TECHNICAL DATA SHEET

AlphaLISA® Research Reagents

Human Total Histone2A Cellular Detection Kit

Product number: AL725 HV/C/F

Lot number: 2688548

Manufacturing date: March 3, 2020

Research Use Only. Not for use in diagnostic procedures.

Contents

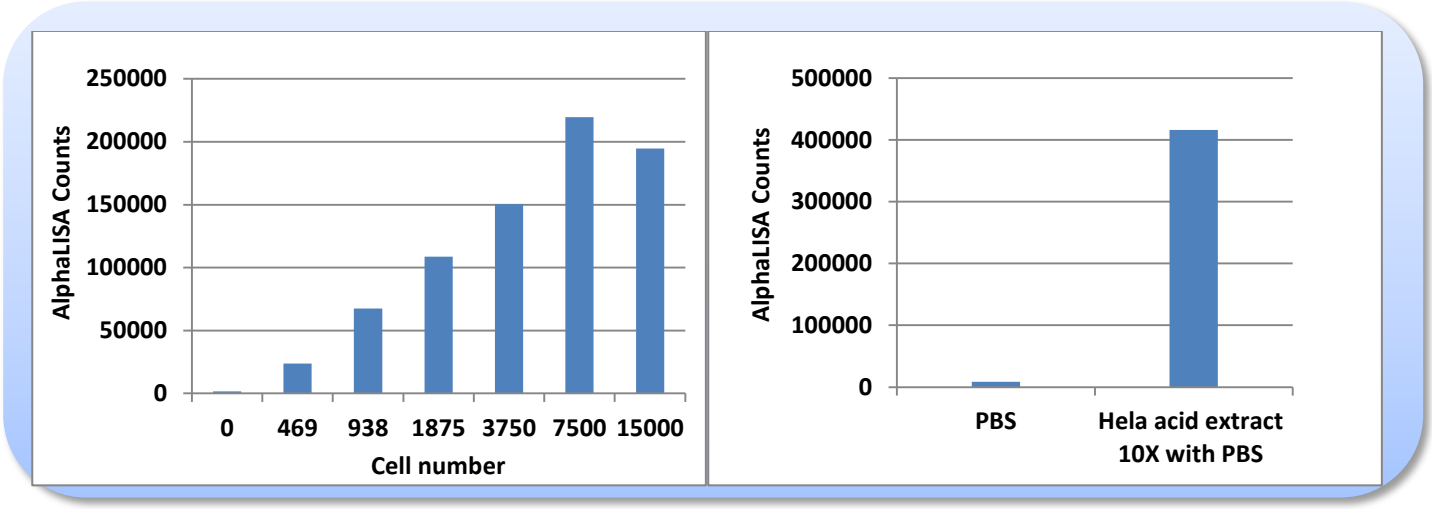
Product Information

Application: This kit is designed for the detection of total human H2A in cell extracts or cells in culture medium, using a homogeneous AlphaLISA assay (no wash steps).

Kit contents: The kit contains 6 components: AlphaLISA Acceptor beads coated with an anti-H2A (C-terminus) antibody, Streptavidin-coated Donor beads, Biotinylated anti-Histone 2A antibody (epitope near residue T77), Cell-Histone™ Lysis (1X), Extraction (1X) and Detection (10X) buffers.

Storage: The kit components must be stored dark at +4˚C.

Stability: This kit is stable for at least 6 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.



Hela cells	S/B
Hela acid extract	40 fold
Hela cell assay 7500 cells/well	100 fold

Figure. 1. Typical data generated in a cell-based assay (left) or with HeLa cell extract (right). Assays were performed using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader with Alpha option 2102.

Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum and minimum signals were measured on the EnVision Multilabel Plate Reader with Alpha option using the protocol described in this technical data sheet. We certify that these results meet our quality release criteria. Maximum counts may vary between bead lots and the instrument used, with no impact on ratio of signal to noise.

QC release specifications of the biotinylated antibody are based on spectrophotometric analysis of the labeled antibody.

Labeling Ratio: 12.2 biotin/Ab

Signal/background Ratio: ≥ 92 fold for HeLa acid extract assay

AlphaLISA Detection of Epigenetic Marks in Cellular Extracts and Cells

Histone H2A is one of the five main histone proteins involved in the structure of chromatin in eukaryotic cells. Histones H2A, H2B, H3 and H4 are known as the core histones, while histones H1 is known as the linker histone. Two of each of the core histones assemble to form one octameric nucleosome core and the linker histone H1 binds the nucleosome at the entry and exit sites of the DNA. The nucleosome core is formed of two H2A-H2B dimers and a H3-H4 tetramer. H2A is important for packaging DNA into chromatin. Since H2A packages DNA molecules into chromatin, the packaging process will affect gene expression. H2A plays a major role in determining the overall structure of chromatin and regulating gene expression. The AlphaLISA detection of epigenetic marks in cellular extracts is performed as follows: cells cultured in the presence of compounds are lysed with the Cell-Histone Lysis buffer. Histones are then extracted from the nucleosomes by the addition of the Cell-Histone Extraction buffer. The AlphaLISA anti-H2A (C-terminus) Acceptor beads and Biotinylated anti-Histone H2A (epitope near T77) antibodies are then added for the capture of histone proteins. After incubation, Streptavidin Donor beads are added for the capture of the biotinylated antibody. In the presence of histone proteins bearing the mark of interest, the beads come into proximity. Excitation of the Donor beads provokes the release of singlet oxygen molecules that trigger a cascade of energy transfer reactions in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm.

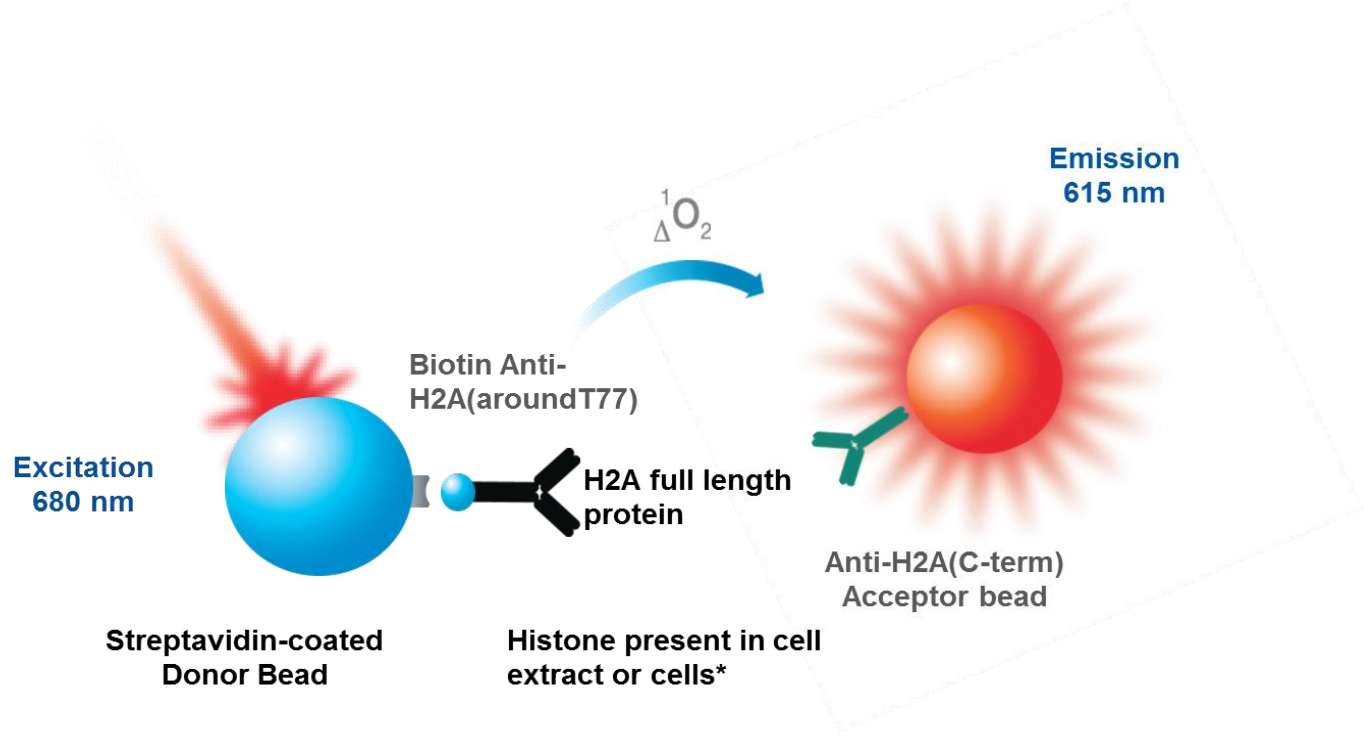


Figure 2. AlphaLISA Celluar Assay principle.* HeLa cells or HeLa cell extract (from Active Motif, cat. no. 36200) can be used as positive controls.

Precautions

- The AlphaScreen® Donor beads are light-sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
- All biological materials should be handled as potentially hazardous.
- The Biotinylated Anti-H2A Antibody contains sodium azide. Contact with skin or inhalation should be avoided. Sodium azide should not be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaLISA signal.

Kt Content: Reagents and Materials

Kit components	AL725 (100 assay points*)	AL725 (500 assay points*)	AL725 (5 000 assay points*)
AlphaLISA Histone H2A (C-term) Acceptor beads stored in PBS, 0.05% Kathon, pH 7.2	20 µL @ 5 mg/mL (1 brown tube, white cap)	100 µL @ 5 mg/mL (1 brown tube, white cap)	1 mL @ 5 mg/mL (1 brown tube, white cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon, pH 7.4	40 µL @ 5 mg/mL (1 brown tube, black cap)	200 µL @ 5 mg/mL (1 brown tube, black cap)	1 mL @ 5 mg/mL (1 brown tubes, black caps)
Biotinylated Antibody anti-Histone 2A (around T77) stored in PBS, 0.1% Tween-20, 0.05% NaN_3, pH 7.4	30 µL @ 500 nM (1 tube, black cap)	150 µL @ 500 nM (1 tube, black cap)	1.5 mL @ 500 nM (1 tube, black cap)
Cell-Histone Lysis buffer	1 mL	5 mL	30 mL
Cell-Histone Extraction buffer	2 mL	20 mL	100 mL
Cell-Histone Detection (10X) buffer	1.0 mL	2.0 mL	20 mL

* The number of assay points is based on an assay volume of 50 µL in 384-well assay plates using the kit components at the recommended concentrations.

Note: The Cell-Histone Detection (10X) buffer may appear slightly cloudy. However, this will not affect assay performance.

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaLISA signal. Note that sodium azide from the Biotinylated Antibody stock solution will not interfere with the AlphaLISA signal (0.0001% final in the assay).

Additional Reagents and Materials

The following items are recommended for the assays:

Item	Supplier	Catalog number
CulturPlate-384, white opaque with lid	PerkinElmer	6007680 (50/box) 6007688 (160/box)
Breathable Sealing Tape, Sterile	Corning Sigma	3345 CLS3345
TopSeal™-A Adhesive Sealing film	PerkinElmer	6050195
EnSpire® or EnVision® Multilabel Alpha Reader	PerkinElmer	Please consult our website

The following reagents might be required for particular applications:

Item	Supplier	Catalog number
AlphaScreen SureFire® GAPDH Assay Kit	PerkinElmer	**TGRGDS**
ATPlite® Luminescence Assay System	PerkinElmer	6016941 (1 000 assay points) 6016947 (5 000 assay points)
AlphaLISA TruHits kit	PerkinElmer	AL900D (1 000 assay points) AL900M (10 000 assay points)
OptiPlate-384, white opaque	PerkinElmer	6007290 (50/box) 6007299 (200/box)
CulturPlate-1536, white opaque with lid	PerkinElmer	6004680 (50/box) 6004688 (160/box)
ProxiPlate-384 TC	PerkinElmer	6008230 (50/box) 6008238 (160/box)
Halt Protease Inhibitor Cocktail (100X)	Thermo Scientific	87786 (1 mL)
HeLa acid extract as positive control	Active Motif	36200

Recommendations

General recommendations:

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to prewet the tip.
- Centrifuge quickly all tubes before use to improve recovery of content (2 000 ×*g*, 10-15 sec). Resuspend all reagents

by gentle mixing before use.

- Use Milli-Q® grade H_2O (18 MΩ•cm) to dilute Cell-Histone Detection (10X) buffer.

- When reagents are added in the microplate, make sure the liquids are at the bottom of the well by tapping or swirling the plate gently on a smooth surface.

- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Sealing Film to reduce evaporation during incubation with the Alpha beads. Microplates can be read with the TopSeal-A Film.

- The AlphaLISA signal is detected with an EnVision Multilabel Reader equipped with the ALPHA option using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).

- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation time and temperature should be used for each plate.

Specific recommendations

- Biotin present in culture medium may interfere with the binding of the biotinylated antibody to Streptavidin Donor beads. Use biotin-free culture medium when following the Universal Protocol. Most cells will not be affected by overnight incubation in biotin-free medium. If longer incubation time is required, reduce biotin in culture medium combining biotin-containing and biotin-free medium to find a condition not affecting cell growth or the Alpha signal.
- Evaporation can be problematic with cells cultured in microtiter plates. For overnight incubation, it is recommended to add warm PBS or sterile water to unused wells. For longer incubation periods, a sterile breathable sealing membrane can be further added to the plate, together with the lid.
  Alternatively, cells can be cultured in larger wells, or in a larger volume of culture medium. The following modifications to the Universal Protocol will then apply:

    1. Following compound treatment, transfer the desired number of cells resuspended in 15 µL PBS to an OptiPlate-384 microtiter plate. Continue with the Lysis step of the Universal Protocol.
    2. Lyse adherent cells directly in the culture plate by upscaling the volume of Cell-Histone Lysis buffer to the volume of medium or PBS in the well. Transfer 20 µL of cell lysate to an OptiPlate-384 microtiter plate. Add the Cell-Histone Extraction buffer as indicated in the Universal Protocol. See also the Wash Protocol section for adherent cells.

- The Cell-Histone Lysis buffer does not include protease inhibitors. Protease inhibitors can be added to the lysis buffer if cellular lysates are not to be used immediately.
- Effects on the Alpha signal of fetal bovine serum (FBS) and phenol red present in the culture medium are minimal. However, medium without phenol red or with reduced FBS concentration can be used to maximize signal and increase assay window with the Universal Protocol.
- Assay specificity can be demonstrated by competing the binding of Acceptor beads to modified histones using peptides carrying marks of interest. See the Control Assays section for details.

Universal Protocol for Adherent and Suspension Cells (No Wash)

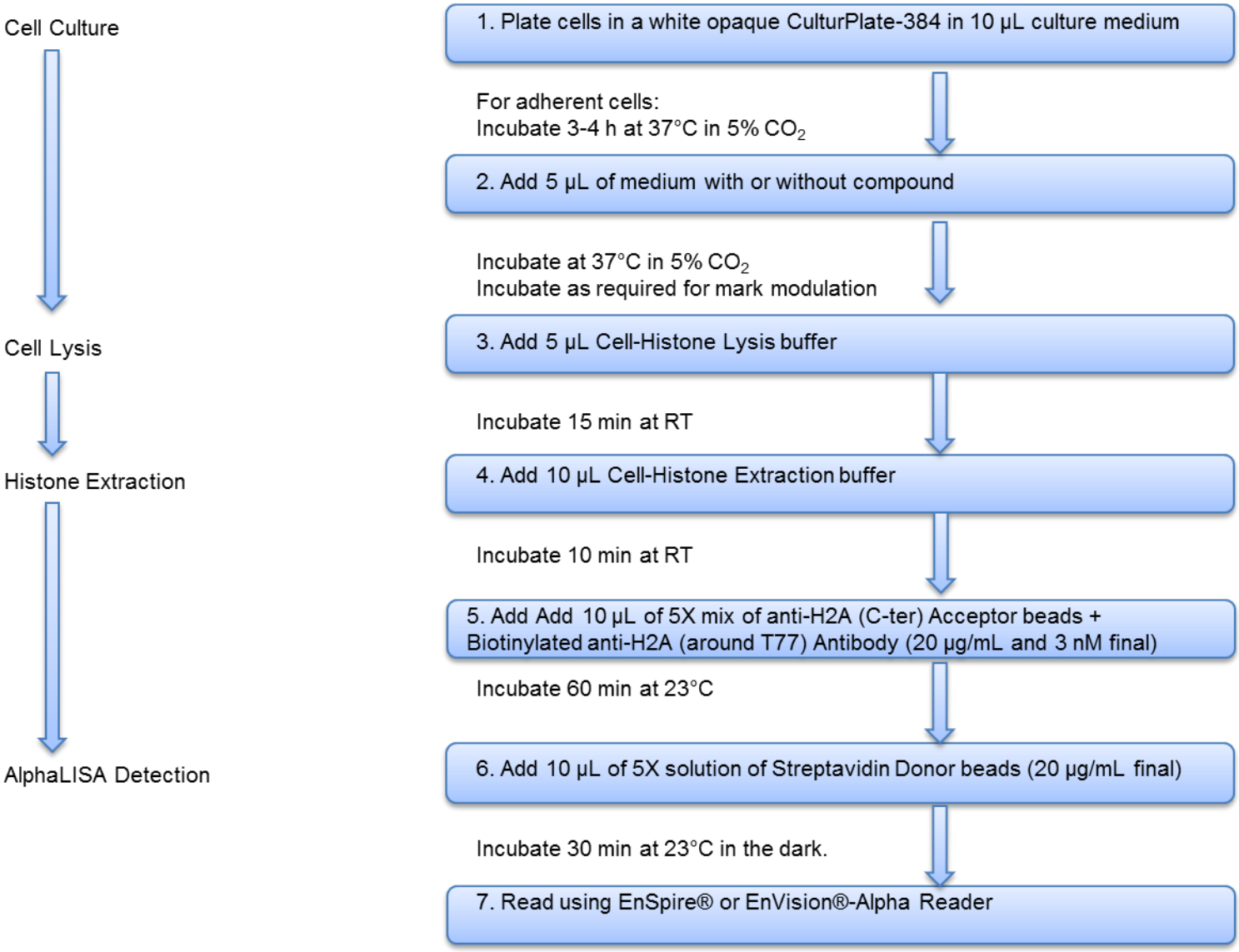
Cell Culture, Lysis and Histone Extraction

- Dispense 10 µL cells per well in culture medium in white opaque CulturPlate-384 microplate.
- Incubate adherent cells for 3-4 h at 37ºC in a 5% CO_2 atmosphere to allow cell adhesion or seed the previous day in incubate overnight. Skip this step for cells growing in suspension.
- Add 5 µL of medium or compound prepared in medium at 3X its final concentration.
- Incubate cells for the time required for histone mark modulation. For incubations longer than overnight, seal plate with sterile breathable membrane and add back the plastic lid.
- Add 5 µL of Cell-Histone Lysis buffer.
- Incubate for 15 min at room temperature (RT).
- Add 10 µL of Cell-Histone Extraction solution.
- Incubate for 10 min at RT.

AlphaLISA Detection

- Dilute the 10X Cell-Histone Detection buffer to 1X with water.
- Prepare just before use, a 5X mix of AlphaLISA anti-H2A (C-ter) Acceptor beads + Biotinylated anti-H2A antibody in 1X Cell-Histone Detection buffer by diluting the anti-H2A Acceptor beads to 100 µg/mL and the biotinylated antibody to 15 nM. Final concentration of beads and antibody are 20 µg/mL and 3 nM, respectively.
    - For example, prepare a 5X mix by adding 20 µL Acceptor beads and 30 µL biotinylated antibody to 950 µL of 1X Cell-Histone Detection buffer. Adjust volumes according to the number of assay points.
- Add 10 µL of the 5X mix containing AlphaLISA anti-H2A Acceptor beads + Biotinylated anti-H2A antibody.
- Cover with TopSeal-A film and incubate 60 min at 23°C.
- Working under subdued light, prepare a 5X solution of Streptavidin Donor beads in 1X Cell-Histone Detection buffer by diluting the beads to 100 µg/mL. Final concentration for Donor beads is 20 µg/mL.
    - For example, add 20 µL Donor beads (5mg/ml) to 980 µL of 1X Cell-Histone Detection buffer. Store dilution in the dark until use. Adjust volumes according to the number of assay points.
- Under subdued light, add 10 µL of the 5X solution of Streptavidin Donor beads.
- Cover with TopSeal-A film and incubate 30 min at 23°C in the dark.
- Read on an Envision® Multilabel or EnSpire Alpha Reader.

Assay Diagram: Universal Protocol (No Wash)



Wash Protocol for Adherent Cells

Removal of the original culture medium is recommended when cells are grown in medium containing biotin, high FBS or DMSO concentrations, or when compounds might interfere with the Alpha signal detection. Optionally, medium removal can be followed with a gentle wash of the cell layer with PBS.

Cell Culture, Lysis and Histone Extraction

- Dispense cells in culture medium in a white opaque CulturPlate-384 microplate.
- Incubate cells for 3-4 h at 37ºC in a 5% CO_2 atmosphere to allow cell adhesion.
- Add medium, or compound prepared in culture medium at the desired concentration.
- Incubate for the time required for histone mark modulation. For incubations longer than overnight, seal plate with a sterile breathable membrane and add back the plastic lid.
- Dilute the Cell-Histone Lysis buffer 4-fold in H_2O.
    - For example, add 500 µL of Cell-Histone Lysis buffer to 1.5 mL of H_2O.
- Remove culture medium. Optionally, wash gently the cell layer with PBS, and then remove PBS from the wells.
- Add 20 µL of the 4-fold diluted Cell-Histone Lysis buffer.
- Incubate for 15 min at room temperature (RT).
- Add 10 µL of Cell-Histone Extraction buffer.
- Incubate for 10 min at RT.
- Then follow the Universal Protocol for Adherent and Suspension Cells (No Wash).

Control Assays

Histone Protein Competitions

Specificity of the Alpha signal can be confirmed by performing protein or peptide competition experiments, adding the protein or peptide of interest to cellular extracts before the addition of Acceptor beads and biotinylated antibody.

You will need to modify the Universal Protocol as follows:

- Prepare 10X protein solutions by diluting protein serially in 1X Cell-Histone Detection buffer from 35 µM to 49 nM (final concentrations 3.5 µM to 4.9 nM).
- Prepare just before use, a 10X mix of anti-H2A (C-term) Acceptor beads + Biotinylated anti-H2A antibody in 1X Cell-Histone Detection buffer by diluting the anti-H2A Acceptor beads to 200 µg/mL and the biotinylated antibody to 30 nM. Final concentration of beads and antibody are 20 µg/mL and 3 nM, respectively.
    - For example, prepare a 10X mix by adding 40 µL Acceptor beads and 60 µL biotinylated antibody to 900 µL of 1X Cell-Histone Detection buffer. Adjust volumes according to the number of assay points.
- Follow Universal Protocol and stop at the end of the Extraction steps.
- Add 5 µL of 10X protein dilutions to the wells
- Add 5 µL of 10X mix containing AlphaLISA anti-H2A Acceptor beads+ Biotinylated anti-Histone H2Aantibody.
- Cover with TopSeal-A film and incubate 60 min at 23°C.
- Continue with the preparation and addition of the Donor beads as described in the Universal Protocol.

Tube	Volume of protein	Volume of 1X Cell-Histone Detection buffer (µL)	[protein] (M) in the buffer
A	100 µL of 70 µM stock	100	3.5E-05
B	100 µL of tube A	200	1.2E-05
C	100 µL of tube B	200	3.9E-06
D	100 µL of tube C	200	1.3E-06
E	100 µL of tube D	200	4.4E-07
F	100 µL of tube E	200	1.5E-07
G	100 µL of tube F	200	4.9E-08
I	0	200	0

Histone Protein Competition Data

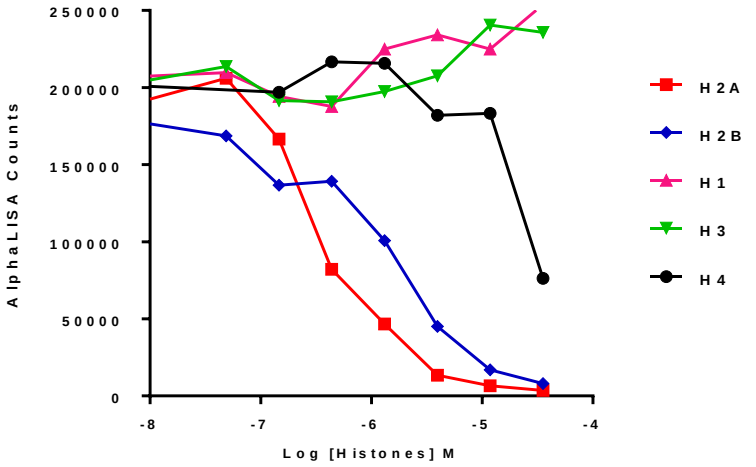


Figure 3. HeLa cells were seeded at a density of 7500 cells per well. Serial dilutions of histone H2A and other histones H1, H2B, H3 and H4 were added to the wells at concentrations ranging from 49 nM to 35 µM just before the addition of the AlphaLISA detection reagents. The H2A protein competed with high affinity for the interaction between the Acceptor beads and histone proteins and H2B competed similarly due to the complex formation of H2A blocking the anti-H2A antibody binding to H2A. Histones H1, H3 and H4 don't compete with the reaction.

Assay Normalization

Results can be normalized using one of the following methods, seeding cells in separate wells:

- ATPlite Luminescence Assay System (PerkinElmer)
- AlphaScreen SureFire GAPDH Assay Kit (PerkinElmer)
- Evaluation of cell density by Imaging Microscopy

Compound Interference

When using the no wash Universal Protocol, compounds remain present for the AlphaLISA detection step. In some cases, these could interfere with the Alpha signal.

Compound Interference can be detected using different methods:

1. The AlphaScreen TruHits kit will allow you to determine if and how your compound interferes with the generation of the Alpha assay signal. Follow the instructions provided with the kit.
2. You may also include control wells in your culture plate where cells are plated without compound in a volume of 10 µL of culture medium.
   - Grow cells as you normally would.
   - Proceed with cell Lysis and histone Extraction as described in the Universal Protocol.
   - Following the Extraction step, add compound prepared in 5 µL culture medium.
   - Proceed with the Alpha Detection as indicated in the Universal Protocol.
   - Compare signal of the control wells with that of untreated wells.

If compound interference is detected, decrease compound concentration to reduce interference, or use the Wash Protocol.

Troubleshooting Guide

You will find below recommendations for common situations that you might encounter with your AlphaLISA Epigenetics Cellular detection assay. If further assistance is needed, do not hesitate to contact our technical support team for assistance..

Issue	Recommendations and Comments
No signal increase in cell titration experiment	• Endogenous mark levels might be too low for detection in your cell line. Try increasing mark level by compound treatment (e.g. 20 mM sodium butyrate overnight for acetylated marks and H3K4me2). • It is recommended to include in your experiment a control for the assay. • Try one of the cell lines and assay conditions shown in the Typical Data section. • Universal Protocol: use biotin-free culture medium, or culture medium with reduced biotin concentration.
No mark modulation upon compound treatment	• Verify by Western blot that the mark is actually modulated by compound. • Treat cells for different time periods and at different compound concentrations. • Try a different cell line or different compounds • Verify the Alpha signal specificity by peptide titration (see Control Assays section).
Modulation of mark in Alpha does not correlate with Western blot data	• Specificity of Western blot antibody could be different from that of Acceptor beads. • Verify the Alpha signal specificity by peptide titration (see Control Assays section).
Signal lower in the Universal Protocol than in the Wash Protocol	• Use biotin-free culture medium. • Use culture medium without phenol red and/or with lower FBS concentration. • Make sure treating compound is not interfering with the Alpha signal.(see Control Assays section)
High variation between replicates or low Z' values	• Universal Protocol: make sure that reagents are at the bottom of the well by tapping or swirling the plate gently on a smooth surface after each addition. Be particularly careful when adding the 5 µL Cell-Histone Lysis buffer to the wells. • Rotate the plate to 120 rpm after Cell-Histone Lysis buffer addition to ensure efficient mixing of assay components. • Wash Protocol: use if possible an automated plate washer, or remove medium carefully without disrupting the cell layer. Culture poorly adhering cells (e.g. HEK-293) on poly-lysine coated plates. • All protocols: lengthen incubation time with the Alpha Donor beads up to 18 hours before measuring the Alpha signal.

Insufficient assay window	• Universal Protocol: use medium without phenol red or with lower • FBS concentration to maximize signal. • All protocols: lengthen incubation time with the Alpha Donor beads up to 18 h with before measuring the Alpha signal.

You will find detailed recommendations for common situations you might encounter with your AlphaLISA Assay kit at:

http://www.perkinelmer.com/in/resources/technicalresources/applicationsupportknowledgebase/alphalisa-alphascreen-no-washassays/alpha_troubleshoot.xhtml

This product is not for resale or distribution except by authorized distributors.

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