TECHNICAL NOTE

AlphaLISA #14

AlphaLISA Di-Methyl-Histone H3 Lysine 4 (H3K4me2) Cellular Detection Kit

AlphaLISA®

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This AlphaLISA® immunodetection assay monitors changes in the levels of di-methylated histone H3 lysine 4 (H3K4me2) in cellular extracts.

- AL716C: 500 assay points
- AL716F: 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 changes in the levels of H3K4me2 after treatment of cells with sodium butyrate and Trichostatin A (TSA), two non-selective histone deacetylase (HDAC) inhibitors. 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.

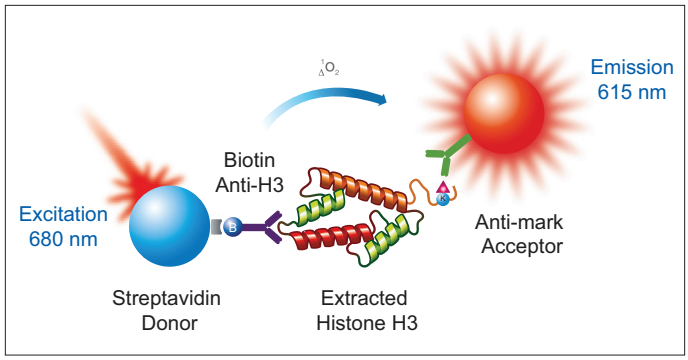

Figure 1. Schematic representation of the AlphaLISA cellular assay for the detection of modified histone proteins.

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Detection of Histone H3 Di-methylated on Lysine 4 in Cellular Extracts:

Reagents needed for the assay:

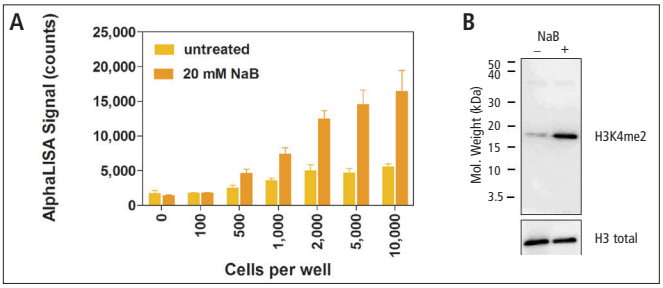
AlphaLISA Di-Methyl-Histone H3 Lysine 4 (H3K4me2) Cellular Detection Kit	PerkinElmer # AL716
HeLa cells	ATCC # CCL-2.2™
White opaque CulturPlate™-384	PerkinElmer # 6007680
TopSeal™-A film	PerkinElmer # 6050195
Trichostatin A (TSA)	Sigma T8552
Sodium butyrate (NaB)	Sigma B5887
Western Lightning™ CDP-*Star*® with Nitro-Block II™ Enhancer	PerkinElmer # NEL616001KT
Anti-Rabbit IgG (Goat), Alkaline Phosphatase Conjugate	PerkinElmer # NEF814001EA

Culture medium for HeLa cells: MEM/EBSS (HyClone # SH30024.02) supplemented with 10% FBS.

Standard Protocol

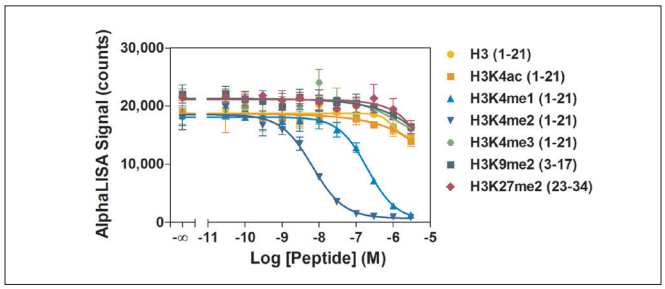
- Distribute 10 µL of cells in the wells of a CulturPlate-384 microtiter plate.
- Incubate adherent cells for 3-4 h at 37 °C in a 5% CO_2 atmosphere to allow cell adhesion. Skip this step for cells growing in suspension.
- Add 5 µL of culture medium or modulator prepared in medium at 3X its final concentration.
- 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



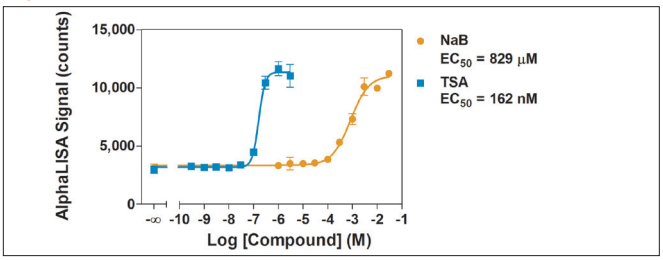
A) AlphaLISA detection of H3K4me2 modulation. HeLa cells were seeded at densities ranging from 100 to 10,000 cells per well in 384-well culture plates and treated overnight with 20 mM sodium butyrate (NaB). B) For Western Blot analysis of H3K4me2 mark modulation, 3 µg of cell lysate was separated by SDS-PAGE on a 10%-20% gradient gel. Following transfer to nitrocellulose, Histone H3 proteins methylated at lysine 4 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



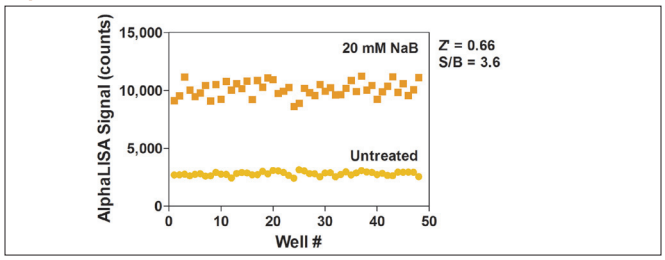
HeLa cells were seeded at a density of 5,000 cells/well and treated overnight with 20 mM NaB. Serial dilutions of histone H3-derived peptides bearing various epigenetic marks were added to the wells at concentrations ranging from 30 pM to 3 µM just before the addition of the AlphaLISA detection reagents. Additional peptides were tested in separate experiments (not shown). The H3K4me2 peptide competed with high affinity for the interaction between the Acceptor beads and histone proteins with an IC_{50} value of 6.6 nM while the H3K4me1 peptide showed 30-fold lower affinity with an IC_{50} value of 200 nM.

Experiment 3: Inhibition Curves



HeLa cells were seeded at a density of 5,000 cells per well and treated overnight with two non-selective HDAC inhibitors, TSA (from 300 pM to 3 µM) and NaB (from 3 µM to 30 mM), in medium containing 0.5% DMSO. TSA showed a 5,000-fold higher potency than NaB at increasing the general levels of H3K4me2 marks in HeLa cells.

Experiment 4: Z'-factor Determination



HeLa cells were seeded at a density of 5,000 cell per well and treated overnight with 20 mM NaB in medium containing 0.5% DMSO. The Z'-factor value compares NaB-treated and untreated cells.

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