TECHNICAL NOTE

AlphaLISA G9a Histone H3-Lysine N-methyltransferase Assay

AlphaLISA #2

AlphaLISA®

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This AlphaLISA immunodetection assay measures the di-methylation of a biotinylated Histone H3 (1-21) peptide at lysine 9.

Anti-methyl-Histone H3 Lysine 9 (H3K9me2) AlphaLISA® Acceptor Beads

- AL117C: 250 µg, 500 assay points*
- AL117M: 5 mg, 10,000 assay points*
- AL117R: 25 mg, 50,000 assay points*

**0.5 µg/assay point*

Peptidic Substrate Sequence:

ARTKQTAR**K**STGGKAPRKQLA-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.

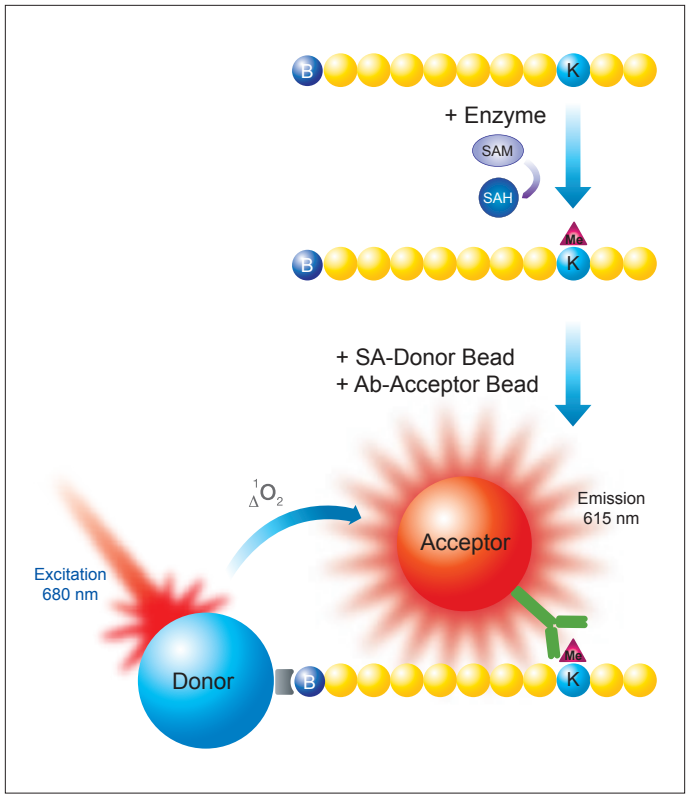

Figure 1. Schematic representation of the AlphaLISA detection of a modified histone peptide.

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Development of a G9a Histone H3-Lysine N-methyltransferase Assay

Reagents needed for the assay:

Anti-methyl-Histone H3 Lysine 9 (H3K9me2) AlphaLISA Acceptor Beads	PerkinElmer # AL117
Alpha Streptavidin Donor beads	PerkinElmer # 6760002
Histone H3 (1-21) peptide, biotinylated	AnaSpec # 61702
AlphaLISA 5X Epigenetics Buffer 1 Kit	PerkinElmer # AL008
G9a (human), recombinant	BPS Bioscience # 51001
White opaque OptiPlate™-384 microplates	PerkinElmer # 6007299
TopSeal™-A films	PerkinElmer # 6005185
S-(5'-Adenosyl)-L-methionine chloride (SAM)	Sigma # A7007
Sinefungin	Sigma # S8559
S-(5'-Adenosyl)-L-homocysteine (SAH)	Sigma # A9384
BIX 01294	Sigma # B9311

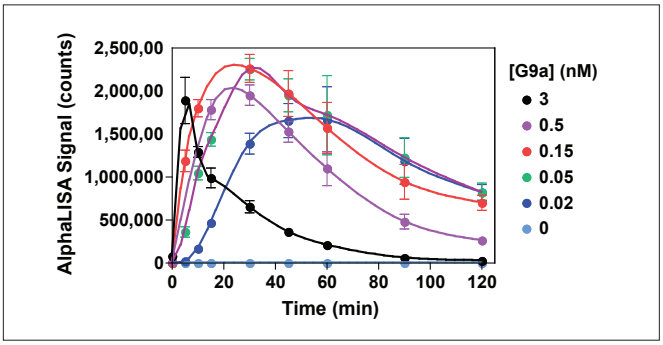
SAM is prepared at 30 mM in 5 mM H_2SO_4/10% ethanol (v/v) in H_2O, aliquoted and stored at -80 °C.

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

Standard Protocol

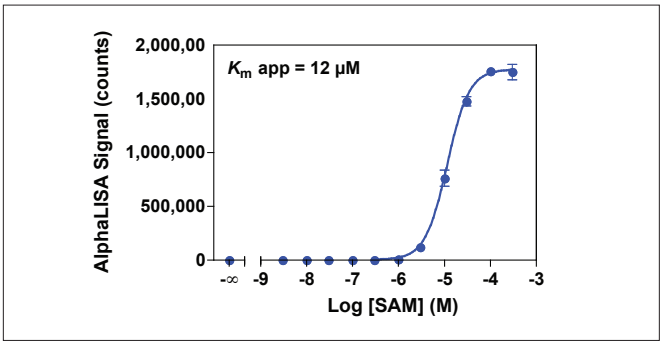
- Dilute G9a enzyme, inhibitors, SAM and biotinylated peptide substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384 microplate:
  - 5 µL inhibitor (2X) or Assay Buffer
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of biotinylated Histone H3 (1-21) peptide/SAM mix (4X). For SAM 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 a 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



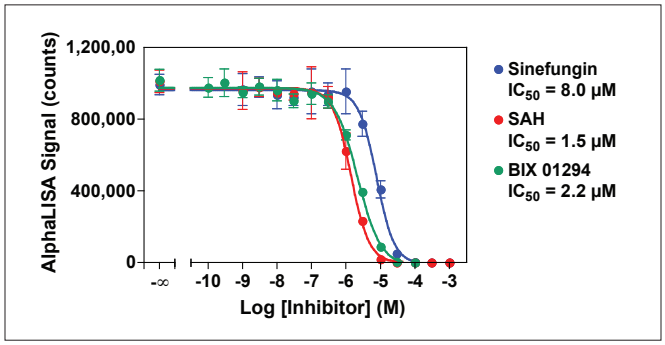
Enzymatic progress curves were performed by incubating G9a at concentrations ranging from 0.02 to 3 nM with 100 nM biotinylated H3 (1-21) peptide substrate and 100 µM SAM. Acceptor beads were added to stop the reactions at the indicated times. Donor beads were added 60 min later and signal was read after 30 min. A 30 min reaction time using 0.05 nM enzyme was selected for all subsequent experiments. Signal decrease observed at higher enzyme concentration or reaction time is due to the generation of peptides tri-methylated at lysine 9, which are not detected by the anti-methyl-Histone H3 at lysine 9 (H3K9me2) AlphaLISA Acceptor Beads.

Experiment 2: SAM Titration



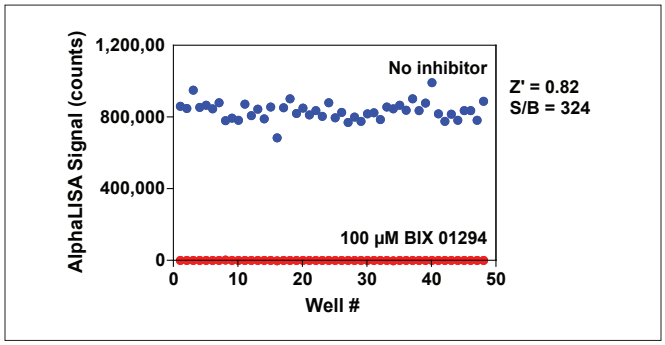
Serial dilutions of SAM ranging from 3 nM to 300 µM were added to 0.05 nM G9a and 100 nM biotinylated H3 (1-21) peptide substrate. A 15 µM SAM concentration was selected for subsequent experiments.

Experiment 3: Enzyme Inhibition



Serial dilutions of sinefungin and SAH ranging from 1 nM to 1 mM and of BIX 01294 ranging from 100 pM to 100 µM were pre-incubated for 10 min with 0.05 nM G9a. Enzymatic reactions were initiated by the addition of 100 nM biotinylated H3 (1-21) peptide substrate plus 15 µM SAM. Enzymatic reactions contain 2% DMSO.

Experiment 4: Z'-factor Determination



G9a (0.05 nM) was pre-incubated with or without 100 µM BIX 01294 for 10 min. Enzymatic reactions were initiated by the addition of 100 nM biotinylated H3 (1-21) peptide substrate plus 15 µM SAM. Enzymatic reactions contain 2% DMSO.

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