TECHNICAL NOTE

AlphaLISA #4

AlphaLISA SET7/9 N-methyltransferase Assay using Full-length Histone H3

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AlphaLISA®

This AlphaLISA immunodetection assay measures the methylation at lysine 4 of full-length histone H3 using a biotinylated anti-histone H3 (C-ter) antibody and anti-H3K4me1-2 Acceptor beads.

AlphaLISA® Biotinylated Anti-Histone H3 (C-ter) 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 an epigenetic enzymatic assay using a full-length histone H3 substrate. Detection of the modified substrate is achieved through the recognition of the carboxy-terminal (C-ter) sequence of human histone H3 by a biotinylated anti-histone H3 (C-ter) antibody captured by Streptavidin (SA) Alpha Donor beads, combined with AlphaLISA Acceptor beads conjugated to an antibody (Ab) directed against the epigenetic mark of interest. 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 substrate modification.

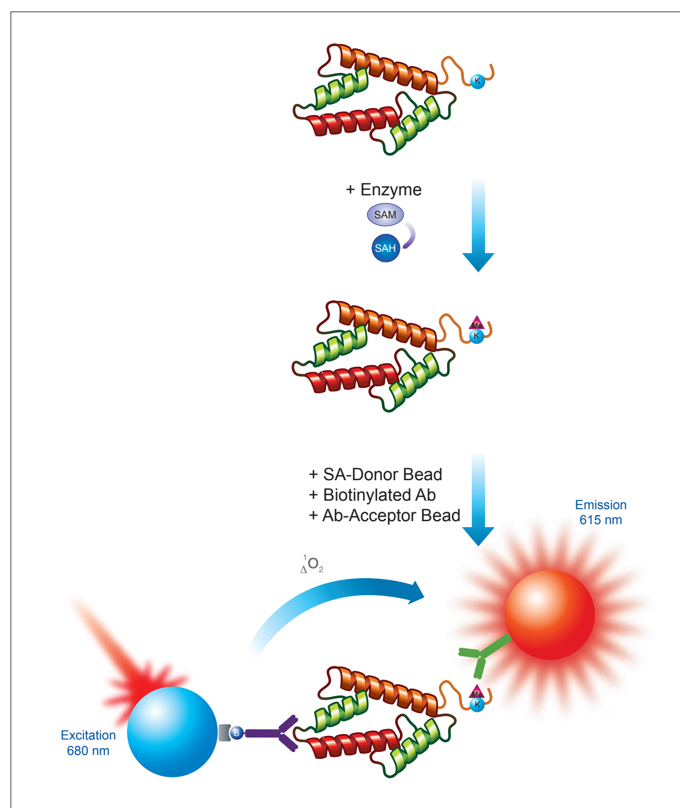

Figure 1. Schematic representation of AlphaLISA detection of a modified full-length histone H3.

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Development of a SET7/9 Histone H3-Lysine N-methyltransferase Assay

Reagents needed for the assay:

AlphaLISA Biotinylated Anti-Histone H3 (C-ter) Antibody	PerkinElmer # AL118
Anti-methyl-Histone H3 Lysine 4 (H3K4me1-2) AlphaLISA Acceptor Beads	PerkinElmer # AL116
Alpha Streptavidin Donor beads	PerkinElmer # 6760002
Recombinant Histone H3 (C110A)	Active Motif # 31207
SET7/9 (human), recombinant	Enzo # ALX-201-178-C100
White opaque OptiPlate™-384	PerkinElmer # 6007299
TopSeal™-A films	PerkinElmer # 6005185
S-(5'-Adenosyl)-L-methionine chloride (SAM)	Sigma # A7007
Poly-L-lysine	Sigma # P1399
Sinefungin	Sigma # S8559
S-(5'-Adenosyl)-L-homocysteine (SAH)	Sigma # A9384

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 8.8, 5 mM $MgCl_2$, 1 mM DTT, 0.01% Tween-20

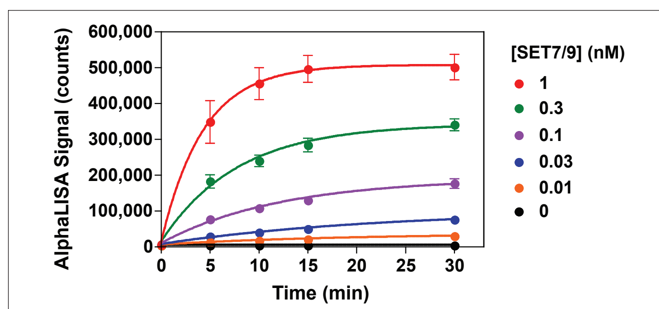
High Salt Buffer: 50 mM Tris-HCl pH 7.4, 1 M NaCl , 0.1% Tween-20, 0.3% poly-L-lysine

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

Standard Protocol

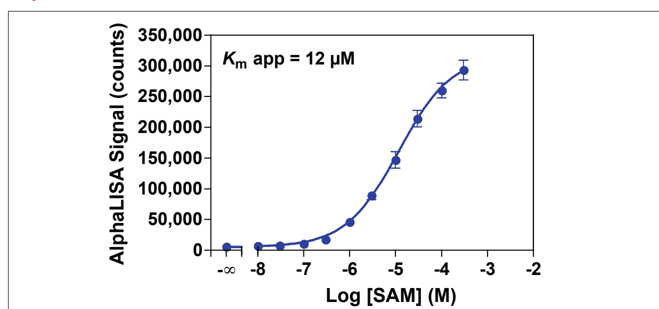
- Dilute SET7/9 enzyme, inhibitors, SAM and full-length histone H3 substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 5 µL of inhibitor (2X) or Assay Buffer
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of histone H3/SAM mix (4X)
- Cover the plate with TopSeal-A film and incubate at room temperature (RT).
- Add 5 µL of High Salt Buffer. *Addition of High Salt Buffer stops the SET7/9 enzymatic reaction.*
- Cover with TopSeal-A film and incubate 15 min at RT.
- Prepare a 5X mix of Acceptor beads and biotinylated anti-H3 antibody at 100 µg/mL and 5 nM, respectively, in 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 anti-H3 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 (final concentration of 20 µg/mL in 25 µL total assay volume) in subdued light.
- Add 5 µL of 5X Donor beads in subdued light.
- Cover with TopSeal-A film and incubate in subdued light 30 min at RT.
- Read signal in Alpha mode with an EnVision® or EnSpire® reader.

Experiment 1: Enzyme Titration and Time-Course



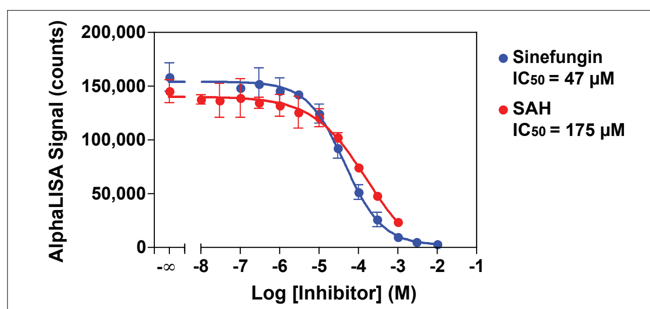
Enzymatic progress curves were performed by incubating SET7/9 at concentrations ranging from 0.01 to 1 nM with 300 nM histone H3 substrate and 100 µM SAM. High salt buffer was added to stop the reactions at the indicated times. After 15 min, a mixture of Acceptor beads and biotinylated anti-H3 antibody was added and product detection was carried out for 60 min. Donor beads were finally added and signal was read after 30 min. A 10 min reaction time using 0.3 nM enzyme was selected for all subsequent experiments.

Experiment 2: SAM Titration



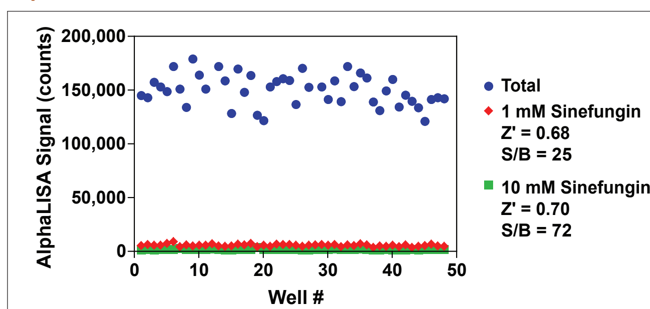
Serial dilutions of SAM ranging from 10 nM to 300 µM were added to 0.3 nM SET7/9 and 300 nM histone H3 substrate. A 10 µM SAM concentration was selected for subsequent experiments.

Experiment 3: Enzyme Inhibition



Serial dilutions of sinefungin ranging from 100 nM to 10 mM, and S-adenosylhomocysteine (SAH) ranging from 10 nM to 1 mM, were pre-incubated for 10 min with 0.3 nM SET7/9. Enzymatic reactions were initiated by the addition of 300 nM histone H3 substrate plus 10 µM SAM. Enzymatic reactions contain 1% DMSO.

Experiment 4: Z'-factor Determination



SET7/9 (0.3 nM) was pre-incubated with or without either 1 mM or 10 mM sinefungin for 10 min. Enzymatic reactions were initiated by the addition of 300 nM histone H3 substrate plus 10 µM SAM. Enzymatic reactions contain 1% DMSO.

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