTA ranging from 10 nM to 1 mM, 1 nM to 100 µM, 100 nM to 3 mM, and 10 nM to 1 mM, respectively, were pre-incubated for 10 min with 20 nM DOT1L. Enzymatic reactions were initiated by the addition of 0.25 ng oligonucleosomes plus 2 µM SAM. Enzymatic reactions contain 1% DMSO.

Experiment 4: Z′-factor Determination



DOT1L (20 nM) was pre-incubated with or without 1 mM SAH for 10 min. Enzymatic reactions were initiated by the addition of 0.25 ng oligonucleosomes plus 2 µM SAM. Enzymatic reactions contain 1% DMSO.

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