TECHNICAL NOTE

AlphaLISA SIRT1 Histone H3-Lysine 4 deacetylase assay.

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This AlphaLISA immunodetection assay measures the deacetylation of a biotinylated Histone H3 (1-21) peptide acetylated at lysine 4.

Anti-unmodified Histone H3 Lysine 4 (H3K4) AlphaLISA™ acceptor beads

- AL119C: 250 µg, 500 assay points*
- AL119M: 5 mg, 10,000 assay points*
- AL119R: 25 mg, 50,000 assay points*

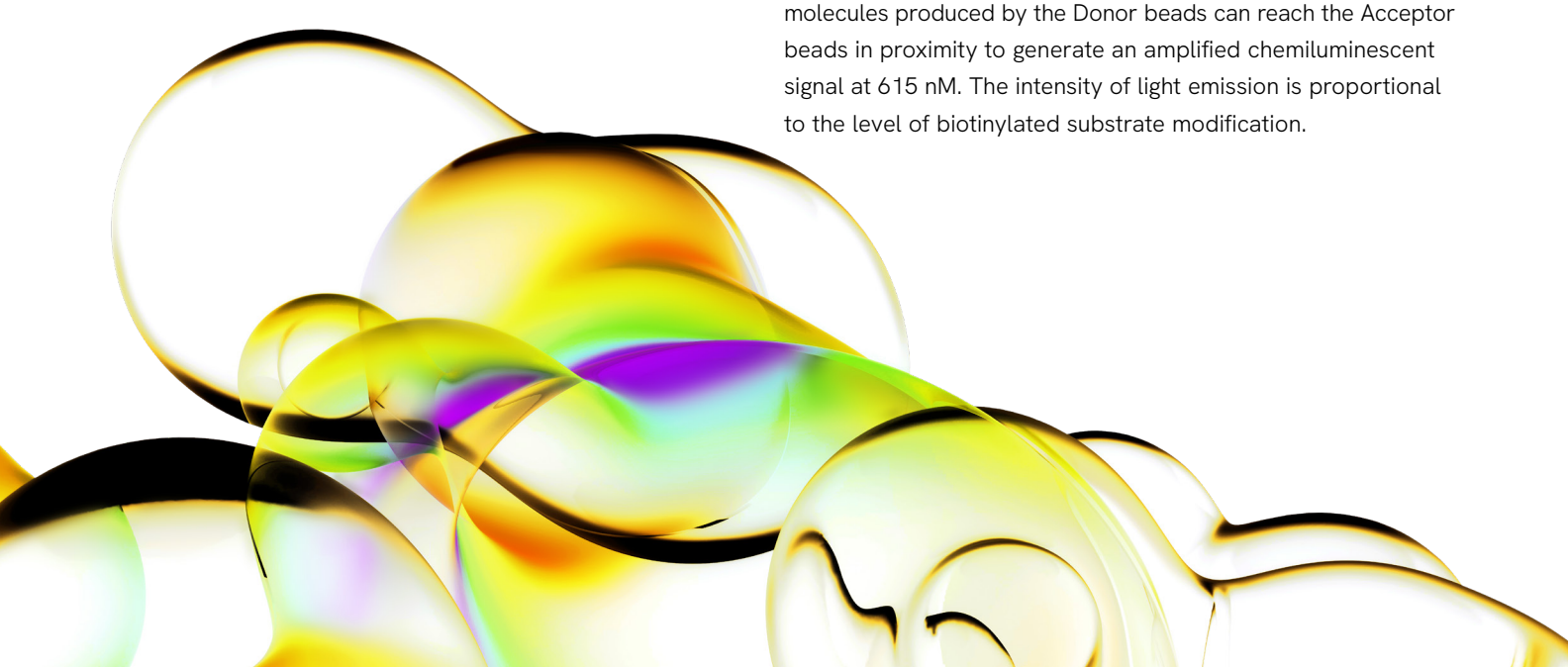
**0.5 µg/assay point*

Peptidic substrate sequence:

ART**K**(**ac**)QTARKSTGGKAPRKQLA-GG-K(Biotin)-OH

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 an SIRT1 enzymatic assay using a biotinylated Histone H3K4ac peptide as substrate. Detection of the deacetylated product was performed by the addition of Streptavidin (SA) Alpha Donor beads and AlphaLISA Acceptor beads conjugated to an antibody (Ab) directed against the unmodified H3K4 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 light emission is proportional to the level of biotinylated substrate modification.



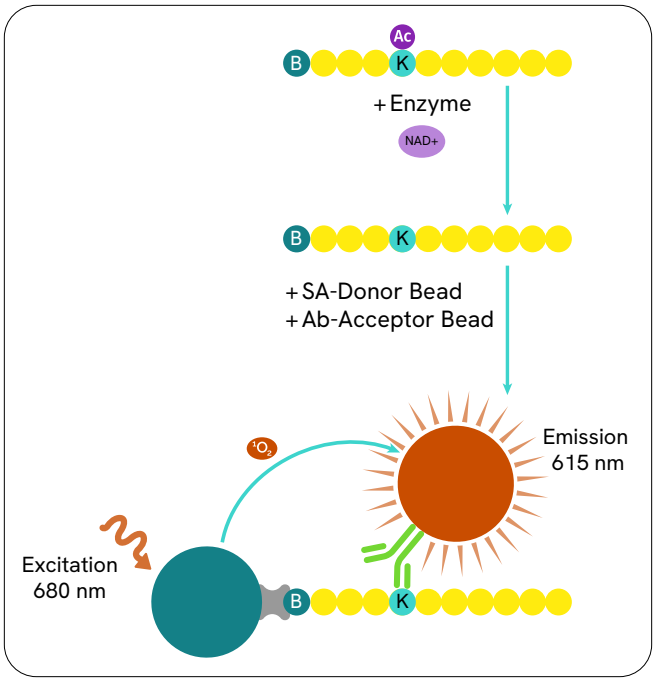


Figure 1: Schematic representation of the AlphaLISA detection of an unmodified histone peptide.

Development of a SIRT1 Histone H3-Lysine 4 deacetylase assay:

Reagents needed for the assay:

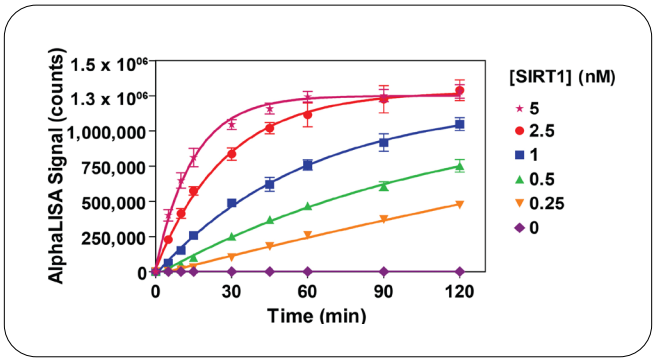
Anti-unmodified Histone H3 lysine 4 (H3K4) AlphaLISA Acceptor beads	Revvity # AL119
Histone H3 (1-21), H3K4ac peptide, biotinylated	AnaSpec # 65207
Alpha Streptavidin Donor beads	Revvity # 6760002
AlphaLISA 5X Epigenetics Buffer 1 Kit	Revvity # AL008
Sirtuin 1 (human), recombinant	BPS BioScience # 50012
EX-527	Tocris # 2780
Suramin	Calbiochem # 574625
Nicotinamide	Sigma # N3376
β-Nicotinamide adenine dinucleotide hydrate (NAD^+)	Sigma # N1636
White opaque OptiPlate™-384	Revvity # 6007299
TopSeal™-A films	Revvity # 6005185

Assay Buffer: 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM DTT, 0.01% Tween-20 and 0.01% BSA.

Standard protocol

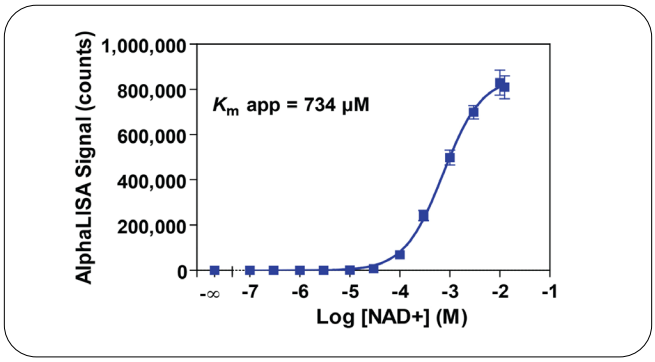
- Dilute SIRT1 enzyme, inhibitors, biotinylated Histone H3K4ac peptide substrate and NAD^+ in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of inhibitor (4X) or Assay buffer
  - Incubate 5 min at RT
  - 2.5 µL of biotinylated Histone H3K4ac peptide substrate (4X)
  - 2.5 µL of NAD^+ (4X)
- 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 a 5X Stop Solution Mix containing 250 µM of EX-527 and 100 µg/mL of Acceptor Beads in 1X Epigenetics Buffer 1 (final concentration of 50 µM EX-527 and 20 µg/mL Acceptor Beads in 25 µL total assay volume).
  - 5 µL of Stop Solution Mix
- Cover with TopSeal-A film and incubate for 60 min at RT.
- Prepare a 2.5X Streptavidin Donor beads solution at 50 µg/mL in 1X Epigenetics Buffer 1 (final concentration of 20 µg/mL in 25 µL total assay volume) in subdued light.
  - 10 µL of Streptavidin Donor beads
- Cover with TopSeal-A film and incubate in subdued light for 30 min at RT.
- Read signal in Alpha mode with the EnVision™ or EnSpire™ reader.

Experiment 1: Enzyme titration and time-course



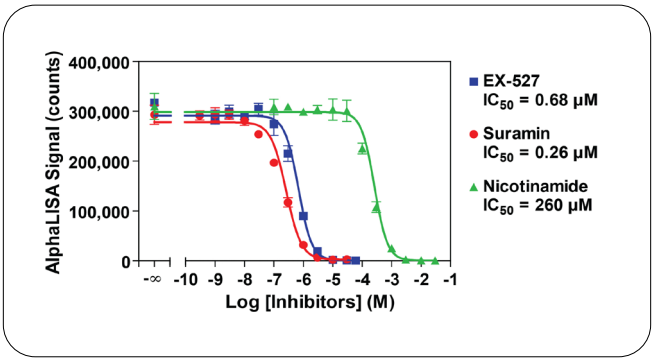
Enzymatic progress curves were performed by incubating SIRT1 at concentrations ranging from 0.25 to 5 nM with 200 nM biotinylated Histone H3K4ac peptide substrate and 1 mM NAD^+. A mix of Acceptor beads and EX-527 was added to stop the reaction at the indicated times. Streptavidin Donor beads were added 60 min later and signal was read after 30 min. A 30 min reaction time using 1 nM enzyme was selected for all subsequent experiments.

Experiment 2: NAD^+ titration



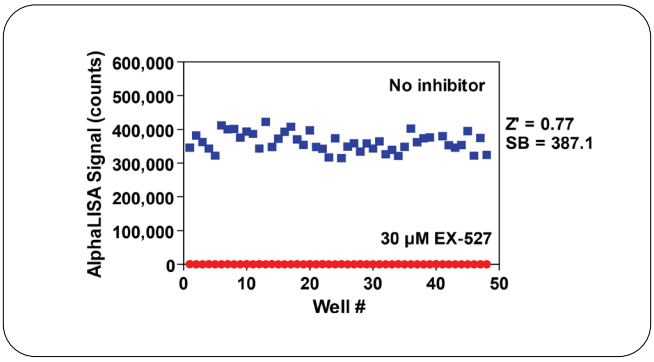
Serial dilutions of NAD^+ ranging from 100 nM to 12.5 mM were added to 1 nM SIRT1 and 200 nM biotinylated Histone H3K4ac peptide substrate. A 800 µM NAD^+ concentration was selected for subsequent experiments.

Experiment 3: Enzyme inhibition



Serial dilutions of EX-527 ranging from 1 nM to 60 µM, serial dilutions of suramin ranging from 0.3 nM to 30 µM and serial dilutions of nicotinamide ranging from 100 nM to 30 mM were pre-incubated for 5 min with 1 nM of SIRT1. Enzymatic reactions were initiated by the addition of 200 nM biotinylated Histone H3K4ac peptide substrate plus 800 µM NAD^+. Enzymatic reactions contain 1% DMSO.

Experiment 4: Z′-factor determination



SIRT1 (1 nM) was pre-incubated with or without 30 µM EX-527 for 5 min. Enzymatic reactions were initiated by the addition of 200 nM biotinylated Histone H3K4ac peptide substrate plus 800 µM NAD^+. Enzymatic reactions contain 1% DMSO.

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