TECHNICAL NOTE

AlphaLISA p300 Histone H3-Lysine Acetyltransferase Assay

AlphaLISA #3

AlphaLISA®

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

Anti-acetyl-Histone H3 Lysine 9 (H3K9ac) AlphaLISA® Acceptor Beads

- AL114C: 250 µg, 500 assay points*
- AL114M: 5 mg, 10,000 assay points*
- AL114R: 25 mg, 50,000 assay points*

**0.5 µg/assay point*

Peptidic Substrate Sequence:

ARTKQTAR**K**STGGKAPRKQLA-GG-K(BIOTIN)-NH2

AlphaLISA Assays

The AlphaLISA technology allows performing no-wash homogeneous proximity immunoassays using Alpha Donor and AlphaLISA Acceptor beads. In this technical note, we present the optimization of an epigenetic enzymatic assay using a biotinylated histone H3-derived peptide as substrate. Detection of the modified substrate was performed by the addition of Streptavidin (SA) Alpha Donor beads and AlphaLISA Acceptor beads conjugated to an antibody (Ab) directed against the epigenetic 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 light emission is proportional to the level of biotinylated substrate modification.

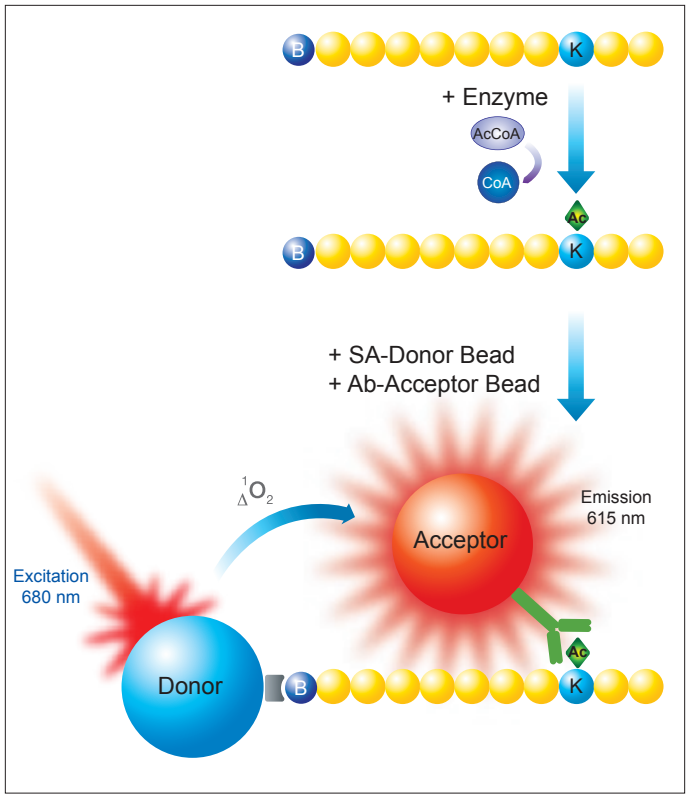

Figure 1. Schematic representation of the AlphaLISA detection of a modified histone peptide.

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Development of a p300 Histone H3-Lysine Acetyltransferase Assay

Reagents needed for the assay:

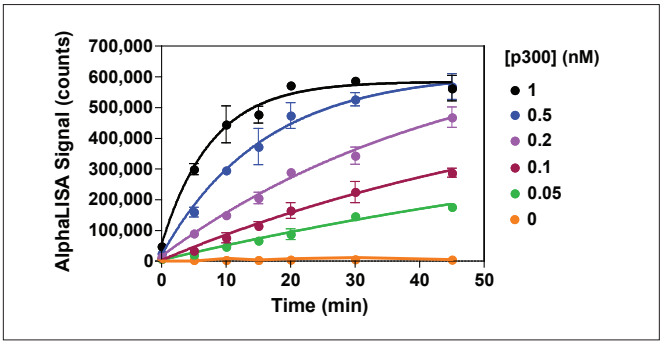
Anti-acetyl-Histone H3 Lysine 9 (H3K9Ac)	
AlphaLISA Acceptor Beads	PerkinElmer # AL114
Alpha Streptavidin Donor beads	PerkinElmer # 6760002
Histone H3 (1-21) peptide, biotinylated	AnaSpec # 61702
AlphaLISA 5X Epigenetics Buffer 1 Kit	PerkinElmer # AL008
p300 (human), recombinant	Enzo # BML-SE451
White opaque OptiPlate™-384	PerkinElmer # 6007299
TopSeal™-A films	PerkinElmer # 6005185
Acetyl coenzyme A (acetyl CoA)	Sigma # A2056
Trichostatin A (TSA)	Sigma # T8552
Anacardic Acid	Calbiochem # 172050
Garcinol	Sigma # G5173

Acetyl CoA is prepared at 56.9 mM in H_2O, aliquoted and stored at -80 °C.
Assay Buffer: 50 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 1 mM DTT, 0.01% Tween-20, 0.01% BSA, 330 nM TSA

Standard Protocol

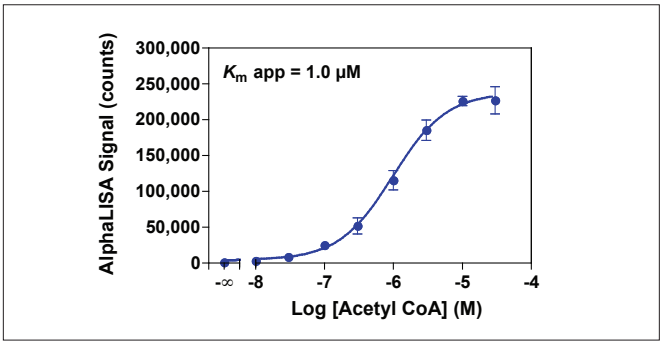
- Dilute p300 enzyme, acetyl CoA, inhibitors and biotinylated peptide substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 5 µL of inhibitor (2X) or Assay Buffer
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of biotinylated Histone H3 (1-21) peptide/acetyl CoA mix (4X). For acetyl CoA titration, add acetyl CoA dilutions independently of substrate.
- 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 Acceptor beads/anacardic acid mix by diluting the Acceptor beads to 100 µg/mL and anacardic acid to 250 µM in 1X Epigenetics Buffer 1 (final concentrations of 20 µg/mL and 50 µM, respectively, in 25 µL total assay volume).
- Add 5 µL of Acceptor beads/ anacardic acid mix. *Addition of anacardic acid stops the enzymatic reaction*.
- Cover with TopSeal-A film and incubate for 60 min at RT.
- Prepare Streptavidin Donor beads at 50 µg/mL in 1X Epigenetics Buffer 1 (final concentration of 20 µg/mL in 25 µL total assay volume) in subdued light.
- Add 10 µL Streptavidin Donor beads in subdued light.
- Cover with TopSeal-A film and incubate in the dark for 30 min at RT.
- Read signal in Alpha mode with EnVision® or EnSpire® readers.

Experiment 1: Enzyme Titration and Time-Course



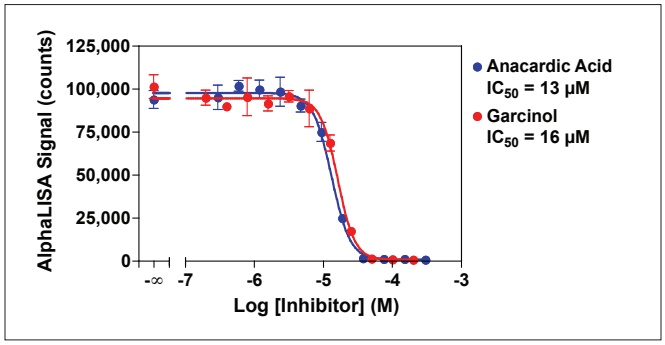
Enzymatic progress curves were performed by incubating p300 at concentrations ranging from 0.05 to 1 nM with 50 nM biotinylated H3 (1-21) peptide substrate and 25 µM acetyl CoA. A mix of Acceptor beads and anacardic acid was added to stop the reactions at the indicated times. Donor beads were added 60 min later and signal was read after 30 min. A 15 min reaction time using 0.2 nM enzyme was selected for all subsequent experiments.

Experiment 2: Acetyl CoA Titration



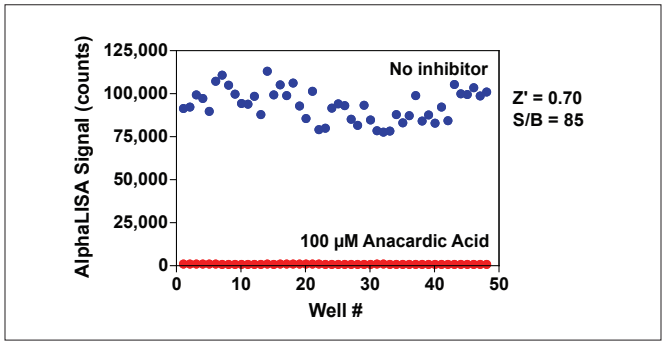
Serial dilutions of acetyl CoA ranging from 10 nM to 30 µM were added to 0.2 nM p300 and 50 nM biotinylated H3 (1-21) peptide substrate. A 3 µM acetyl CoA concentration was selected for subsequent experiments.

Experiment 3: Enzyme Inhibition



Serial dilutions of anacardic acid ranging from 300 nM to 300 µM and garcinol ranging from 200 nM to 200 µM were pre-incubated for 10 min with 0.2 nM p300. Enzymatic reactions were initiated by the addition of 50 nM biotinylated H3 (1-21) peptide substrate plus 3 µM acetyl CoA. Enzymatic reactions contain 1% DMSO.

Experiment 4: Z'-factor Determination



p300 (0.2 nM) was pre-incubated with or without 100 µM anacardic acid for 10 min. Enzymatic reactions were initiated by the addition of 50 nM biotinylated H3 (1-21) peptide substrate plus 3 µM acetyl CoA. Enzymatic reactions contain 1% DMSO.

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