AlphaLISA LSD1 Histone H3-Lysine 4 demethylase assay.

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This AlphaLISA immunodetection assay measures the demethylation of a biotinylated Histone H3 (1-21) peptide mono-methylated 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*

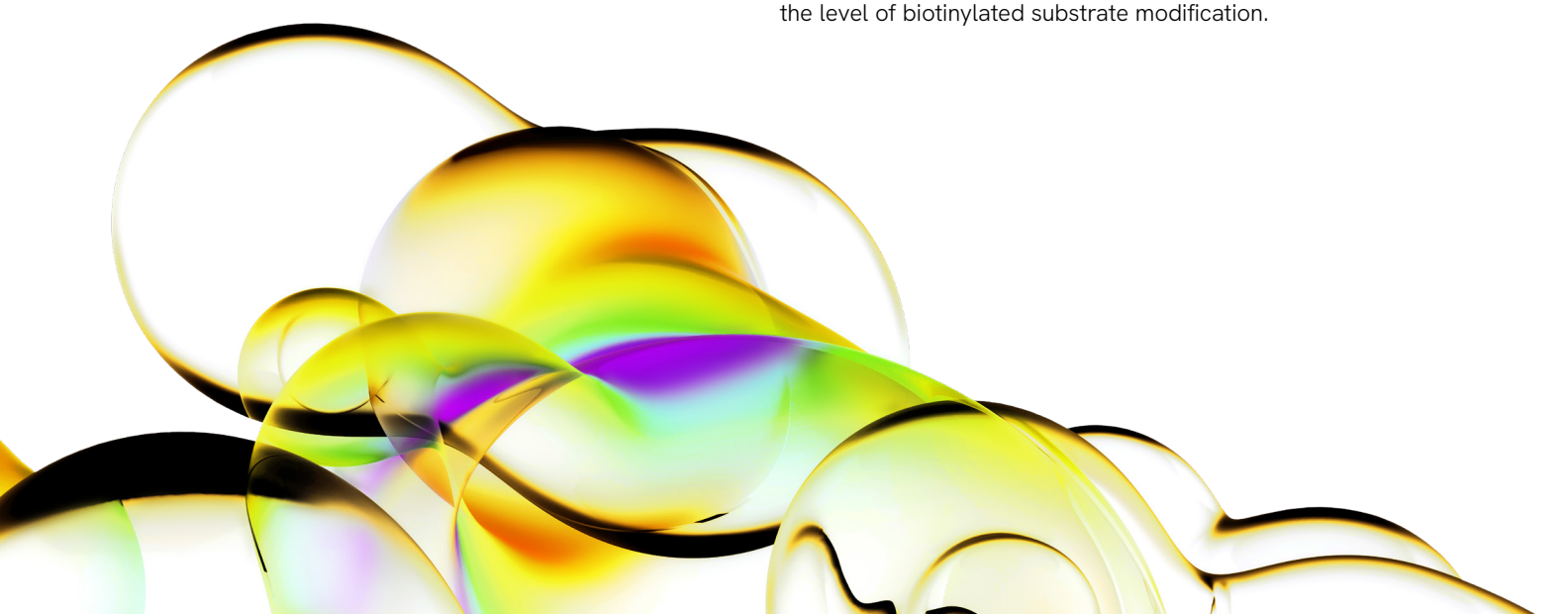
**0.5 µg/assay point*

Peptidic substrate sequence:

ART**K**(**me1**)QTARKSTGGKAPRKQLA-GG-K(Biotin)-NH_2

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 epigenetic enzymatic assay using a biotinylated Histone H3-derived peptide as substrate. Detection of the un-modified substrate was performed by the addition of Streptavidin (SA) Alpha Donor beads and AlphaLISA Acceptor beads conjugated to an anti-body (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.



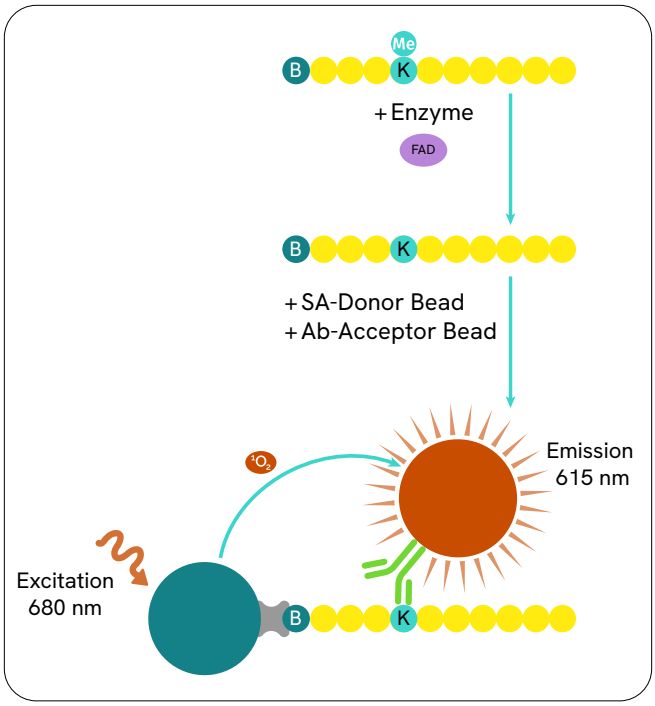


Figure 1: Schematic representation of AlphaLISA detection of an un-modified histone peptide.

Development of a LSD1 Histone H3-Lysine 4 demethylase assay:

Reagents needed for the assay:

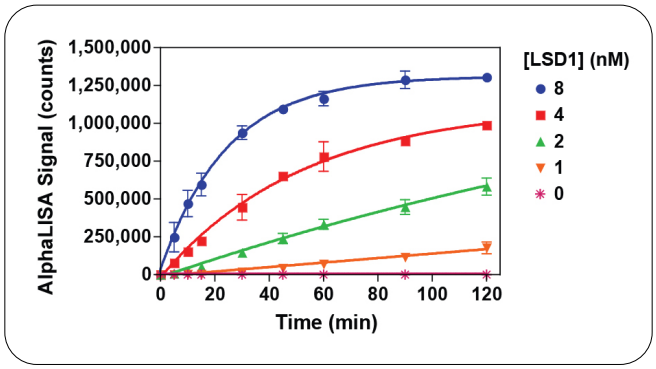
Anti-H3K4 unmodified AlphaLISA Acceptor beads	Revvity # AL119
Alpha Streptavidin Donor beads	Revvity # 6760002
Histone H3 (1-21), H3K4(me1) peptide, biotinylated	AnaSpec # 64355
AlphaLISA 5X Epigenetics buffer 1 kit	Revvity # AL008
LSD1 (human), recombinant	BPS BioScience # 50100
White opaque OptiPlate™-384	Revvity # 6007299
TopSeal™-A films	Revvity # 6005185
Trans-2-Phenylcyclopropylamine (Tranylcypromine)	Sigma # P8511

Assay Buffer: 50 mM Tris-HCl pH 9.0, 50 mM NaCl, 1 mM DTT and 0.01% Tween-20.

Standard protocol

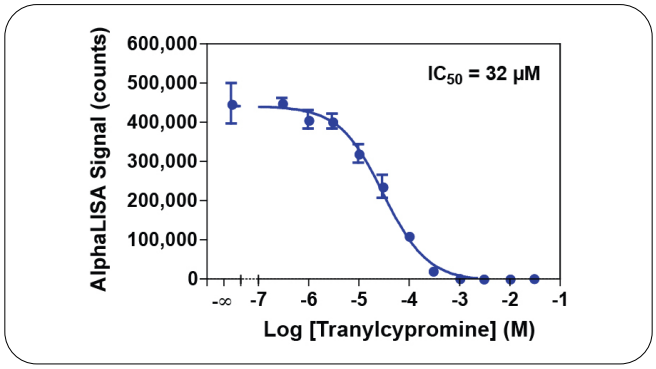
- Dilute LSD1 enzyme, tranylcypromine (inhibitor) and biotinylated peptide substrate in Assay Buffer just before use.
- Add to the wells of a white OptiPlate-384:
  - 5 µL of inhibitor (2X) or Assay Buffer
  - 2.5 µL of enzyme (4X)
  - 2.5 µL of biotinylated Histone H3K4me1 peptide (4X)
- Cover the plate 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 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



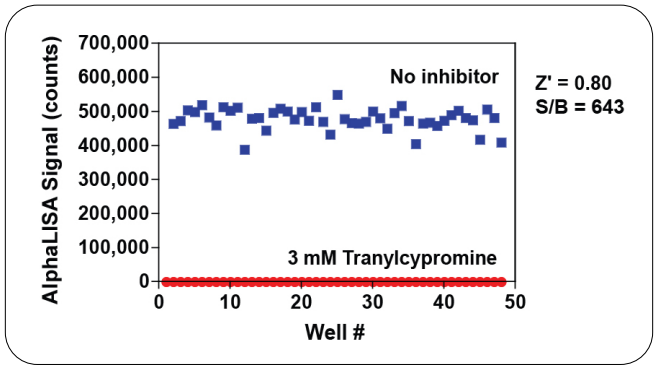
Enzymatic progress curves were performed by incubating LSD1 at concentrations ranging from 1 to 8 nM with 80 nM biotinylated Histone H3K4me1 peptide substrate. Acceptor beads were added at the indicated times. Donor beads were added 60 min later and signal was read after 30 min. A 60 min reaction time using 2 nM enzyme was selected for all subsequent experiments.

Experiment 2: Enzyme inhibition



Serial dilutions of tranylcypromine from 300 nM to 30 mM were pre-incubated for 10 min with 2 nM LSD1. Enzymatic reactions were initiated by the addition of 80 nM biotinylated Histone H3K4me1 peptide substrate. Enzymatic reactions contain 1% DMSO.

Experiment 3: Z′-factor determination



LSD1 (2 nM) was pre-incubated with or without 3 mM tranylcypromine for 10 min. Enzymatic reactions were initiated by the addition of 80 nM biotinylated Histone H3K4me1 peptide substrate. Enzymatic reactions contain 1% DMSO.

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