TECHNICAL NOTE

AlphaLISA PRMT1 Histone H4-Arginine 3 N-methyltransferase assay

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Revvity, Inc.

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This AlphaLISA™ immunodetection assay measures the methylation of a biotinylated histone H4 (1-21) peptide at arginine 3.

AlphaLISA Anti-methyl-Histone H4 Arginine 3 acceptor beads

- AL150C: 250 µg, 500 assay points*
- AL150M: 5 mg, 10,000 assay points*
- AL150R: 25 mg, 50,000 assay points*

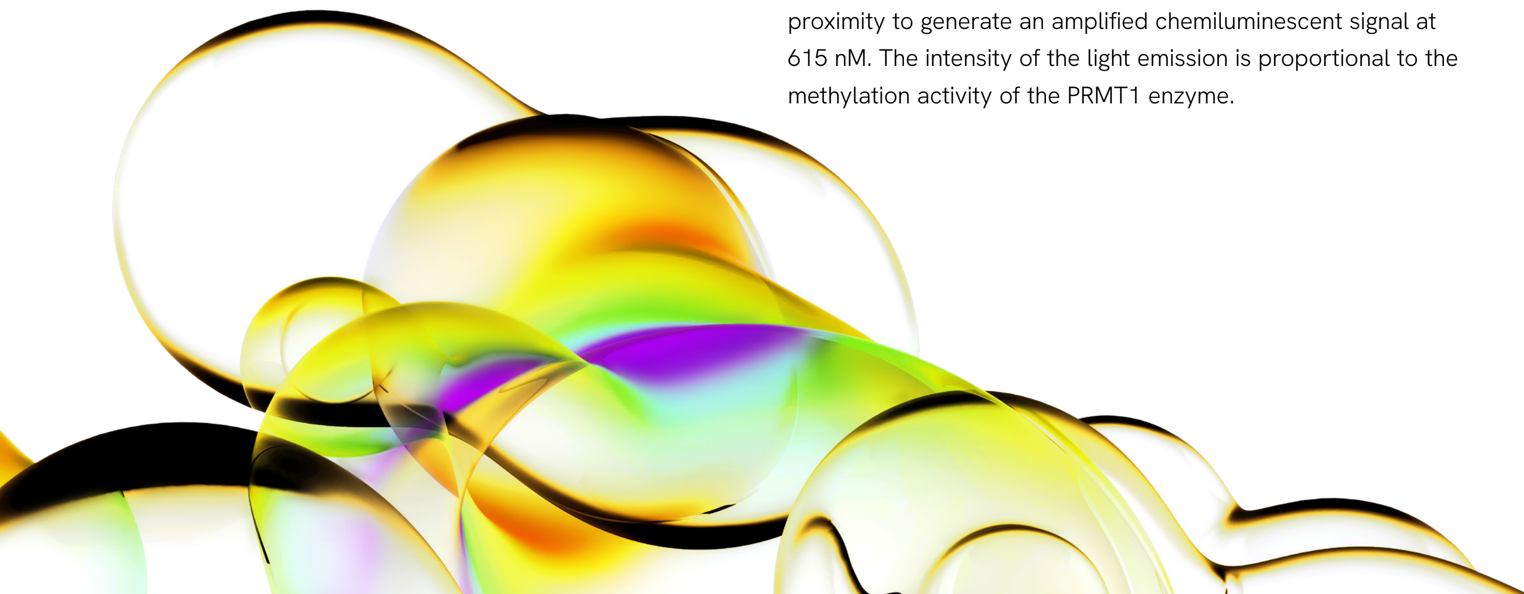
**0.5 µg/assay point*

Peptidic substrate sequence:

Ac-SG**R**GKGGKGLGKGGAKRHRKVGG-K(Biotin)

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 PRMT1 enzymatic assay using a biotinylated histone H4-derived peptide as substrate. Detection of the histone H4 arginine 3 methylated product is achieved through the addition of Streptavidin (SA) Alpha Donor beads and AlphaLISA Acceptor beads conjugated to an antibody (Ab) directed against the 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 the light emission is proportional to the methylation activity of the PRMT1 enzyme.



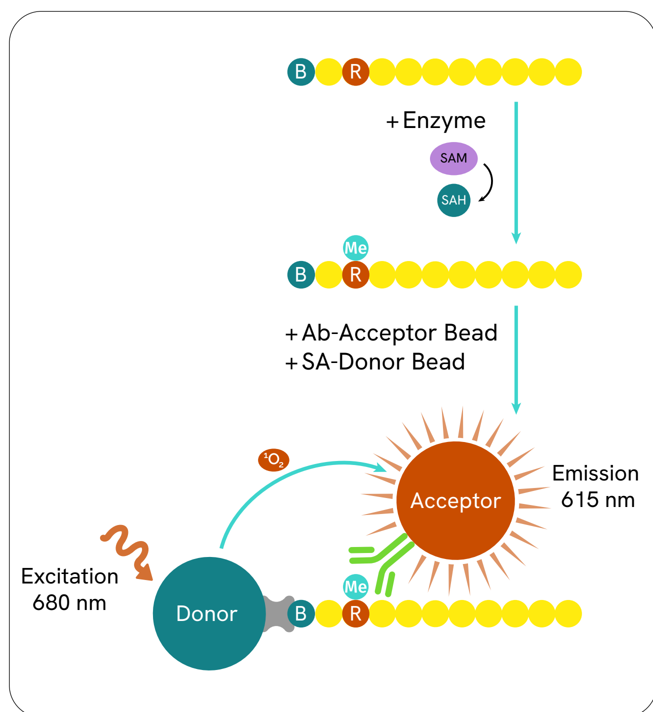


Figure 1: Schematic representation of the AlphaLISA detection of a methylated histone peptide (B: biotin group; R: arginine residue; Me: methyl group).

Development of a PRMT1 Histone H4-Arginine 3 N-methyltransferase assay

Reagents needed for this assay:

Anti-methyl-Histone H4 Arginine 3 (H4R3me) AlphaLISA Acceptor Beads	Revvity # AL150
Alpha Streptavidin Donor beads	Revvity # 6760002
Histone H4 (1-21) peptide, biotinylated	AnaSpec # 62555
PRMT1, recombinant	BPS BioScience # 51041
AlphaLISA 5X Epigenetics Buffer 1 Kit	Revvity # AL008
White opaque OptiPlate™-384	Revvity # 6007290
TopSeal™-A film	Revvity # 6050195
S-(5′-Adenosyl)-L-methionine chloride (SAM)	Sigma # A7007
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. Assay Buffer: 30 mM Tris-HCl, pH 8.0, 1 mM DTT, 0.01% BSA, 0.01% Tween-20

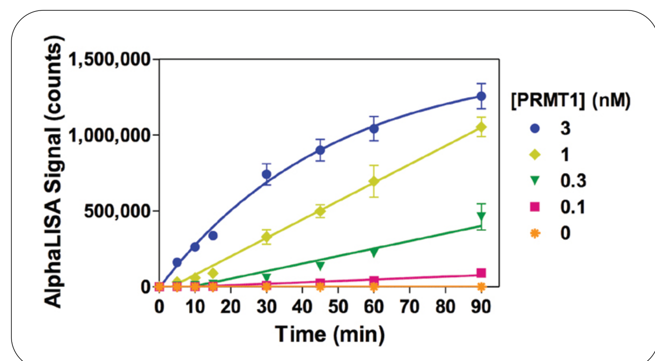
Standard protocol

- Dilute PRMT1 enzyme, SAM, inhibitors and biotinylated H4 (1-21) 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)
  - Incubate for 10 min at room temperature (RT).
  - 2.5 µL of biotinylated histone H4 (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 RT.
- Prepare 1X Epigenetics Buffer 1 as recommended in the buffer technical data sheet.
- Prepare 5X Acceptor beads mix at 100 µg/mL and 0.5 mM sinefungin in 1X Epigenetics Buffer 1. (final concentration of 20 µg/mL and 0.1 mM respectively in 25 µL total assay volume).
- Add 5 µL of Acceptor beads mix. *Addition of the sinefungin-containing Acceptor beads mix stops the enzymatic reaction.*
- Cover with TopSeal-A film and incubate 60 min at RT.
- Prepare 2.5X Streptavidin Donor beads at 50 µg/mL in 1X Detection Buffer 1 in subdued light. (final concentration of 20 µg/mL in 25 µL total assay volume).
- Add 10 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™ Multilabel Plate Reader or EnVision™ Multimode Plate Reader.

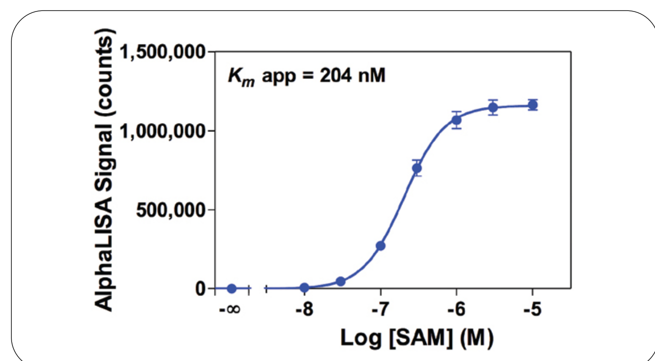
Results

Experiment 1: Enzyme titration and time course



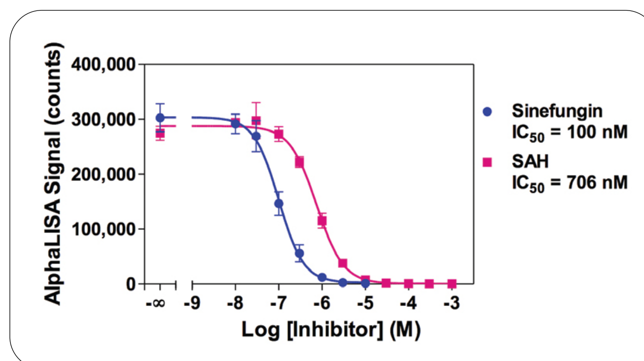
Enzymatic progress curves were performed by incubating PRMT1 at concentrations ranging from 0.1 to 3 nM with 10 nM biotinylated H4 (1-21) peptide substrate and 100 µM SAM. The sinefungin-containing Acceptor beads mix was added to stop the reactions at the indicated times. Donor beads were added 60 min later and signal was read after 30 min. A 60 min reaction time using 1 nM enzyme was selected for all subsequent experiments.

Experiment 2: SAM titration



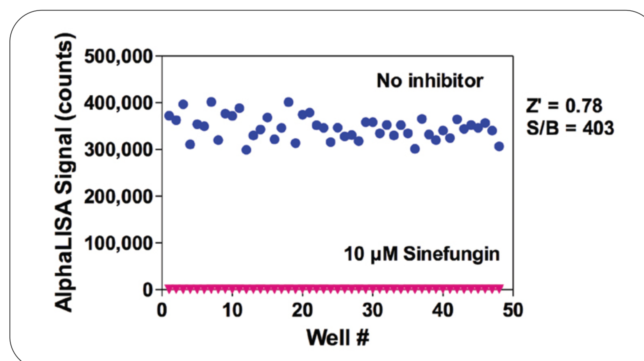
Serial dilutions of SAM ranging from 10 nM to 10 µM were added to 1 nM PRMT1 and 10 nM biotinylated H4 (1-21) peptide substrate. A 300 nM SAM concentration was selected for subsequent experiments.

Experiment 3: Enzyme inhibition



Serial dilutions of sinefungin and SAH ranging from 10 nM to 10 µM and 10 nM to 1 mM, respectively, were pre-incubated for 10 min with 1 nM PRMT1. Enzymatic reactions were initiated by the addition of 10 nM biotinylated H4 (1-21) peptide substrate and 300 nM SAM. Enzymatic reactions contain 1% DMSO.

Experiment 4: Z′-factor determination



PRMT1 (1 nM) was pre-incubated with or without 10 µM sinefungin for 10 min. Enzymatic reactions were initiated by the addition of 10 nM biotinylated H4 (1-21) peptide substrate and 300 nM SAM. Enzymatic reactions contain 1% DMSO.

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