TECHNICAL NOTE

AlphaLISA HDAC1 Histone H3-Lysine 9 Deacetylase Assay

AlphaLISA #17

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AlphaLISA®

This AlphaLISA immunodetection assay measures the deacetylation of a biotinylated histone H3 (1-21) peptide acetylated at lysine 9.

Anti-unmodified-Histone H3 Lysine 9/Lysine 27 (H3K9/K27) AlphaLISA® Acceptor Beads

- AL138C: 250 µg, 500 assay points*
- AL138M: 5 mg, 10,000 assay points*
- AL138R: 25 mg, 50,000 assay points*

**0.5 µg/assay point*

Peptidic Substrate Sequence:

ARTKQTAR-**K(ac)**-STGGKAPRKQLA-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 HDAC1 enzymatic assay using a biotinylated histone H3K9ac 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 H3K9 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 deacetylation activity of the HDAC1 enzyme.

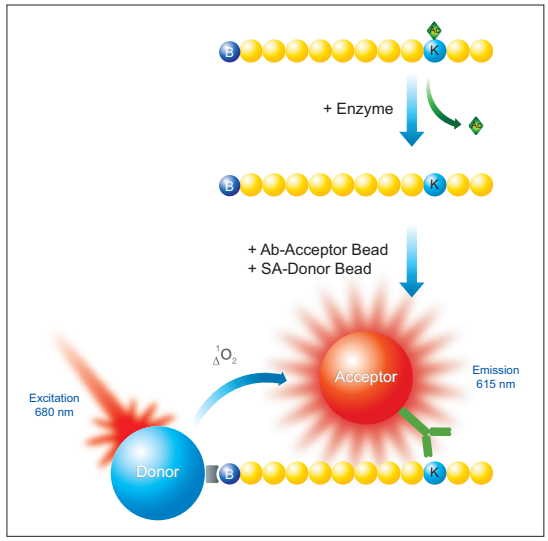

Figure 1. Schematic representation of the AlphaLISA detection of a deacetylated histone peptide (B: biotin group; K: lysine residue; Ac: acetyl group).

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Development of a HDAC1 Histone H3-Lysine 9 Deacetylase Assay

Reagents needed for the assay:

Anti-unmodified-Histone H3 Lysine 9/Lysine 27 (H3K9/K27) AlphaLISA® Acceptor beads	PerkinElmer # AL138
Alpha Streptavidin Donor beads	PerkinElmer # 6760002
Histone H3 (1-21) lysine 9 acetylated peptide, biotinylated (H3K9ac)	AnaSpec # 64361
AlphaLISA 5X Epigenetics Buffer 1 Kit	PerkinElmer # AL008
HDAC1 (human), recombinant	Cayman # 10009231
White opaque OptiPlate™-384	PerkinElmer # 6007290
TopSeal™-A film	PerkinElmer # 6050195
Trichostatin A	Sigma # T8552
SAHA	Cayman # 10009929

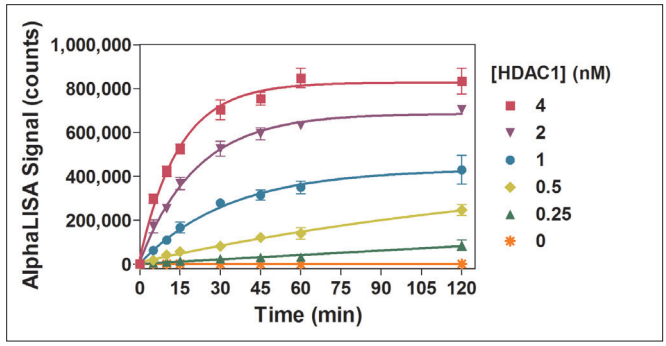
Assay Buffer: 50 mM Tris-HCl pH 8.0, 1 mM DTT, 0.01% Tween-20, 0.01% BSA

Standard Protocol

- Dilute HDAC1 enzyme, inhibitors and biotinylated histone H3K9ac 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 5 min at room temperature (RT).
  - 2.5 µL of biotinylated H3K9ac peptide (4X)
- 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® or EnSpire® readers.

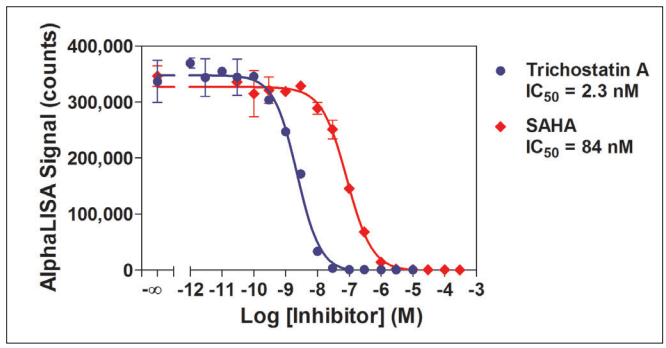
Results

Experiment 1: Enzyme Titration and Time-Course



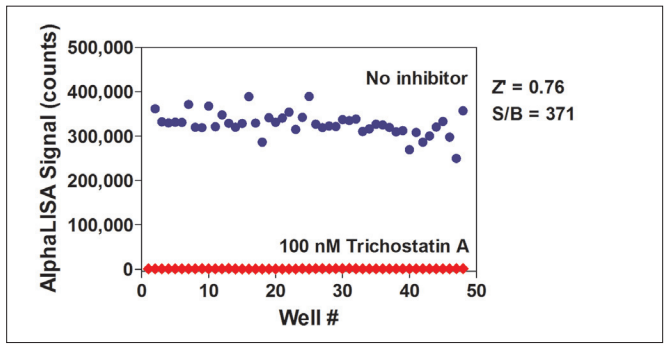
Enzymatic progress curves were performed by incubating HDAC1 at concentrations ranging from 0.25 to 4 nM with 50 nM biotinylated H3K9ac peptide substrate. Acceptor beads were added at the indicated times. Donor beads were added 60 min later and signal was read after 30 min. A 30 min reaction time using 1.5 nM enzyme was selected for all subsequent experiments.

Experiment 2: Enzyme Inhibition



Serial dilutions of Trichostatin A ranging from 1 pM to 10 µM and SAHA ranging from 30 pM to 300 µM were pre-incubated for 5 min with 1.5 nM HDAC1. Enzymatic reactions were initiated by the addition of 50 nM biotinylated H3K9ac peptide substrate. Enzymatic reactions contain 2% DMSO.

Experiment 3: Z'-factor Determination



HDAC1 (1.5 nM) was pre-incubated with or without 100 nM Trichostatin A for 5 min. Enzymatic reactions were initiated by the addition of 50 nM biotinylated H3K9ac peptide substrate. Enzymatic reactions contain 2% DMSO.

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