revvity

TECHNICAL NOTE

AlphaLISA HDAC1 Histone H3-Lysine 27 deacetylase assay.

Authors

Julie Blouin
Mathieu Arcand
Mireille Caron
Anne Labonté
Claire Normand
Lucille Beaudet
Jaime Padrós

Revvity, Inc.

For research purposes only.
Not for use in diagnostic procedures.

This AlphaLISA immunodetection assay measures the deacetylation of a biotinylated Histone H3 (21-44) peptide acetylated at lysine 27.

Anti-acetyl Histone H3 Lysine 27 (H3K27ac) AlphaLISA™ acceptor beads

- AL120C: 250 µg, 500 assay points*
- AL120M: 5 mg, 10,000 assay points*
- AL120R: 25 mg, 50,000 assay points*

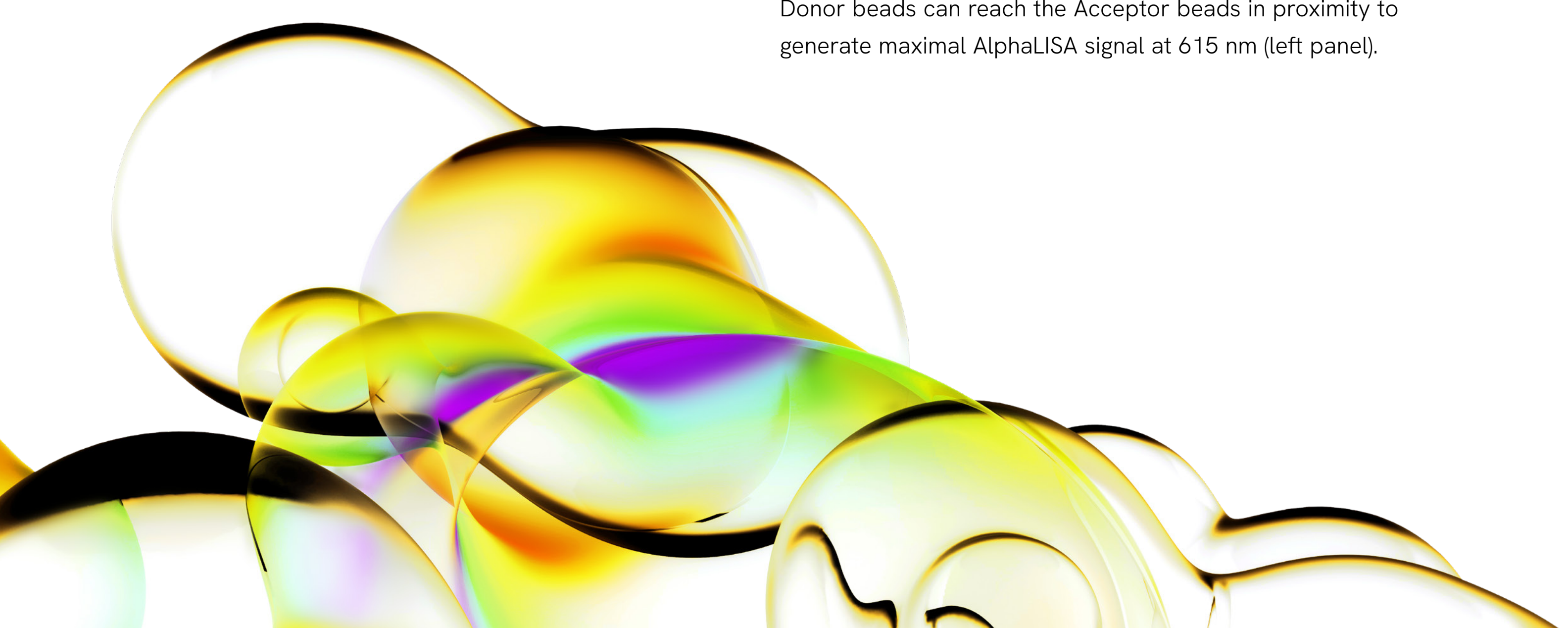
**0.5 µg/assay point*

Peptidic substrate sequence:

ATKAAR**K**(**ac**)SAPATGGVKKPHRYRP-GG-K(Biotin)-OH

AlphaLISA assays

AlphaLISA technology is a powerful and versatile platform that offers highly sensitive, no-wash immunoassays using Alpha Streptavidin Donor and AlphaLISA Acceptor beads. In this technical note, we present the optimization of a signal decrease HDAC1 assay using as substrate a biotinylated Histone H3-derived peptide acetylated at lysine 27. In the absence of enzyme, the anti-H3K27ac Acceptor beads bind the acetylated residue on the peptide. 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 maximal AlphaLISA signal at 615 nm (left panel).



When the enzyme is added to the reaction, the peptide substrate is deacetylated and the anti-H3K27ac Acceptor beads do not recognize the biotinylated peptide anymore, leading to a signal decrease (right panel). This signal decrease is proportional to the deacetylation activity of the HDAC1 enzyme.

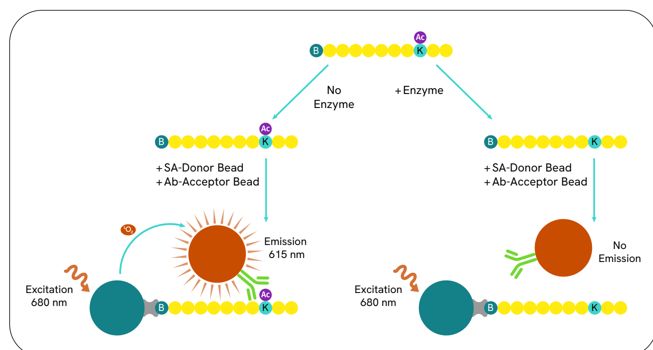


Figure 1: Schematic representation of the AlphaLISA detection of a modified histone peptide.

Development of a HDAC1 Histone H3-Lysine 27 deacetylase assay

Reagents needed for the assay:

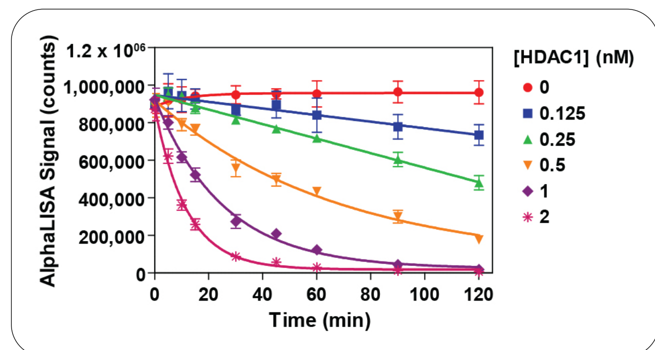
Anti-acetyl Histone H3 lysine 27 (H3K27ac) AlphaLISA Acceptor beads	Revvity # AL120
Histone H3 (21-44), H3K27ac peptide, biotinylated	AnaSpec # 64846
Alpha Streptavidin Donor beads	Revvity # 6760002
AlphaLISA 5X Epigenetics Buffer 1 Kit	Revvity # AL008
HDAC1 (human), recombinant	Cayman Chemical # 10009231
Trichostatin A	Sigma # T8552
SAHA	Cayman Chemical # 10009929
White opaque OptiPlate™-384	Revvity # 6007299
TopSeal™-A films	Revvity # 6005185

Assay Buffer: 50 mM Tris-HCl pH 8.0, 0.1 mM EDTA, 1 mM DTT, 0.01% Tween-20 and 0.01% BSA.

Standard protocol

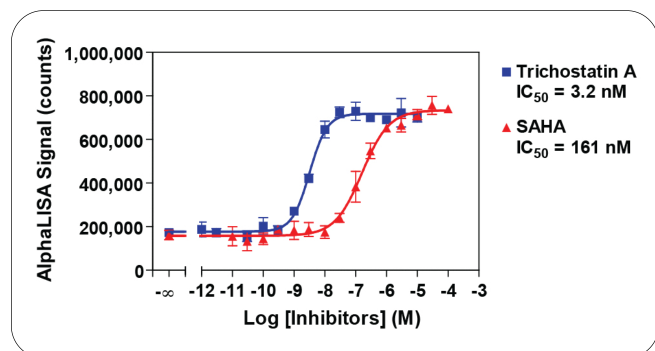
- Dilute HDAC1 enzyme, inhibitors and biotinylated Histone H3K27ac peptide substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of inhibitor (4X) or Assay buffer
  - Incubate 5 min at RT
  - 5 µL of biotinylated Histone H3K27ac peptide substrate (2X)
- 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 Acceptor beads solution at 100 µg/mL in 1X Epigenetics Buffer 1 (final concentration of 20 µg/mL in 25 µL total assay volume).
  - 5 µL of Acceptor beads
    Addition of Acceptor beads prepared in 1X Epigenetics Buffer 1 stops the enzymatic reaction.
- 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



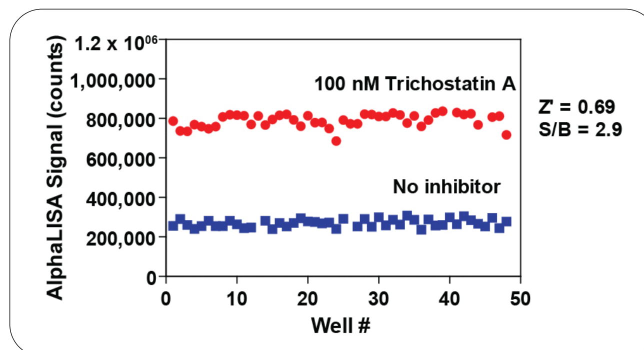
Enzymatic progress curves were performed by incubating HDAC1 at concentrations ranging from 0.125 to 2 nM with 3 nM biotinylated Histone H3K27ac peptide substrate. Acceptor beads were added at the indicated times. Donor beads were added 60 min later and signal was read after 30 min. A 30 min reaction time using 1 nM enzyme was selected for all subsequent experiments.

Experiment 2: Enzyme inhibition

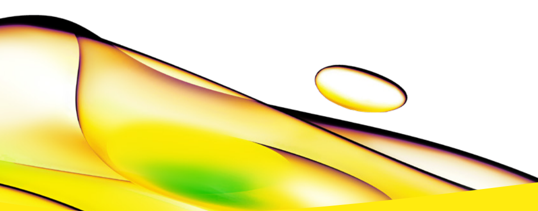


Serial dilutions of Trichostatin A ranging from 1 pM to 10 µM and serial dilutions of SAHA ranging from 10 pM to 100 µM were pre-incubated for 5 min with 1 nM of HDAC1. Enzymatic reactions were initiated by the addition of 3 nM biotinylated Histone H3K27ac peptide substrate. Enzymatic reactions contain 1% DMSO.

Experiment 3: Z′-factor determination



HDAC1 (1 nM) was pre-incubated with or without 100 nM Trichostatin A for 5 min. Enzymatic reactions were initiated by the addition of 3 nM biotinylated Histone H3K27ac peptide substrate. Enzymatic reactions contain 1% DMSO.



revvity

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com
Copyright ©2024, Revvity, Inc. All rights reserved.