AlphaLISA PRMT6 Histone H3-Arginine N-methyltransferase assay.

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Revvity, Inc.

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This AlphaLISA immunodetection assay measures the methylation of a biotinylated histone H3 (1-21) peptide at arginine 2.

Anti-methyl-Histone H3 Arginine 2 (H3R2me) AlphaLISA™ acceptor beads

- AL139C: 250 µg, 500 assay points*
- AL139M: 5 mg, 10,000 assay points*
- AL139R: 25 mg, 50,000 assay points*

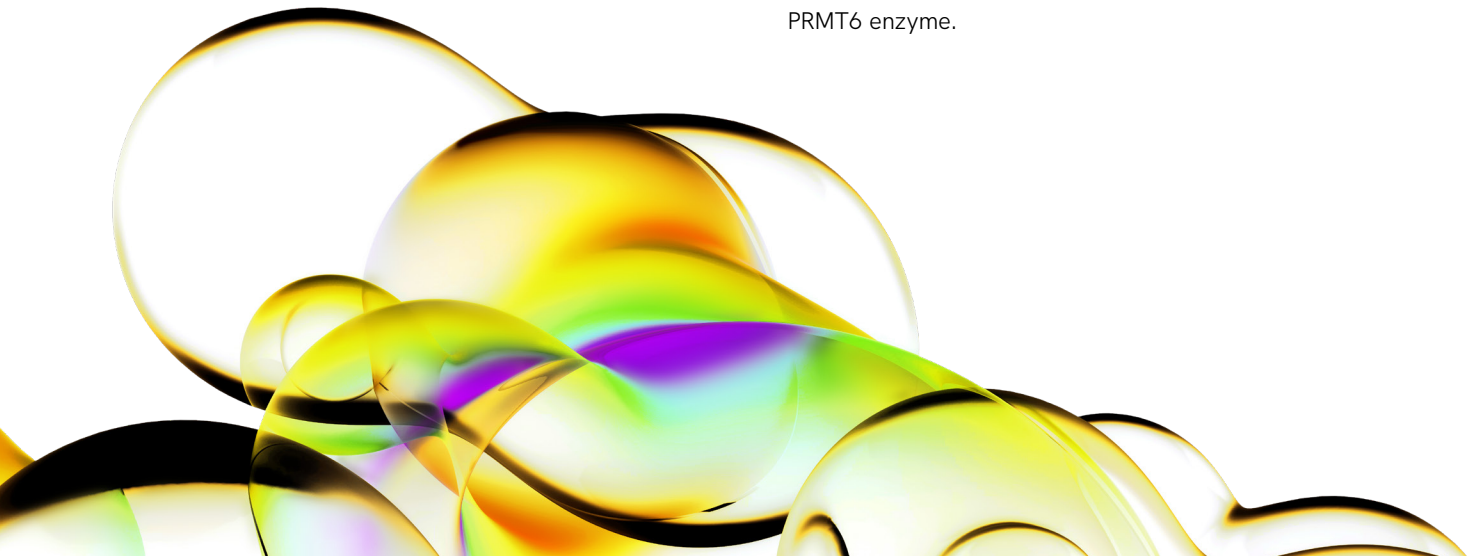
**0.5 µg/assay point*

Peptidic substrate sequence:

A**R**TKQTARKSTGGKAPRKQLA-GG-K(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 PRMT6 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) directed against the methylated H3R2 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 PRMT6 enzyme.



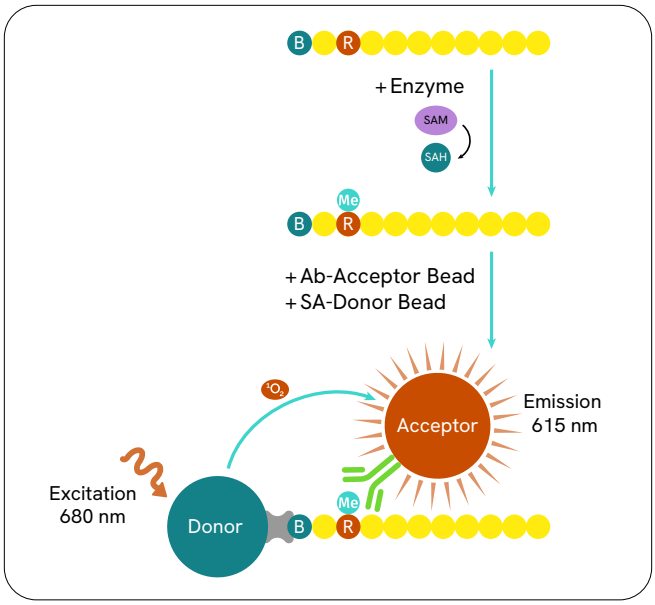


Figure 1: Schematic representation of the AlphaLISA detection of a methylated histone peptide (B: biotin group; R: arginine residue; Me: methyl group).

Development of a PRMT6 H3-Arginine N-methyltransferase assay

Reagents needed for the assay:

AlphaLISA anti-methyl-Histone H3 Arginine 2 (H3R2me) Acceptor beads	Revvity # AL139
Alpha Streptavidin Donor beads	Revvity # 6760002
Histone H3 (1-21) peptide, biotinylated	AnaSpec # 61702
AlphaLISA 5X Epigenetics Buffer 1 Kit	Revvity # AL008
PRMT6 (human), recombinant	BPS BioScience # 51049
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
BIX-01338	Sigma # B5313

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 8.0, 1 mM DTT, 0.01% Tween-20, 0.01% BSA.

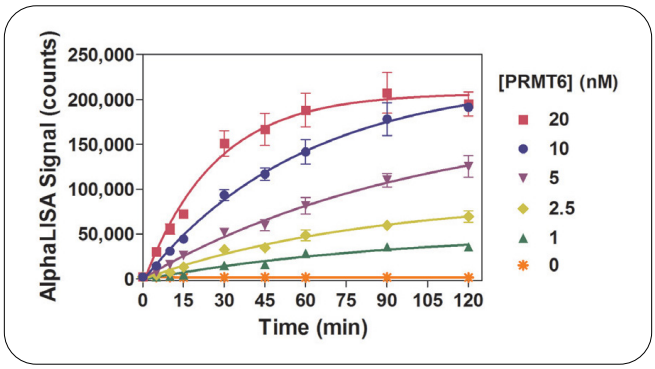
Standard protocol

- Dilute PRMT6 enzyme, SAM, inhibitors and biotinylated histone H3 peptide substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 2.5 µL of inhibitor (4X) or Assay Buffer
  - 5 µL of enzyme (2X)
  - Incubate for 10 min at 37 °C.
  - 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 37 °C.
- 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 room temperature (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 2 hours at RT.
- Read signal in Alpha mode with the EnVision™ or EnSpire™ readers.

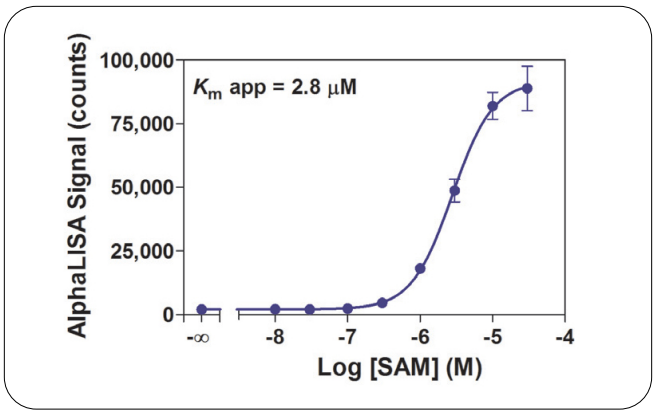
Results

Experiment 1: Enzyme titration and time-course



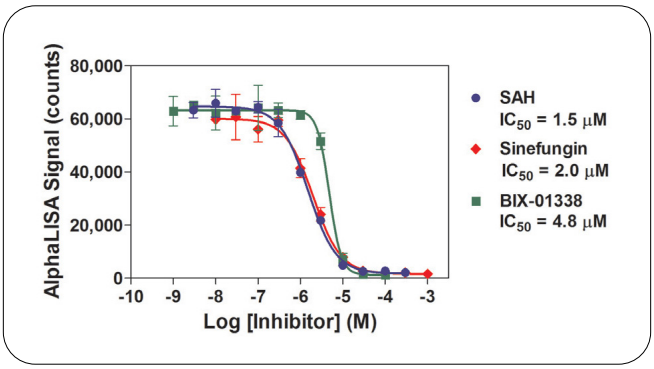
Enzymatic progress curves were performed by incubating PRMT6 at concentrations ranging from 1 to 20 nM with 50 nM biotinylated histone H3 peptide substrate plus 100 µM SAM. Acceptor beads were added at the indicated times. Donor beads were added 60 min later and signal was read after 2 hours. A 30 min reaction time using 10 nM enzyme was selected for all subsequent experiments.

Experiment 2: SAM titration



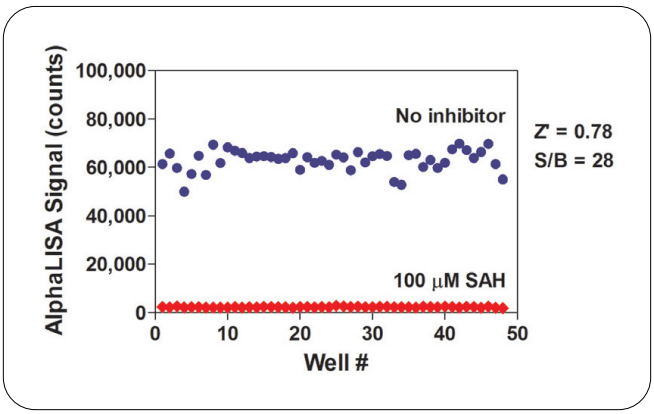
Serial dilutions of SAM ranging from 10 nM to 30 µM were added to 10 nM PRMT6 and 50 nM biotinylated histone H3 peptide substrate. A 5 µM SAM concentration was selected for subsequent experiments.

Experiment 3: Enzyme inhibition



Serial dilutions of SAH, sinefungin and BIX-01338 ranging from 3 nM to 300 µM, 10 nM to 1 mM, and 1 nM to 100 µM, respectively, were pre-incubated for 10 min with 10 nM PRMT6. Enzymatic reactions were initiated by the addition of 50 nM biotinylated histone H3 peptide substrate plus 5 µM SAM. Enzymatic reactions contain 2% DMSO.

Experiment 4: Z′-factor determination



PRMT6 (10 nM) was pre-incubated with or without 100 µM SAH for 10 min. Enzymatic reactions were initiated by the addition of 50 nM biotinylated histone H3 peptide substrate plus 5 µM SAM. Enzymatic reactions contain 2% DMSO.

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