AlphaLISA SET7/9 Histone H3-lysine N-methyltransferase assay.

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This AlphaLISA immunodetection assay measures the mono-methylation of a biotinylated Histone H3 (1-21) peptide at lysine 4.

Anti-methyl-histone H3 lysine 4 (H3K4me1-2) AlphaLISA™ acceptor beads

- AL116C: 250 µg, 500 assay points*
- AL116M: 5 mg, 10,000 assay points*
- AL116R: 25 mg, 50,000 assay points*

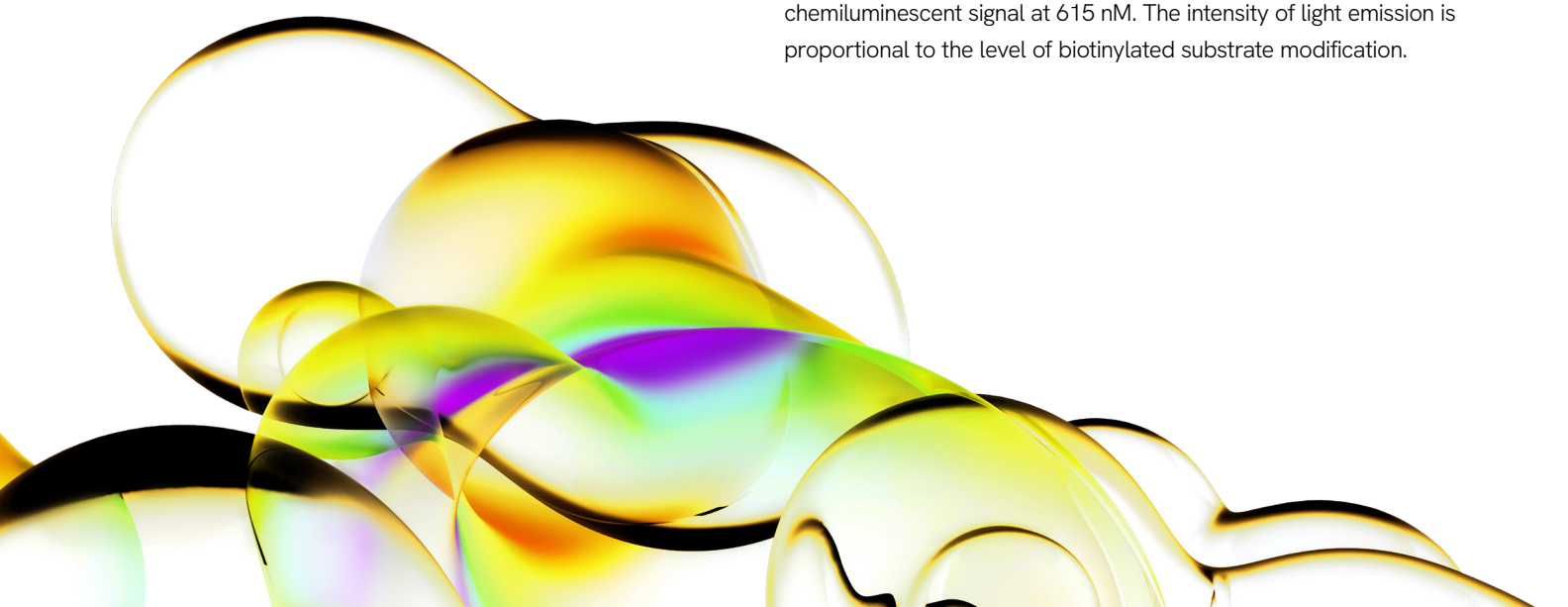
**0.5 µg/assay point*

Peptidic substrate sequence:

ART**K**QTARKSTGGKAPRKQLA-GG-K(BIOTIN)-NH2

AlphaLISA assays

The AlphaLISA technology allows performing no-wash homogeneous proximity immunoassays using Alpha Donor and AlphaLISA Acceptor beads. In this technical note, we present the optimization of an epigenetic enzymatic assay using a biotinylated histone H3-derived peptide as substrate. Detection of the modified substrate was performed by the addition of Streptavidin (SA) Alpha Donor beads and 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 biotinylated substrate modification.



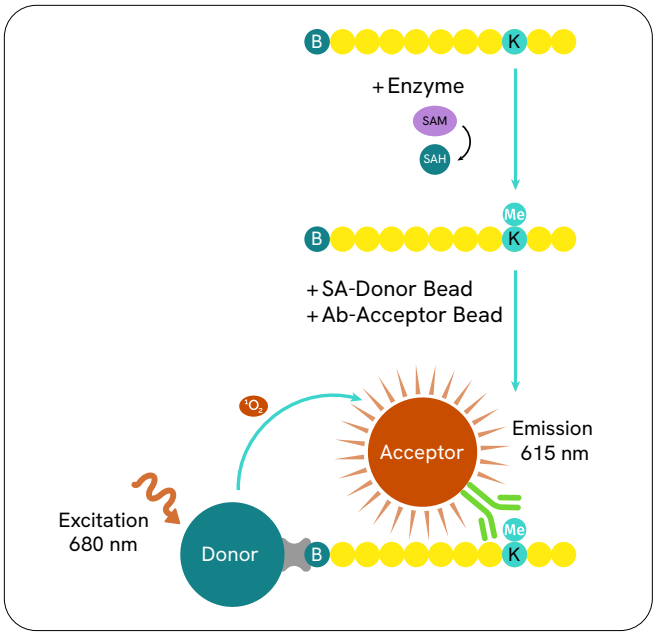


Figure 1: Schematic representation of the AlphaLISA detection of a modified histone peptide.

Development of a SET7/9 Histone H3-lysine N-methyltransferase assay

Reagents needed for the assay:

Anti-methyl-Histone H3 Lysine 4 (H3K4me1-2) AlphaLISA Acceptor beads	Revvity # AL116
Alpha Streptavidin Donor beads	Revvity # 6760002
Histone H3 (1-21) peptide, biotinylated	AnaSpec # 61702
AlphaLISA 5X Epigenetics Buffer 1 Kit	Revvity # AL008
SET7/9 (human), recombinant	Enzo # ALX-201-178-C100
White opaque OptiPlate™-384	Revvity # 6007299
TopSeal™-A films	Revvity # 6005185
S-(5′-Adenosyl)-L-methionine chloride (SAM)	Sigma # A7007
Sinefungin	Sigma # S8559
S-(5′-Adenosyl)-L-homocysteine (SAH)	Sigma # A9384

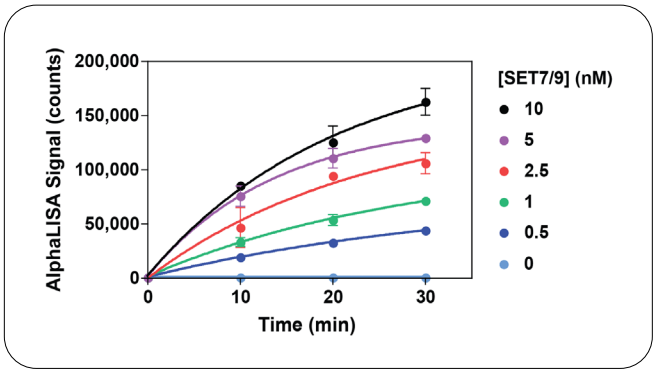
SAM is prepared at 30 mM in 5 mM H_2SO_4/10% ethanol (v/v) in H_2O, aliquoted and stored at -80 °C.

Assay Buffer: 50 mM Tris-HCl, pH 8.8, 5 mM $MgCl_2$, 1 mM DTT, 0.01% Tween-20

Standard protocol

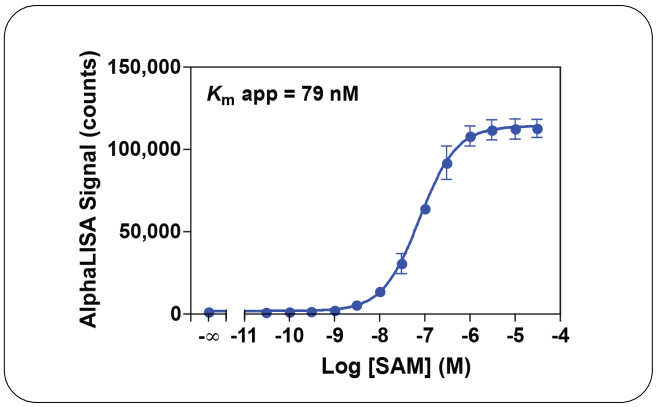
- Dilute SET7/9 enzyme, inhibitors, SAM and biotinylated peptide substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 5 µL of inhibitor (2X) or Assay Buffer
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of biotinylated Histone H3 (1-21) peptide/ SAM mix (4X). For SAM titration, add SAM 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 Acceptor beads. *Addition of Acceptor beads prepared in Epigenetics Buffer 1 stops the enzymatic reaction.*
- Cover with TopSeal-A film and incubate for 60 min at RT.
- Prepare Streptavidin Donor beads at 50 µg/mL in 1X Epigenetics Buffer 1 (final concentration of 20 µg/mL in 25 µL total assay volume) in subdued light.
- Add 10 µL Donor beads in subdued light.
- Cover with TopSeal-A film and incubate in the dark for 30 min at RT.
- Read signal in Alpha mode with EnVision™ or EnSpire™ readers.

Experiment 1: Enzyme titration and time-course



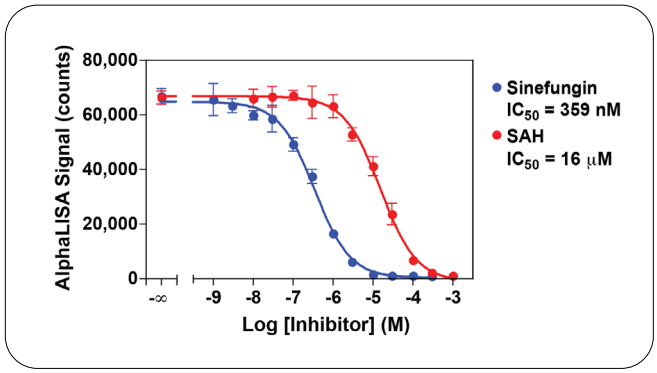
Enzymatic progress curves were performed by incubating SET7/9 at concentrations ranging from 0.5 to 10 nM with 50 nM biotinylated H3 (1-21) peptide substrate and 100 µM SAM. Acceptor beads were added to stop the reactions at the indicated times. Donor beads were added 60 min later and signal was read after 30 min. A 30 min reaction time using 1 nM enzyme was selected for all subsequent experiments.

Experiment 2: SAM titration



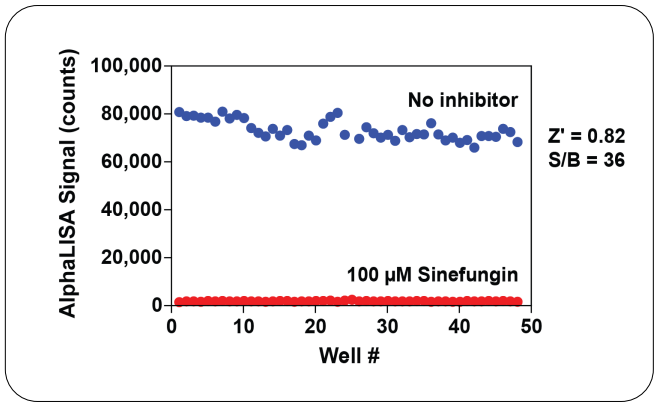
Serial dilutions of SAM ranging from 30 pM to 30 µM were added to 1 nM SET7/9 and 50 nM biotinylated H3 (1-21) peptide substrate. A 100 nM SAM concentration was selected for subsequent experiments.

Experiment 3: Enzyme inhibition



Serial dilutions of sinefungin ranging from 1 nM to 300 µM and SAH ranging from 10 nM to 1 mM were pre-incubated for 10 min with 1 nM SET7/9. Enzymatic reactions were initiated by the addition of 50 nM biotinylated H3 (1-21) peptide substrate plus 100 nM SAM. Enzymatic reactions contain 1% DMSO.

Experiment 4: Z′-factor determination



SET7/9 (1 nM) was pre-incubated with or without 100 µM sinefungin for 10 min. Enzymatic reactions were initiated by the addition of 50 nM biotinylated H3 (1-21) peptide substrate plus 100 nM SAM. Enzymatic reactions contain 1% DMSO.

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