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.](image)
Detection of Histone H3 Di-methylated on Lysine 4 in Cellular Extracts:

Reagents needed for the assay:

- AlphaLISA Detection of H3K4me2 (H3K4me2) Cellular Detection Kit
- HeLa cells
- White opaque CulturPlate™-384
- TopSeal™-A film
- Trichostatin A (TSA)
- Sodium butyrate (NaB)
- Western Lightning™ CDP-Star®
- Anti-Rabbit IgG (Goat), Alkaline Phosphatase Conjugate
- Histone H3 proteins

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

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 with 20 mM NaB in medium containing 0.5% DMSO. The Z’-factor value compares NaB-treated and untreated cells.

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 30 nM, in medium containing 0.5% DMSO. TSA showed a 5,000-fold higher potency than NaB at increasing the general levels 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 and Western Lightning™ CDP-Star® with Nitro-Block II™ Enhancer.

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.

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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