AlphaLISA PRMT4 (CARM1) Histone H3-Arginine N-methyltransferase assay

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This AlphaLISA™ immunodetection assay measures the methylation of a biotinylated histone H3 (21-44) peptide at arginine 26.

Anti-methyl-Arginine AlphaLISA acceptor beads

- AL151C: 250 µg, 500 assay points*
- AL151M: 5 mg, 10,000 assay points*
- AL151R: 25 mg, 50,000 assay points*

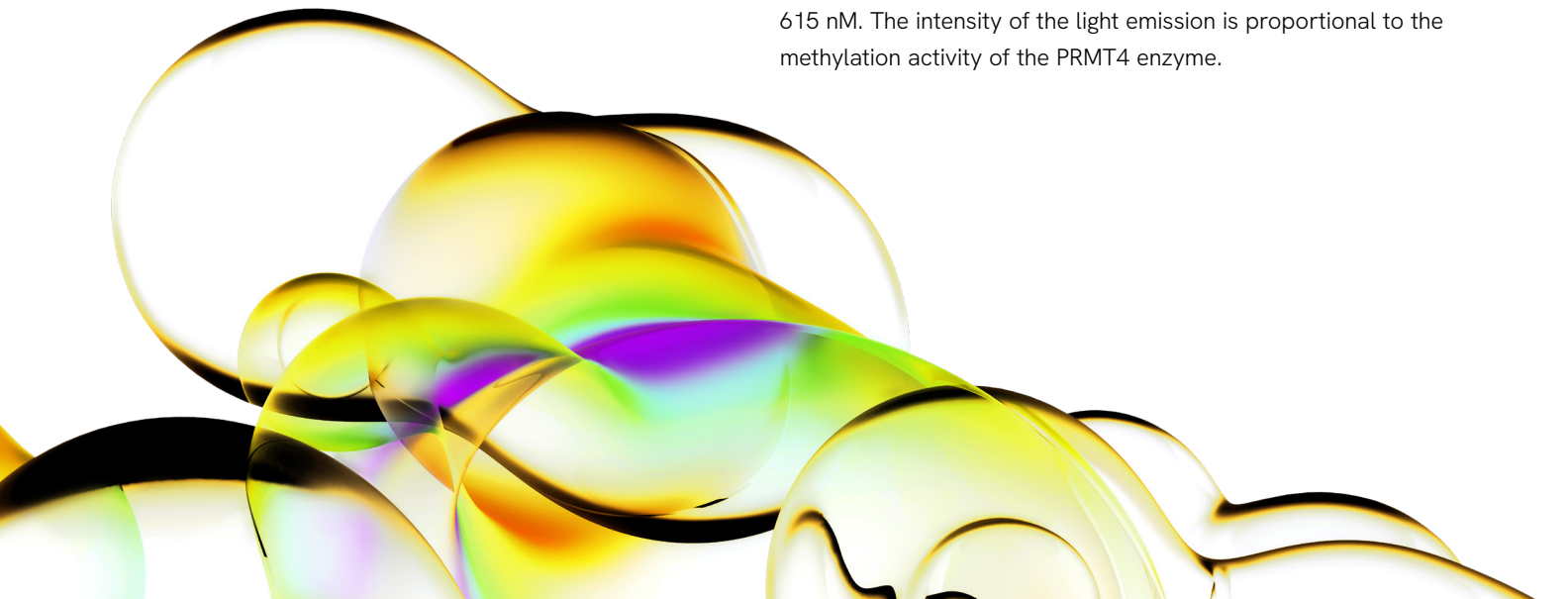
**0.5 µg/assay point*

Peptidic substrate sequence:

ATKAA**R**KSAPSTGGVKKPHRYRPG-GK(Biotin)-NH_2

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 PRMT4 (CARM1) enzymatic assay using a biotinylated histone H3-derived peptide as substrate. Detection of the methylated product was performed by the addition of Streptavidin (SA) Alpha Donor beads and AlphaLISA Acceptor beads conjugated to an antibody (Ab) binding to the methylated H3R26 residue. 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 PRMT4 enzyme.



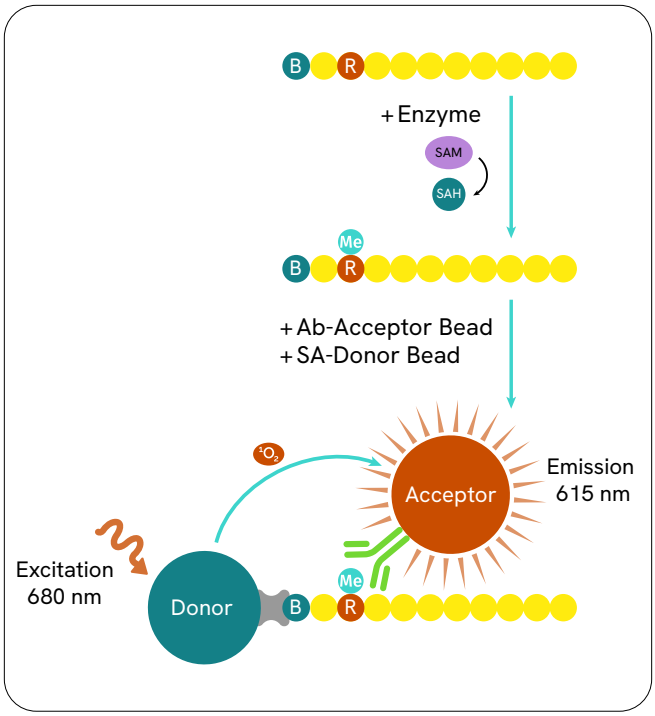


Figure 1: Schematic representation of the AlphaLISA detection of a methylated histone peptide (B: biotin group; R: arginine residue; Me: methyl group).

Development of a PRMT4 (CARM1) Histone H3-Arginine N-methyltransferase assay

Reagents needed for this assay:

AlphaLISA anti-methyl-Arginine Acceptor Beads	Revvity # AL151
Alpha Streptavidin Donor beads	Revvity # 6760002
Histone H3 (21-44) peptide, amide, biotinylated	AnaSpec # 64641
AlphaLISA 5X Epigenetics Buffer 1 Kit	Revvity # AL008
PRMT4 (human CARM1), recombinant	Reaction Biology # HMT-11-120
White opaque OptiPlate™-384	Revvity # 6007290
TopSeal™-A film	Revvity # 6050195
S-(5′-Adenosyl)-L-methionine chloride (SAM)	Sigma # A7007
Sinefungin	Sigma # S8559
AMI-5	EMD Millipore # 539211

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: 25 mM Tris-HCl, pH 8.0, 1 mM DTT, 0.01% BSA, 0.01% Tween-20.

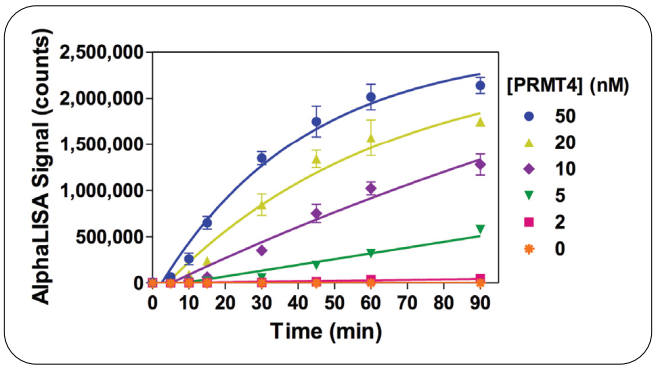
Standard protocol

- Dilute PRMT4 enzyme, SAM, inhibitors and biotinylated histone H3 (21-44) 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 H3 (21-44) 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 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 of Acceptor beads. *Addition of Acceptor beads prepared in Epigenetics Buffer 1 stops the enzymatic reaction.*
- Cover with TopSeal-A film and incubate 60 min at RT.
- Prepare Streptavidin Donor beads at 50 µg/mL in 1X Epigenetics 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 EnSpire™ Multimode Plate Reader.

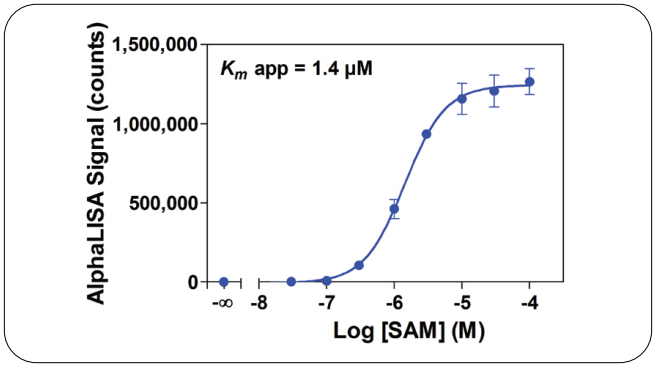
Results

Experiment 1: Enzyme titration and time course



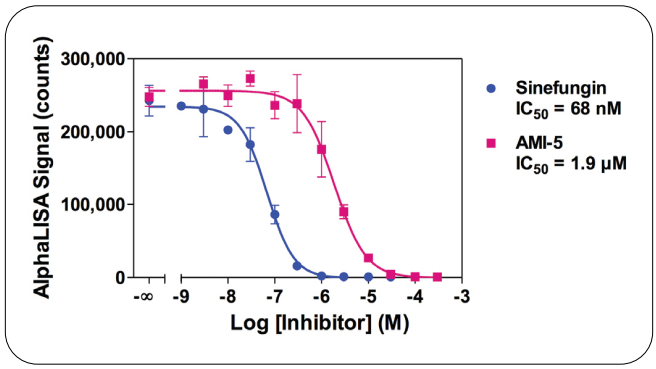
Enzymatic progress curves were performed by incubating PRMT4 at concentrations ranging from 2 to 50 nM with 10 nM biotinylated histone H3 peptide substrate plus 100 µM SAM. Acceptor beads were added at the indicated times. Donor beads were added 60 min later and signal was read after 30 min. A 60 min reaction time using 10 nM enzyme was selected for all subsequent experiments.

Experiment 2: SAM titration



Serial dilutions of SAM ranging from 30 nM to 100 µM were added to 10 nM PRMT4 and 10 nM biotinylated histone H3 peptide substrate. A 2 µM SAM concentration was selected for the next experiment.

Experiment 3: Enzyme inhibition



Serial dilutions of sinefungin and AMI-5 ranging from 1 nM to 30 µM and 3 nM to 300 µM, respectively, were pre-incubated for 10 min with 10 nM PRMT4. Enzymatic reactions were initiated by the addition of 10 nM biotinylated histone H3 peptide substrate plus 2 µM SAM. Enzymatic reactions contain 1% DMSO, which decreases significantly total signal.

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