TECHNICAL NOTE

AlphaLISA EZH2 Histone H3-Lysine 27 N-methyltransferase assay.

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This AlphaLISA immunodetection assay measures the dimethylation of a biotinylated Histone H3 (21-44) peptide at lysine 27.

Anti-di/mono-methyl-Histone H3 Lysine 27 (H3K27me2-1) AlphaLISA™ acceptor beads

- AL121C: 250 µg, 500 assay points*
- AL121M: 5 mg, 10,000 assay points*
- AL121R: 25 mg, 50,000 assay points*

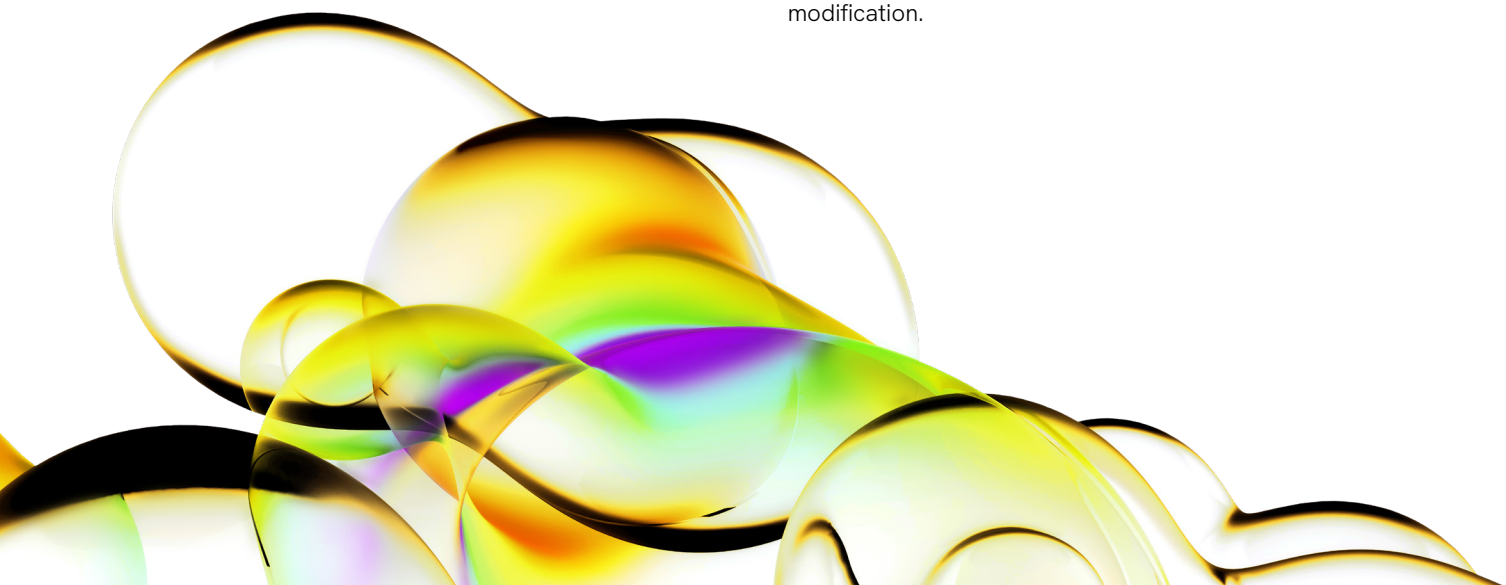
**0.5 µg/assay point*

Peptidic substrate sequence:

ATKAAR**K**SAPATGGVKKPHRYRP-GG-K(Biotin)-OH

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 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.



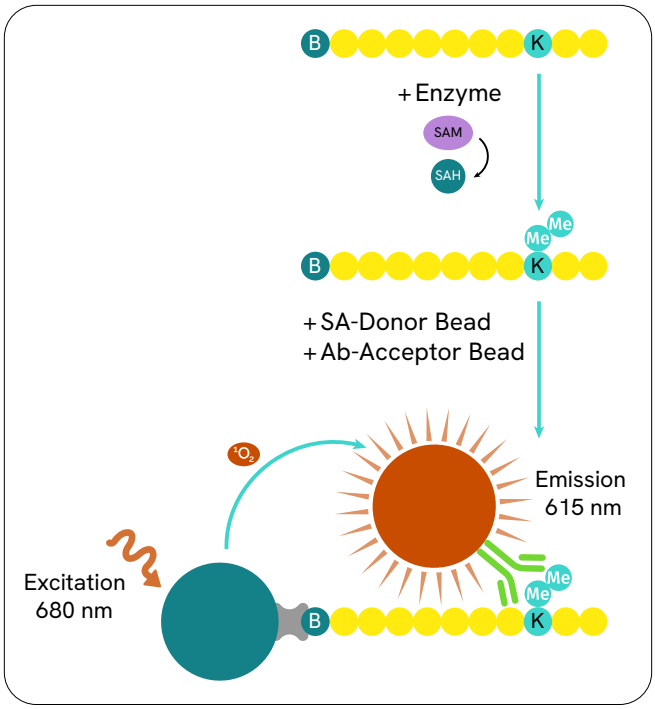


Figure 1: Schematic representation of AlphaLISA detection of a modified histone peptide.

Development of an EZH2 Histone H3-Lysine 27 N-methyltransferase assay:

Reagents needed for the assay:

Anti-di/mono-methyl-Histone H3 Lysine 27 (H3K27me2-1) AlphaLISA Acceptor beads	Revvity # AL121
Alpha Streptavidin Donor beads	Revvity # 6760002
Histone H3 (21-44) peptide, biotinylated	AnaSpec # 64440
AlphaLISA 5X Epigenetics Buffer 1 Kit	Revvity # AL008
EZH2/EED/SUZ12/RbAp48/ AEBP2 Complex	BPS BioScience # 51004
White opaque OptiPlate™-384	Revvity # 6007299
TopSeal™-A film	Revvity # 6005185
Sinefungin	Sigma # S8559
S-(5′-Adenosyl)-L-methionine chloride (SAM)	Sigma # A7007

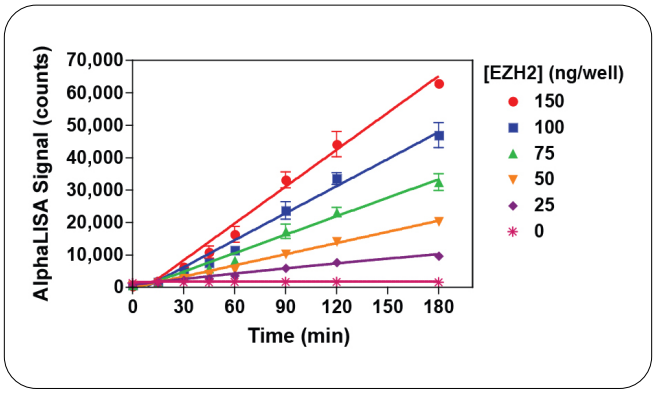
SAM is prepared at 30 mM in 5 mM H_2SO_4/10% ethanol (v/v) in H_2O, aliquoted and stored at -80 °C.

Assay Buffer: 50 mM Tris-HCl pH 9.0, 50 mM NaCl, 1 mM DTT, 0.01% Tween-20 and 0.01% BSA.

Standard protocol

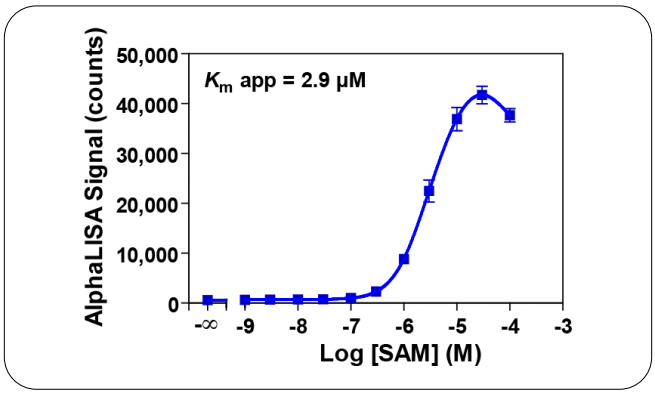
- Dilute EZH2 enzyme complex, SAM, sinefungin (inhibitor) and biotinylated peptide substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of inhibitor (4X) or Assay Buffer
  - 5 µL of biotinylated Histone H3 (21-44) peptide/SAM Mix (2X).
    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 a 1.67X Detection Mix by diluting Acceptor and Donor Beads at 33.4 µg/mL in 1X Epigenetics Buffer 1 in subdued light (final concentration of 20 µg/mL in 25 µL total assay volume).
  - 15 µL of Detection mix in subdued light
    Addition of Detection Mix prepared in Epigenetics Buffer 1 stops the enzymatic reaction.
- Cover with TopSeal-A film and incubate for 60 min at RT.
- Read signal in Alpha mode with the EnVision™ or EnSpire™ reader.

Experiment 1: Enzyme titration and time-course



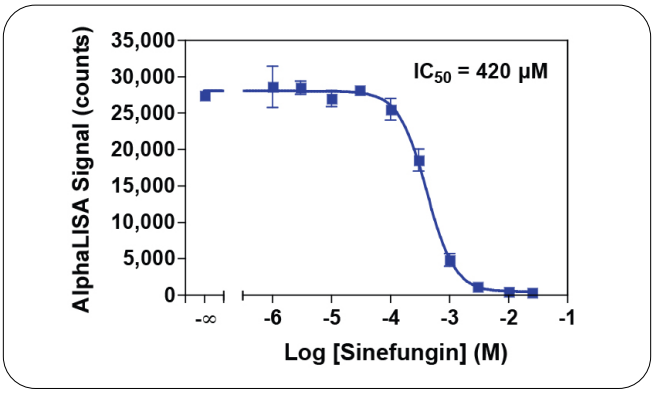
Enzymatic progress curves were performed by incubating EZH2 complex at concentrations ranging from 25 to 150 ng/well with 100 nM biotinylated Histone H3 (21-44) peptide substrate and 100 µM SAM. Detection Mix was added at the indicated times. Signal was read 60 min after the addition of Detection Mix. A 120 min reaction time using 150 ng/well enzyme complex was selected for all subsequent experiments.

Experiment 2: SAM titration



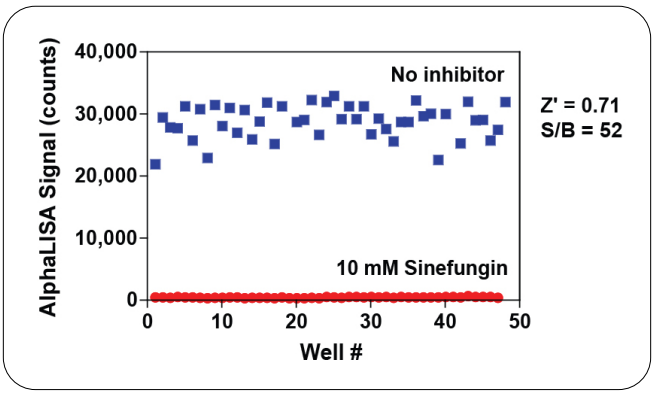
Serial dilutions of SAM ranging from 1 nM to 100 µM were added to 150 ng/well EZH2 complex and 100 nM biotinylated Histone H3 (21-44) peptide substrate. A 3 µM SAM concentration was selected for subsequent experiments.

Experiment 3: Enzyme inhibition



Serial dilutions of sinefungin ranging from 1 µM to 30 mM were pre-incubated for 10 min with 150 ng/well EZH2 complex. Enzymatic reactions were initiated by the addition of 100 nM biotinylated Histone H3 (21-44) peptide substrate plus 3 µM SAM. Enzymatic reactions contain 1% DMSO.

Experiment 4: Z′-factor determination



EZH2 complex (150 ng/well) was pre-incubated with or without 10 mM sinefungin for 10 min. Enzymatic reactions were initiated by the addition of 100 nM biotinylated Histone H3 (21-44) peptide substrate plus 3 µM SAM. Enzymatic reactions contain 1% DMSO.

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