This LANCE Ultra immunodetection assay measures the demethylation of a biotinylated Histone H3 (1-21) peptide mono-methylated at lysine 4.

**Europium-anti-unmodified Histone H3 Lysine 4 (H3K4) Antibody**
- TRF0404-D: 10 µg, 1,562 assay points*
- TRF0404-M: 100 µg, 15,625 assay points*
*40 fmol/assay point

**Peptidic Substrate Sequence:**
ARTK(me1)QTARKSTGGKAPRKQLA-GG-K(Biotin)-NH₂

**LANCE Ultra Assays**

LANCE Ultra time-resolved fluorescence resonance energy transfer (TR-FRET) assays use a proprietary europium chelate donor dye, W1024 (Eu), together with ULight™, a small molecular weight acceptor dye with a red-shifted fluorescent emission.

In this technical note, we present the optimization of an epigenetic enzymatic assay using a biotinylated Histone H3-derived methylated peptide as substrate. The unmodified peptide is captured by the Eu-labeled antibody (Eu-Ab) and ULight-Streptavidin (ULight-SA), which brings the Eu donor and ULight acceptor dye molecules into close proximity. Upon irradiation at 320 or 340 nm, the energy from the Eu donor is transferred to the ULight acceptor dye which, in turn, generates light at 665 nm. The intensity of the light emission is proportional to the level of biotinylated reaction product.

Figure 1. Schematic representation of the LANCE Ultra detection of a modified histone peptide.
Development of a LSD1 Histone H3-Lysine 4 Demethylase Assay:

Reagents needed for the assay:

- Europium-anti-unmodified Histone H3 Lysine 4 (H3K4) Antibody PerkinElmer # TRF0404
- LANCE Ultra ULight-Streptavidin PerkinElmer # TRF0102
- Histone H3 (1-21), H3K4(me1) peptide, biotinylated AnaSpec # 64355
- LANCE Detection Buffer, 10X PerkinElmer # CR97-100
- LSD1 (human), recombinant BPS BioScience # 50100
- White opaque OptiPlate™-384 PerkinElmer # 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 a 4X Detection Mix by diluting the Eu-Ab to 8 nM and ULight-Streptavidin to 200 nM in 1X LANCE Detection Buffer (final concentrations of 2 nM and 50 nM, respectively, in 20 μL total assay volume).
- Prepare a 4X Stop Solution containing 1.2 mM tranylcypromine in 1X LANCE Detection Buffer (final concentration of 300 μM in 20 μL total assay volume).
  - 5 μL of Tranylcypromine Stop Solution and incubate 5 min at RT
  - 5 μL of Detection Mix
- Cover with TopSeal-A film and incubate for 60 min at RT.
- Remove the TopSeal-A film and read signal with the EnVision® Multilabel Reader in TR-FRET mode (excitation at 320 or 340 nm & emission at 665 nm).

Experiment 1: Enzyme Titration and Time-Course

Enzymatic progress curves were performed by incubating LSD1 at concentrations ranging from 1 to 5 nM with 200 nM biotinylated Histone H3K4me1 peptide substrate. Reactions were stopped by the addition of tranylcypromine at indicated times. The Detection Mix was then added and signal was read after 60 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 200 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 200 nM biotinylated Histone H3K4me1 peptide substrate. Enzymatic reactions contain 1% DMSO.