AlphaLISA KAT5 (TIP60) assay

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This AlphaLISA™ immunodetection assay measures the acetylation of a Histone H4 (1-25) peptide at the N-terminal lysine residues.

Anti-Acetyl-Lysine AlphaLISA acceptor beads

- AL143C: 250 µg, 500 assay points*
- AL143M: 5 mg, 10,000 assay points*
- AL143R: 25 mg, 50,000 assay points*

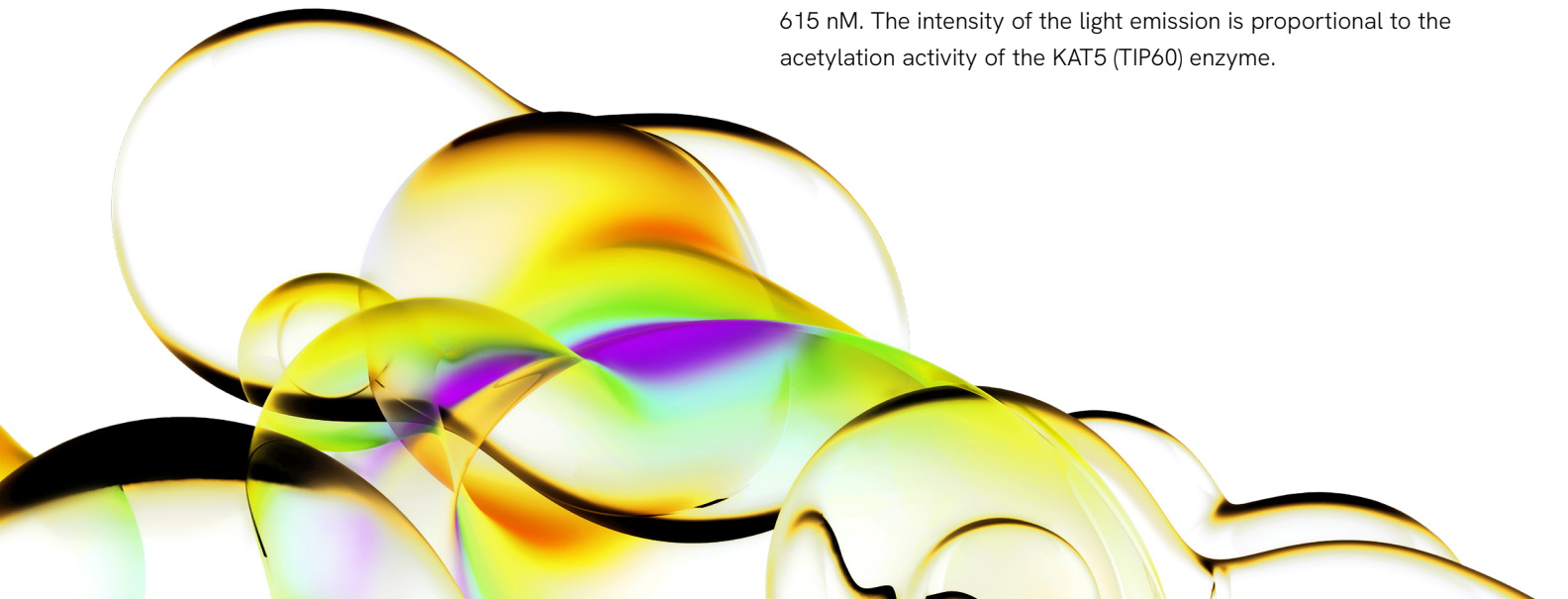
**0.5 µg/assay point*

Peptidic substrate sequence:

SGRG**K**GG**K**GLG**K**GGA**K**RHRKVLRDNGSGS-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 KAT5 (TIP60) enzymatic assay using a biotinylated H4-derived peptide as substrate. Detection of the acetylated product was performed by the addition of Streptavidin (SA) Alpha Donor beads and AlphaLISA Acceptor beads conjugated to an antibody (Ab) directed against acetylated lysine residues. 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 acetylation activity of the KAT5 (TIP60) enzyme.



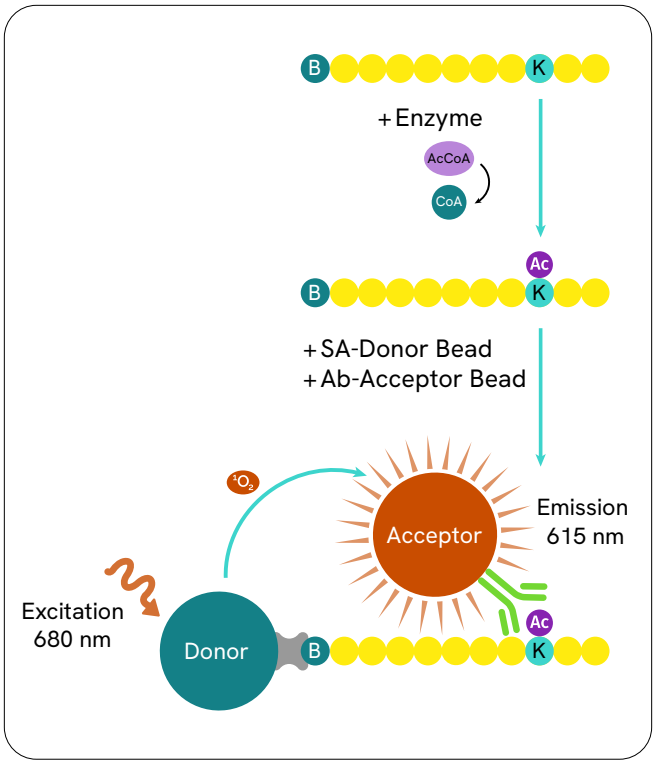


Figure 1: Schematic representation of the AlphaLISA detection of an acetylated histone peptide (B: biotin group; K: lysine residue).

Development of AlphaLISA KAT5 (TIP60) assay

Reagents needed for this assay:

AlphaLISA anti-Acetyl-Lysine Acceptor beads	Revvity # AL143
Alpha Streptavidin Donor beads	Revvity # 6760002
Histone H4 (1-25) peptide, biotinylated	AnaSpec # 65242
AlphaLISA 5X Epigenetics Buffer 1 Kit	Revvity # AL008
KAT5 (TIP60), active	SignalChem # K314-380G
White opaque OptiPlate™-384	Revvity # 6007290
TopSeal™-A film	Revvity # 6050195
Acetyl coenzyme A sodium salt	Sigma # A2056
Anacardic acid	EMD MIllipore # 172050
Garcinol	Sigma # G5173

Assay Buffer: 50 mM TRIS-HCl pH 8.0, 0.1 mM EDTA, 1 mM DTT, 330 nM TSA, 0.01% BSA and 0.01% Tween

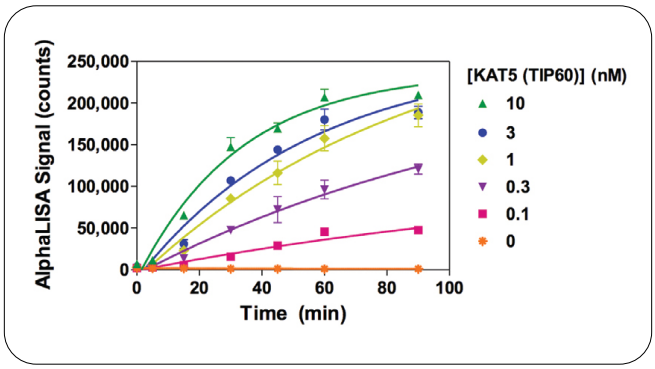
Standard protocol

- Dilute KAT5 (TIP60) enzyme, acetyl CoA, inhibitors and biotinylated H4 (1-25) 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 23 °C.
  - 2.5 µL of biotinylated H4 (1-25)/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 23 °C.
- 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 23 °C.
- 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 23 °C.
- Read signal in Alpha mode with the EnVision™ Multilabel Plate Reader or EnSpire™ Multimode Plate Reader.

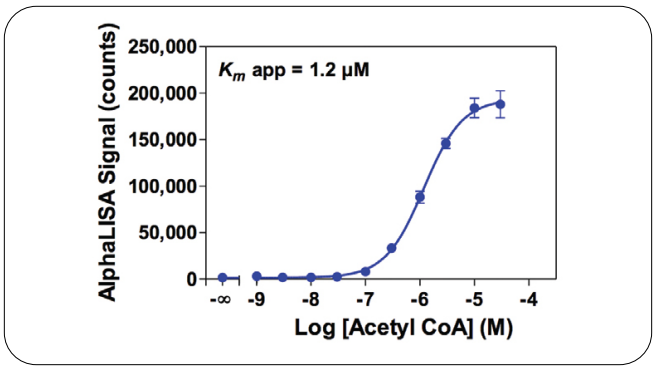
Results

Experiment 1: Enzyme titration and time course



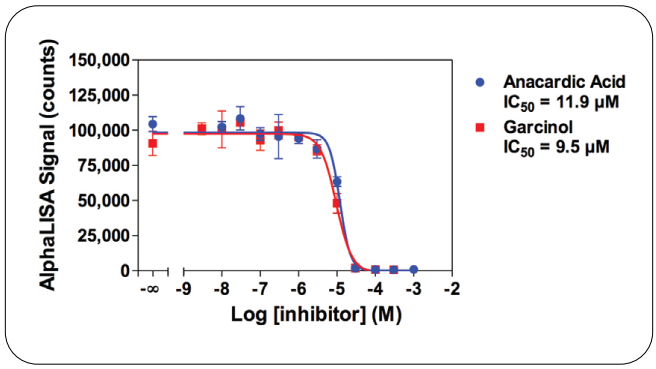
Enzymatic progress curves were performed by incubating KAT (TIP60) at concentrations ranging from 0.1 to 10 nM with 100 nM biotinylated H4 (1-25) substrate. Acceptor beads were added at the indicated times. Donor beads were added 60 min later and signal was read after 30 min. A 60 min reaction time using 1 nM enzyme was selected for all subsequent experiments.

Experiment 2: Acetyl CoA titration



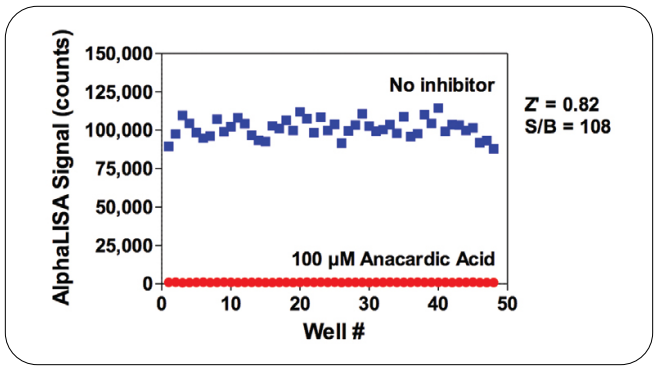
Serial dilutions of acetyl CoA ranging from 1 nM to 30 µM were added to 1 nM KAT5 (TIP60) and 100 nM biotinylated H4 (1-25) peptide substrate. A 1.5 µM acetyl CoA concentration was selected for subsequent experiments.

Experiment 3: Enzyme inhibition



Serial dilutions of anacardic acid and garcinol ranging from 10 nM to 1 mM and 3 nM to 0.3 mM, respectively, were pre-incubated for 5 min with 1 nM KAT5 (TIP60). Enzymatic reactions were initiated by the addition of 100 nM biotinylated H4 (1-25) peptide substrate. Enzymatic reactions contain 1% DMSO.

Experiment 4: Z′-factor determination



KAT5 (TIP60) (1 nM) was pre-incubated with or without 100 µM anacardic acid for 5 min. Enzymatic reactions were initiated by the addition of 100 nM biotinylated H4 (1-25) peptide substrate. Enzymatic reactions contain 1% DMSO.

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