# AlphaLISA JMJD3 Histone H3-Lysine 27 demethylase assay.


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

## Anti-di/mono-methyl-Histone H3 lysine 27 (H3K27me2-1) AlphaLISA™ acceptor beads

- AL121C: 250 µg, 500 assay points*
- AL121M: 5 mg, 10,000 assay points*
- AL121R: 25 mg, 50,000 assay points*

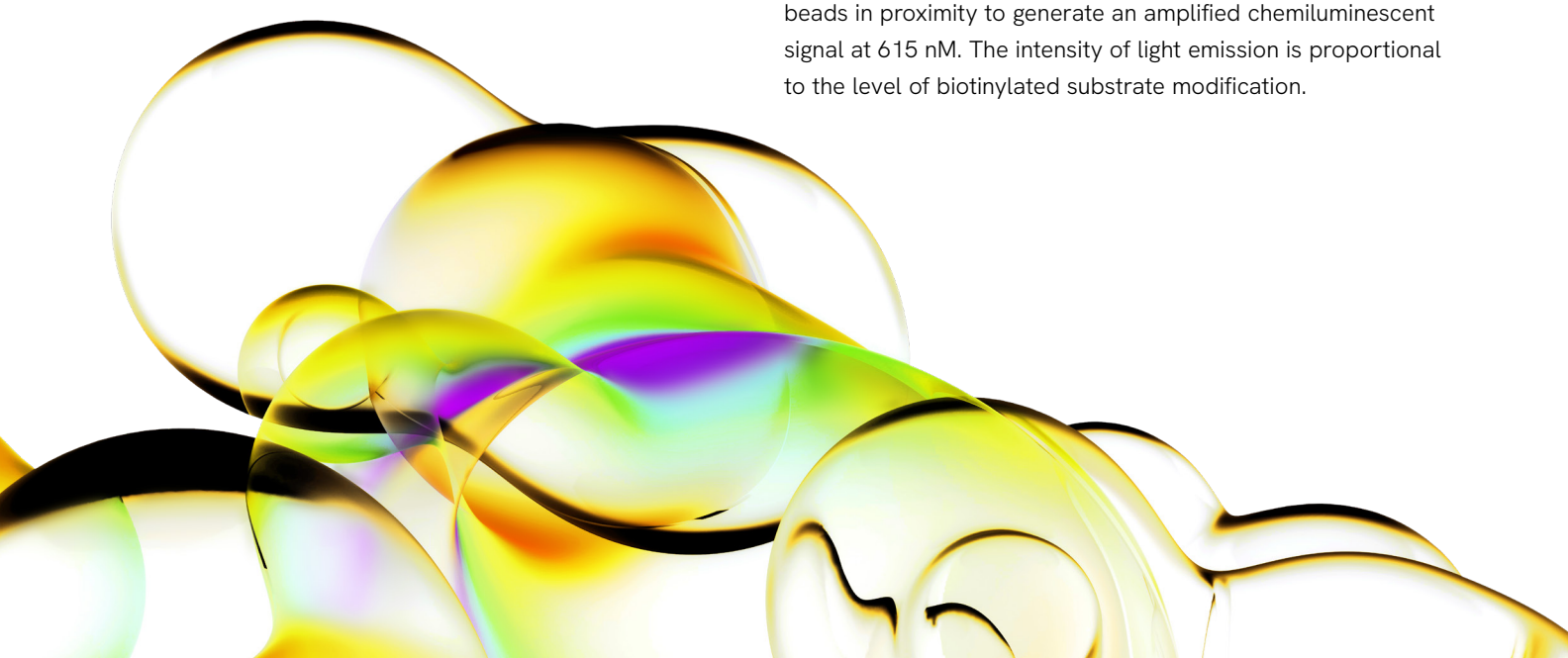
**0.5 µg/assay point*

## Peptidic substrate sequence:

ATKAAR**K(me3)**SAPATGGVKKPHRYRP-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 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 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.



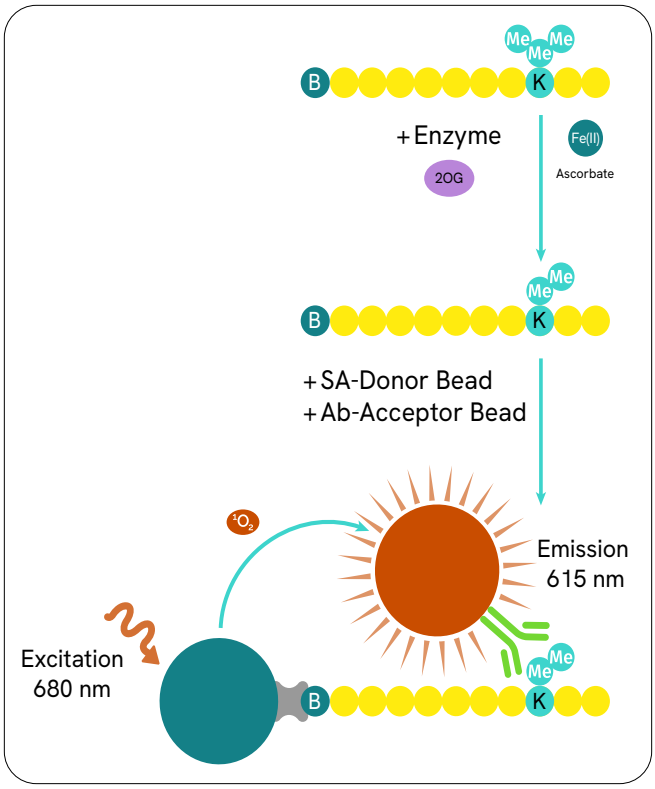



Figure 1: Schematic representation of AlphaLISA detection of a modified histone peptide

## Development of a JMJD3 Histone H3-Lysine 27 demethylase assay:

**Reagents needed for the assay:**

| | |
|---|---|
| Anti-di/mono-methyl-Histone H3 Lysine 27 (H3K27me2-1) AlphaLISA Acceptor beads | Revvity # AL121 |
| Alpha Streptavidin Donor beads | Revvity # 6760002 |
| Histone H3 (21-44), H3K27(me3) peptide, biotinylated | AnaSpec # 64367 |
| AlphaLISA 5X Epigenetics Buffer 1 Kit | Revvity # AL008 |
| JMJD3 (active human recombinant protein) | BPS BioScience # 50115 |
| White opaque OptiPlate™-384 | Revvity # 6007299 |
| TopSeal™-A films | Revvity # 6005185 |
| α-Ketoglutaric acid potassium salt (2OG) | Sigma # K2000 |
| (+) Sodium L-ascorbate | Sigma # 11140 |
| Ammonium iron(II) sulfate hexahydrate (Fe(II)) | Sigma # 215406 |
| 2,4-Pyridinedicarboxylic acid (2,4-PDCA) | Sigma # P63395 |

Assay Buffer: 50 mM HEPES pH 7.5, 0.01% Tween-20 and 0.01% BSA.

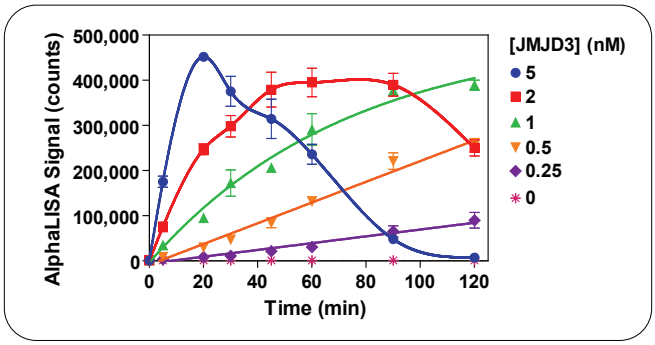
**Standard protocol**

- Dilute JMJD3 enzyme, 2OG, Fe(II), ascorbate, 2,4-PDCA (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 H3K27me3 peptide/2OG/Fe(II)/ascorbate mix (4X).

    *For 2OG titration, add 2OG 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 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

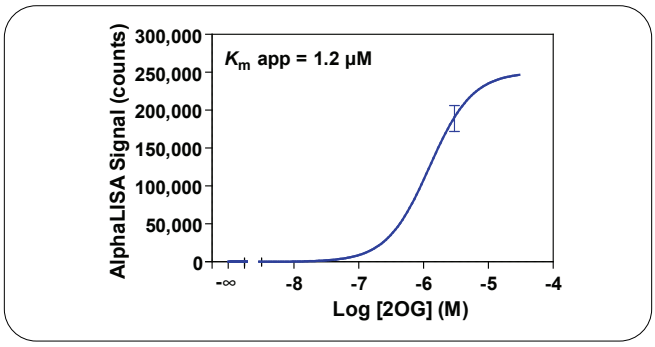
    *Addition of Acceptor beads prepared in 1X Epigenetics Buffer 1 stops the enzymatic reaction.*
- Cover with TopSeal-A film and incubate for 60 min at RT.
- Prepare a 2.5X Streptavidin Donor beads solution at 50 µg/mL in 1X Epigenetics Buffer 1 (final concentration of 20 µg/mL in 25 µL total assay volume) 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



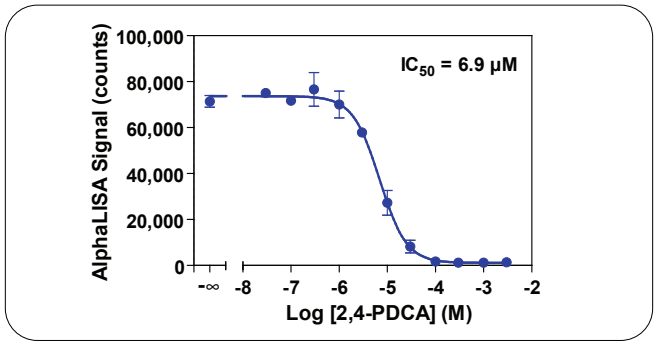

Enzymatic progress curves were performed by incubating JMJD3 at concentrations ranging from 0.25 to 5 nM with 50 nM biotinylated Histone H3K27me3 peptide substrate plus 50 µM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Acceptor beads were added at the indicated times. Donor beads were added 60 min later and signal was read after 30 min. A 45 min reaction time using 1 nM enzyme was selected for all subsequent experiments. Signal decrease observed at higher enzyme concentration or reaction time is due to the generation of peptides un-methylated at lysine 27, which are not detected by the anti-H3K27me2-1 AlphaLISA Acceptor beads.

### Experiment 2: 2OG titration



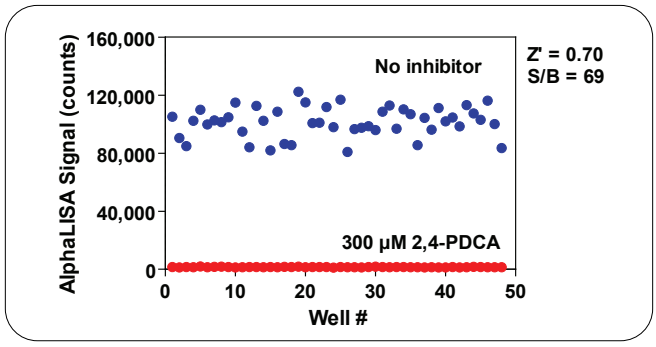

Serial dilutions of 2OG ranging from 10 nM to 30 µM were added to 1 nM JMJD3 and 50 nM biotinylated Histone H3K27me3 peptide substrate plus 5 µM Fe(II) and 100 µM ascorbate. A 1 µM 2OG concentration was selected for subsequent experiments.

### Experiment 3: enzyme inhibition




Serial dilutions of 2,4-PDCA ranging from 30 nM to 3 mM were pre-incubated for 15 min with 1 nM JMJD3. Enzymatic reactions were initiated by the addition of 50 nM biotinylated Histone H3K27me3 peptide substrate plus 1 µM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Enzymatic reactions contain 1% DMSO.

### Experiment 4: Z′-factor determination




JMJD3 (1nM) was pre-incubated with or without 300 µM 2,4-PDCA for 15 min. Enzymatic reactions were initiated by the addition of 50 nM biotinylated Histone H3K27me3 peptide substrate plus 1 µM 2OG, 5 µM Fe(II) and 100 µM ascorbate. Enzymatic reactions contain 1% DMSO.

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