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\text{R}GKGGGLGKGGAKHRKVGG-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.
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 PerkinElmer # AL150
- Alpha Streptavidin Donor beads PerkinElmer # 6760002
- Histone H4 (1-21) peptide, biotinylated AnaSpec # 62555
- PRMT1, recombinant BPS BioScience # 51041
- AlphaLISA SX Epigenetics Buffer 1 Kit PerkinElmer # AL008
- White opaque OptiPlate™-384 PerkinElmer # 6007290
- TopSeal™-A film PerkinElmer # 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$_2$SO$_4$/10% ethanol (v/v) in H$_2$O.

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.

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.