TECHNICAL NOTE

AlphaLISA Tri-Methyl-Histone H3 Lysine 27 (H3K27me3) Cellular Detection Kit

AlphaLISA #16

AlphaLISA®

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This AlphaLISA® immunodetection assay monitors changes in the levels of tri-methylated histone H3 lysine 27 (H3K27me3) in cellular extracts.

- AL722C: 500 assay points
- AL722F: 5,000 assay points

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 an optimized assay for measuring the levels of H3K27me3 in the SU-DHL-6 and OCI-LY-19 lymphoma cells lines. Following a homogeneous histone extraction protocol, the mark of interest is detected by the addition of a biotinylated anti-Histone H3 (C-terminus) antibody and AlphaLISA Acceptor beads conjugated to an antibody (Ab) specific to the mark. The biotinylated antibody is then captured by Streptavidin (SA) Donor beads, bringing the two beads into proximity. Upon laser irradiation of the Donor beads 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.

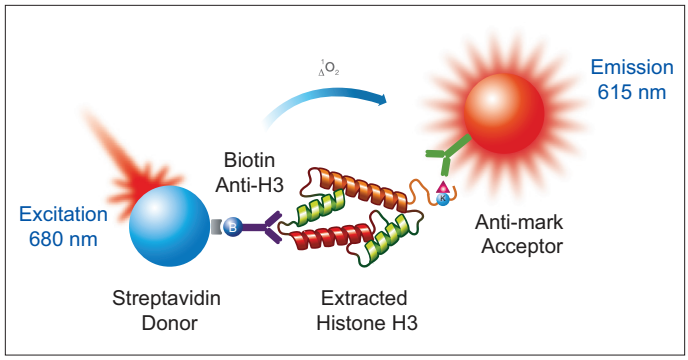

Figure 1. Schematic representation of the AlphaLISA cellular assay for the detection of modified histone proteins.

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Detection of Histone H3 Tri-methylated on Lysine 27 in Cellular Extracts:

Reagents needed for the assay:

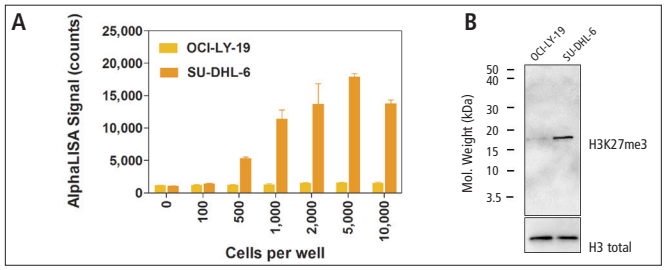
AlphaLISA Tri-Methyl-Histone H3 Lysine 27 (H3K37me3) Cellular Detection Kit	PerkinElmer # AL722
OCI-LY-19 cell line	DSMZ # ACC 528
SU-DHL-6 cell line	DSMZ # ACC 572
White opaque CulturPlate™-384	PerkinElmer # 6007680
TopSeal™-A film	PerkinElmer # 6050195
Western Lightning™ CDP-*Star*® with Nitro-Block II™ Enhancer	PerkinElmer # NEL616001KT
Anti-Rabbit IgG (Goat), Alkaline Phosphatase Conjugate	PerkinElmer # NEF814001EA

Culture medium: Alpha MEM (HyClone # SH30265.02) supplemented with 15% FBS.

Standard Protocol

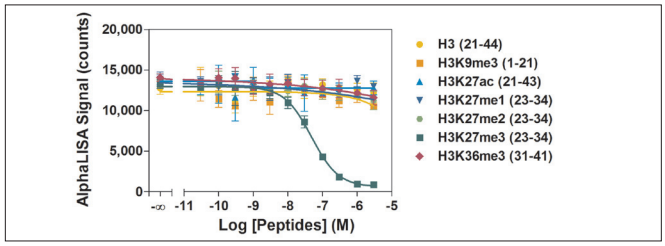
- Distribute 10 µL of cells in the wells of a CulturPlate-384 microtiter plate.
- Incubate adherent cells for 3-4 h at 37 °C in a 5% CO_2 atmosphere to allow cell adhesion. Skip this step for cells growing in suspension.
- For testing modulators, add 5 µL of culture medium or modulator prepared in medium at 3X its final concentration.
- Incubate for 16-21 h at 37 °C in a 5% CO_2 atmosphere.
- Add 5 µL of Cell-Histone™ Lysis buffer.
- Incubate 15 min at room temperature.
- Add 10 µL of Cell-Histone Extraction buffer.
- Incubate 10 min at room temperature.
- Dilute the 10X Cell-Histone Detection buffer to 1X with water.
- Prepare a 5X mix of Acceptor beads at 100 µg/mL and biotinylated anti-Histone H3 at 15 nM in 1X Cell-Histone Detection buffer.
- Add 10 µL of the 5X mix of Acceptor beads/biotin anti-H3 antibody (final concentration 20 µg/mL and 3 nM, respectively).
- Cover with TopSeal-A film and incubate for 60 min at 23 °C.
- Prepare in subdued light a 5X solution of SA Donor beads at 100 µg/mL in 1X Cell-Histone Detection buffer.
- Add 10 µL Donor beads (final concentration 20 µg/mL).
- Cover with TopSeal-A film and incubate for 30 min at 23 °C in the dark.
- Read signal in Alpha mode with the EnVision® or EnSpire® Multilabel plate reader.

Experiment 1: Detection of Histone Mark



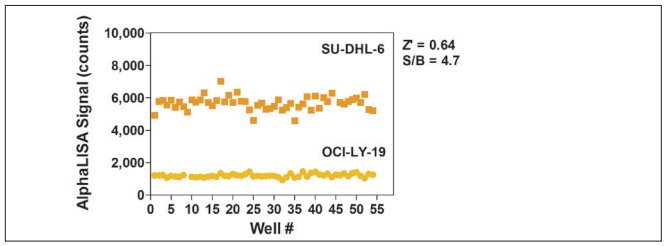
A) For the AlphaLISA detection of H3K27me3 mark, OCI-LY-19 (EZH2 WT homozygote) and SU-DHL-6 (EZH2 WT/Y641N heterozygote) cells were seeded at densities ranging from 100 to 10,000 cells per well in 384-well culture plates. (B) For Western Blot analysis of H3K27me3, 3 µg of cell lysate was separated by SDS-PAGE on a 10%-20% gradient gel. Following transfer to nitrocellulose, histone H3 proteins tri-methylated at lysine 27 were detected using the same antibody present on the Acceptor beads. For total histone H3, an antibody recognizing a histone H3 C-terminal epitope was used. Western blots were revealed using alkaline phosphatase-labeled anti-species secondary antibodies and Western Lightning™ CDP-*Star*® with Nitro-Block II™ Enhancer.

Experiment 2: Specificity of Cellular Detection



SU-DHL-6 cells were seeded at a density of 5,000 cells per well. Serial dilutions of histone H3-derived peptides bearing various epigenetic marks were added to the wells at concentrations ranging from 30 pM to 3 µM just before the addition of the AlphaLISA detection reagents. Additional peptides were tested in separate experiments (not shown). Only the H3K27me3 peptide competed with high affinity for the interaction between the Acceptor beads and histone proteins with an IC_{50} value of 47.8 nM.

Experiment 3: Z'-factor Determination



SU-DHL-6 and OCI-LY-19 cells were seeded at a density of 5,000 cell per well in medium containing 0.5% DMSO. The Z'-factor value compares the detection of the H3K27me3 mark in the two cell lines.

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010021_01 Printed in USA Dec. 2011