TECHNICAL NOTE

AlphaLISA #21

AlphaLISA Di/Mono-Methyl-Histone H3 Lysine 27 (H3K27me2-1) Cellular Detection Kit

AlphaLISA®

Authors

Nancy MacDonald
Jean-Francois Michaud
Jean-Philippe Levesque-Sergerie
Philippe Bourgeois
Lucille Beaudet
Nathalie Rouleau
Mathieu Arcand

PerkinElmer, Inc.
Montreal, Quebec, CAN

- AL721HV: 100 assay points (96-well format, half-area plates)
- AL721C: 500 assay points (384-well format)
- AL721F: 5,000 assay points (384-well format)

This AlphaLISA® immunodetection assay monitors changes in the levels of di-methylated histone H3 lysine 27 (H3K27me2-1) in cellular extracts.

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 changes in the levels of H3K27me2-1. 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.

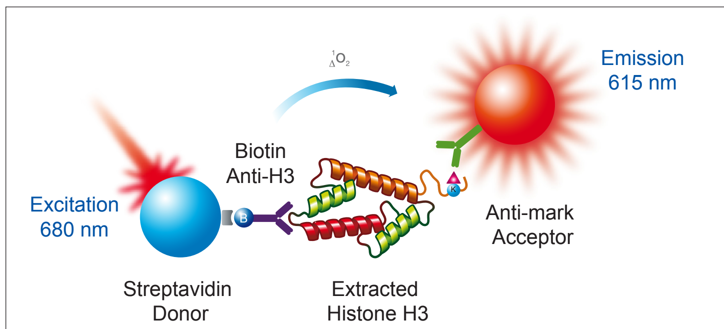

Figure 1. Schematic representation of the AlphaLISA cellular assay for the detection of modified histone proteins.

Detection of Histone H3 Di/Mono-Methylated on Lysine 27 in Cellular Extracts:

Reagents needed for the assay:

Reagent	Source
AlphaLISA Di-Methyl-Histone H3 Lysine 27 (H3K27me2-1) Cellular Detection Kit	PerkinElmer # AL721
AlphaLISA Unmodified Histone H3 Lysine 4 (H3K4) Cellular Detection Kit	PerkinElmer # AL719
HeLa cells	ATCC # CCL-2.2™
OCI-LY-19 cells	DSMZ # ACC 528
SU-DHL-6 cells	DSMZ # ACC 572
White opaque CulturPlate™-384	PerkinElmer # 6007680
TopSeal™-A film	PerkinElmer # 6050195
PolyScreen® PVDF Hybridization Transfer Membrane for mini-gels	PerkinElmer # NEF 1003001PK
Western Lightning™ ECL Pro	PerkinElmer # NEL 120001EA
Anti-Mouse IgG (Goat), HRP-Labeled	PerkinElmer # NEF 822001EA
Anti-Rabbit IgG (Goat), HRP-Labeled	PerkinElmer # NEF 812001EA

Cell-plating medium:
OCI-LY-19 and SU-DHL-6: Alpha MEM (HyClone # SH30265) supplemented with 15% FBS HeLa: MEM/EBSS (HyClone # SH30024) supplemented with 10% FBS.

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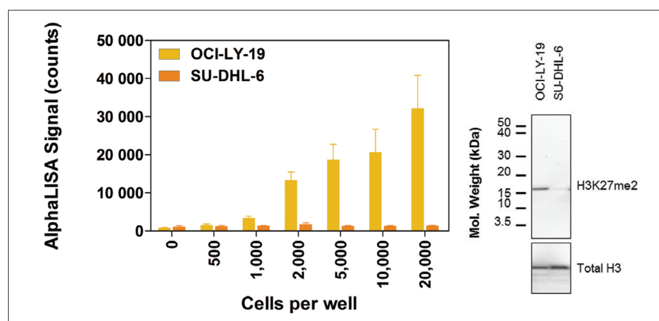
Standard Protocol

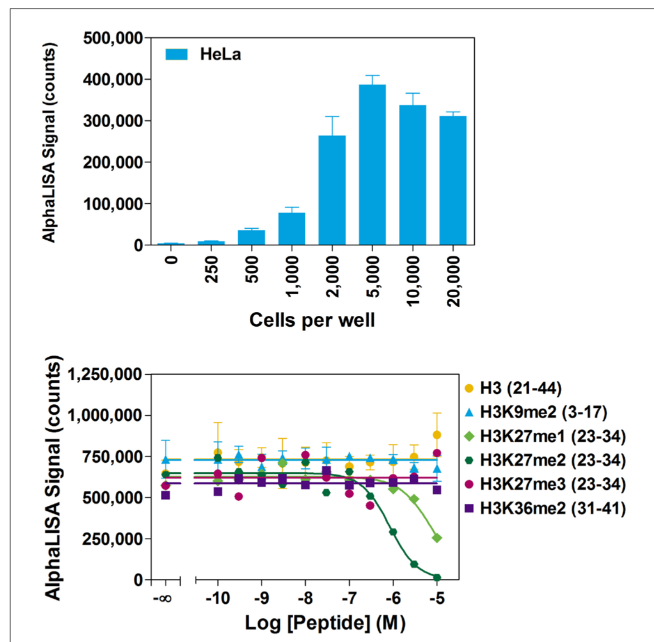
- Distribute 10 µL of cells in the wells of a CulturPlate-384 microtiter plate.
- Fill outer and unfilled wells with sterile water or PBS.
- Incubate adherent cells for 3-4 h at 37 °C in a 5% CO_2 atmosphere to allow cell adhesion. Skip this step for cells growing in suspension.
- Add 5 µL of culture medium or modulator prepared at 3X its final concentration in medium.
- Incubate cells for 16-72 h at 37 °C in a 5% CO_2 atmosphere. For longer incubation, a medium change is recommended.
- 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-histone 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. Plates can be read after overnight (O/N) incubation to maximize signal intensity.

Experiment 1: Detection of Histone Mark



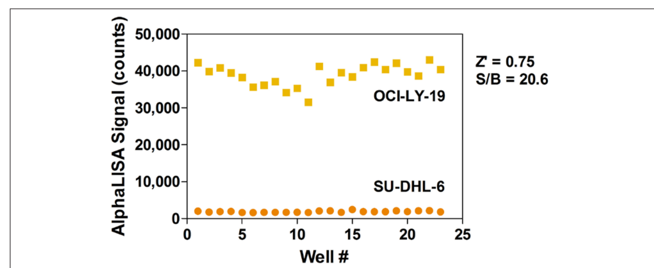
Detection of H3K27me2-1 levels in OCI-LY-19 and SU-DHL-6 cells. A) AlphaLISA detection of H3K27me2-1 in cells seeded at densities ranging from 500 to 20,000 cells per well in CulturPlate-384 microplate and incubated O/N. B) Western blot detection of H3K27me2-1 and total histone H3 levels. Cell lysates normalized to total histone H3 levels were separated by SDS-PAGE on a 10%-20% gradient gel. Following transfer to PolyScreen membrane, histone H3 proteins di/mono-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 HRP-labeled anti-species secondary antibody and Western Lightning ECL Pro substrate.

Experiment 2: Specificity of Cellular Detection



A) AlphaLISA detection of H3K27me2-1 in HeLa cells seeded at densities ranging from 250 to 20,000 cells per well in 384-well culture plates and incubated O/N. B) HeLa cells were seeded at a density of 2,000 cells/well. Serial dilutions of histone H3-derived peptides bearing various epigenetic marks were added to the wells at concentrations ranging from 100 pM to 10 µM immediately before the addition of the AlphaLISA detection reagents. Additional peptides tested but not shown include H3K4me2, H3K27ac, H3K36ac, H3K36me1, H3K36me3 and H3K79me2. The H3K27me2 peptide competed for the interaction between the Acceptor beads and histone H3 proteins with an IC_{50} value of 0.84 µM, while H3K27me1 displayed approximately 10-fold less potency.

Experiment 3: Z'-factor Determination



OCI-LY-19 (with WT EZH2 enzyme) and SU-DHL-6 (with EZH2 Y641N heterozygous mutant) cells were seeded at a density of 5,000 cells per well and incubated for 24 h. The Z'-factor value compares the detection of the H3K27me2-1 mark in the two cell lines.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

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010543_02 Printed in USA Oct 2012