ELISA Kit

Catalog # KHO0831 (96 tests)

Human
Histone H3 [K18Ac]

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Contents and Storage

Storage Store at 2 to 8°C.

Contents

Reagents Provided	*96 Test Kit*
Histone H3 [K18 Ac] Standard: Refer to vial label for quantity and reconstitution volume.	2 vials
Standard Diluent Buffer.* Contains 15 mM sodium azide; red dye**; 25 mL per bottle.	1 bottle
*Histone H3 Antibody-Coated Well*s, 96 wells per plate	1 plate
Rabbit Anti-Histone H3 (Detection Antibody). Contains 15 mM sodium azide; blue dye**; 6 mL per bottle.	1 bottle
Anti-rabbit IgG-Horseradish Peroxidase (HRP) Concentrate, (100x). Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial
HRP Diluent. Contains 3.3 mM thymol; yellow dye**; 25 mL per bottle.	1 bottle
Wash Buffer Concentrate (25x); 100 mL per bottle.	1 bottle
Stabilized Chromogen, Tetramethylbenzidine (TMB); 25 mL per bottle.	1 bottle
Stop Solution; 25 mL per bottle.	1 bottle
Plate Covers, adhesive strips.	3

* If precipitates are found in standard diluent buffer, they should be completely dissolved by warming to room temperature before use.

** In order to help our customers avoid any mistakes in pipetting the ELISAs, we provide colored *Standard Diluent Buffer*, *Detection Antibody*, and *HRP Diluent* to help monitor the addition of solution to the reaction well. This does not in any way interfere with the test results.

Disposal Note: This kit contains materials with small quantities of sodium azide and Proclin® 300. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Proclin® 300 is toxic. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Safety All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Introduction

Intended Use

The Invitrogen Histone H3 [K18Ac] ELISA is designed to detect and quantify the levels of Histone H3 protein acetylated at lysine residue 18. This assay is intended to detect Histone H3 [K18Ac] from human, mouse and rat acid extracted histones. Invitrogen also offers a Histone H3 (Total) ELISA kit (Cat. # KHO0661), which quantifies histone H3 independently of the acetylation status and allows normalization of acetylated histone H3 to total histone H3. Please refer to enclosed protocol for modifications to the Histone H3 (Total) ELISA Kit when using acid extracted histones.

Principle of the Method

The Invitrogen Histone H3 [K18Ac] kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for histone H3 (regardless of acetylation state) has been coated onto the wells of the microtiter strips provided. Samples, including a standard containing histone H3 [K18Ac], control specimens, and unknowns, are pipetted into these wells. During the first incubation, the histone H3 antigen binds to the immobilized (capture) antibody. After washing, a rabbit antibody specific for histone H3 acetylated at lysine 18 is added to the wells. During the second incubation, this antibody serves as a detection antibody by binding to the immobilized histone H3 [K18Ac] protein captured during the first incubation. After removal of excess detection antibody, a horseradish peroxidase-labeled anti-rabbit IgG (anti-rabbit IgG-HRP) is added. This binds to the detection antibody to complete the four-member sandwich. After a third incubation and washing to remove all the excess anti-rabbit IgG-HRP, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of histone H3 [K18Ac] present in the original specimen.

Background Information

Histone H3 protein (H3, MW: 17 kDa), one of the four DNA-bound core histones, is a highly conserved protein which consists of 135 amino acid residues. Cellular genomic DNA is protectively organized as nucleoprotein complexes, known as chromatins. The basic unit of chromatin is the nucleosome, which consists of 146 base pairs of DNA wrapped around an octamer of core histones, including two molecules of H2A, H2B, H3 and H4. Each core histone is composed of a structured domain and an unstructured amino-terminal ‘tail’ of 25-40 residues. Histone tails foster sites for a variety of post-translational modifications, such as acetylation, phosphorylation, methylation, ubiquitination and ADP-ribosylation.

The structure of chromatin dynamically changes, permitting localized decondensation and remodeling that facilitates diverse nuclear processes, such as replication, transcription or DNA repair. An emerging theme in the field of chromatin research has been the significant role that post-translational modifications of histones play in regulating nuclear function. It is becoming increasingly clear that combinations of post-translational modifications of histone tails create specific signals that define the ‘histone code’. The histone code hypothesis postulates that specific protein factors can act on chromatin by recognizing and binding particular histone modifications, which in turn induce localized alterations of chromatin function.

Histone acetyltransferases (HATs) target and modify lysine residues on the core histones. Mammalian cells have over a dozen putative and known HATs that possess different histone specificity. GCN5, the first purified HAT, targets K18, K23 and K27 residues of H3, and K8 of histone H4. CBP/p300 also targets all

core histones, but prefers specific sites on H2A, H2B and H3. Acetylation is generally associated with overall gene activity; however, recent data suggests that post-translational modifications provide binding sites for interacting proteins, similar to the role of phosphorylation in signal transduction. Although more studies need to be done to elucidate the significance of individual histone sites, it is becoming more clear that each site contributes a non-redundant function in gene regulation and nuclear pathways within the cell. For example, histone H3 K18 acetylation (K18Ac) has been shown to be correlated to gene expression globally throughout the genome, as well as locally at individual promoters. Among human diseases, K18 acetylation and K4 dimethylation are prognostic markers for determining clinical outcomes of prostate, lung, kidney, and breast cancers. Similarily, it was found that levels of histone H4 K16Ac and K20 dimethylation is a hallmark of human leukemias and colorectal adenocarcinomas. Therefore, several epigenetic markers are sufficient to characterize certain types of cancers, and potentially even offer prognostic information. Individual sites are important for the recruitment of DNA binding or chromatin binding proteins, such as activators and chromatin remodeling complexes.

It has been observed that acetylation at K18 and K23 tethers recombinant CARM1 to the H3 tail and allows CARM1 to act as a more efficient arginine methyltransferase.

Methods

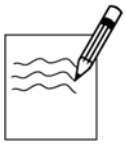
Materials Needed But Not Provided

- Microtiter plate reader (at or near 450 nm) with software
- Calibrated adjustable precision pipettes
- Distilled or deionized water
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Glass or plastic tubes for diluting solutions
- Absorbent paper towels
- Calibrated beakers and graduated cylinders

Procedural Notes

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. Samples above the highest standard should be diluted with *Standard Diluent Buffer*.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. **Do not mix or interchange different reagent lots from various kit lots**.
10. Do not use reagents after the kit expiration date.
11. Absorbances should be read immediately, but can be read up to 2 hours of assay completion. For best results, keep plate covered in the dark.
12. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
13. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
14. Because Stabilized *Chromogen* is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop.

Directions for Washing



- **Incomplete washing will adversely affect the test outcome**. All washing must be performed with Wash Buffer provided.
- Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- If using an automated washer, follow the operating washing instructions carefully.

Procedure For Extraction Of Proteins From Cells Or Tissues

Recommended Formulation of Cell Lysis Buffer:

- 50 mM Tris, pH 7.4
- 250 mM NaCl
- 5 mM EDTA
- 50 mM NaF
- 1 mM Na_3VO_4
- 1% Nonidet P40 (NP40)

This NP40 Cell Lysis Buffer (Invitrogen, Cat. no. FNN0021) needs the following items to be added:

- **1 mM PMSF (stock is 0.3 M in DMSO)**
- **Protease** inhibitor cocktail (e.g., Sigma Cat. # P-2714) (reconstituted according to manufacturer's guideline). Add 500 µL per 5 mL Cell Extraction Buffer.

The Cell Lysis Buffer is stable for 2 to 3 weeks at 4°C or for up to 6 months when aliquoted (without protease inhibitors and PMSF added) and stored at -20°C. When stored frozen, the buffer should be thawed on ice. **Important:** add the protease inhibitors just before using. The stability of protease inhibitor supplemented Cell Lysis Buffer is 24 hours at 4°C. PMSF is very unstable and must be added prior to use, even if added previously.

Recommended Formulation of Acid Extraction Buffer:

- 120 mL H_2S0_4 (concentrated), 0.22 M final concentration
- 20% Glycerol
- 7 µL ß-mercaptoethanol
- 7.87 mL dH_2O

The following protocol has been applied to several cell lines using this Cell Extraction Buffer. Researchers may optimize the cell extraction procedures that work best in their hands.

1. For attached cultures, add approximately 0.75 mL of NP40 Cell Lysis Buffer for every 10^7 cells. Add buffer directly to the monolayer and scrape. For suspension cultures (non-adherent), centrifuge aliquots containing approximately 10^7 cells in 15 to 250 mL tubes and collect cell pellet at 100 x g. Discard supernatants and resuspend the pellet in 0.75 mL of lysis buffer. Transfer the suspension to 2 mL tubes.
2. Centrifuge cells at 16,000 x g for 3 minutes. Carefully aspirate the supernatants.
3. For pellets <5 mL, add 25 mL of the acid extraction solution to the tubes and loosen the pellet. For larger pellets, add 5 volumes of the acid extraction solution. Incubate samples at 4ºC for one hour to overnight.
4. Centrifuge at 16,000 x g for 10 minutes at 4ºC. Discard the pellet and keep the histone containing supernatant.
5. Add 0.5 volume of 1 M NaOH to neutralize the acid solution.
6. These samples are ready for assay. Lysates can be stored at -80ºC.

Preparation of Reagents

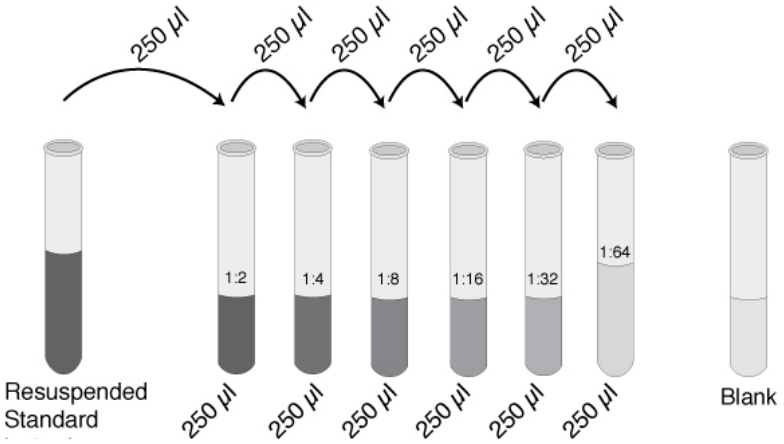
Dilution of Standard

Note: The histone H3 [K18Ac] standard was prepared from K18 acetylated histone H3 recombinant protein. One unit of standard is equivalent to 0.1 ng of acetylated histone H3.

Important

1. Reconstitute *histone H3 [K18Ac] Standard* with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Label as 100 Units/mL histone H3 [K18Ac]. Use the standard within 1 hour of reconstitution.
2. Add 0.25 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 50, 25, 12.5, 6.25, 3.12 and 1.6 Units/mL histone H3 [K18Ac].
3. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

Remaining reconstituted standard should be discarded or frozen in aliquots at -80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Preparing IgG-HRP

Please Note: The *Anti-rabbit IgG-HRP (100x concentrate)* is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow *Anti-rabbit IgG-HRP (100x concentrate)* to reach room temperature. Gently mix. Pipette *Anti-rabbit IgG-HRP (100x concentrate)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µL of this 100x concentrated solution with 1 mL of *HRP Diluent* for each 8-well strip used in the assay. Label as Anti-rabbit IgG-HRP Working Solution.
2. Return the unused *Anti-rabbit IgG-HRP (100x concentrate)* to the refrigerator.

Note

Note: Prepare within 15 minutes of usage, as activity decreases.

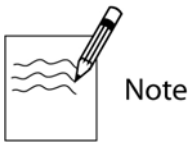
# of 8-Well Strips	Volume of Anti-rabbit IgG-HRP Concentrate	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

Dilution of Wash Buffer

1. Allow the 25x concentrate to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the 25x wash buffer concentrate with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

2. Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

Assay Procedure



Be sure to read the *Procedural Notes* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 100 µL of the *Standard Diluent Buffer* to zero standard wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 100 µL of standards and diluted samples or controls to the appropriate microtiter wells. Standards, samples, and controls will have a red color. Samples prepared in Cell Lysis Buffer must be diluted 1:5 or greater in *Standard Diluent Buffer* (for example, 10 µL sample into 40 µL buffer). While a 1:5 sample dilution has been found to be satisfactory, higher dilutions such as 1:25 or 1:50 may be optimal. The dilution chosen should be optimized for each experimental system. Tap gently on side of plate to mix.
4. **Cover wells with *plate cover* and incubate for 2 hours at room temperature.**
5. Pipette 100 µL *anti-Histone H3 [K18Ac]* (Detection Antibody) solution into each well except the chromogen blank(s). Tap gently on the side of the plate to thoroughly mix.
6. **Cover wells with *plate cover* and incubate for 1 hours at room temperature.**
7. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
8. Add 100 µL *anti-rabbit IgG-HRP* Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **Preparing IgG-HRP)**.
9. Cover wells with the *plate cover* and incubate for **30 minutes at room temperature**.
10. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
11. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
12. Incubate for **30 minutes at room temperature and in the dark. *Please Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader

that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.

13. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
14. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
15. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
16. Read the concentrations for unknown samples and controls from the standard curve. (Samples producing signals greater than that of the highest standard should be diluted in *Standard Diluent Buffer* and reanalyzed. Multiply value(s) obtained for sample(s) by the appropriate dilution factor to correct for the dilution in step 3.

Typical Data (Example)

The following data were obtained for the various standards over the range of 0 to 100 Units/mL histone H3 [K18Ac].

Standard Histone H3 [K18Ac] (Units/mL)	Optical Density (450 nm)
100	3.043
50	1.961
25	1.182
12.5	0.706
6.25	0.399
3.13	0.271
1.57	0.190
0	0.092

Performance Characteristics

Sensitivity

The analytical sensitivity of this assay is <0.8 Unit/mL of histone H3 [K18Ac]. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

The sensitivity of this ELISA was compared to Western blotting using known quantities of histone H3 [K18Ac]. The data presented below show that the sensitivity of the ELISA is approximately 2x greater than that of Western blotting. The bands shown in the Western blotting data were developed using rabbit anti-Histone H3 [K18Ac], and an alkaline phosphatase conjugated anti-rabbit IgG followed by chemiluminescent substrate and autoradiography.

Detection of Histone H3 [K18Ac] by ELISA vs Western Blot:

HeLa cell acid extracted histones:

Western Blot (17 KDa)								
ELISA: O.D. 450 nm	3.07	1.69	0.527	0.222	0.16	0.14	0.12	0.08
TSA Treated HeLa histones	2.5	1.25	0.62	0.3	0.15	0.07	0.3	0

Precision

1. Intra-Assay Precision

Samples of known histone H3 [K18Ac] concentration were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	44.66	10.79	5.14
SD	2.11	0.49	0.36
%CV	4.72	4.54	7.00
SD = Standard Deviation CV = Coefficient of Variation			

2. Inter-Assay Precision

Samples were assayed 48 times in multiple assays to determine precision between assays

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	45.53	10.91	4.83
SD	2.41	0.46	0.37
%CV	5.29	4.22	7.66
SD = Standard Deviation CV = Coefficient of Variation			

Recovery

To evaluate recovery, histone H3 [K18Ac] Standard was spiked at 3 different concentrations into 10% Cell Extraction Buffer. The average recovery was 107%.

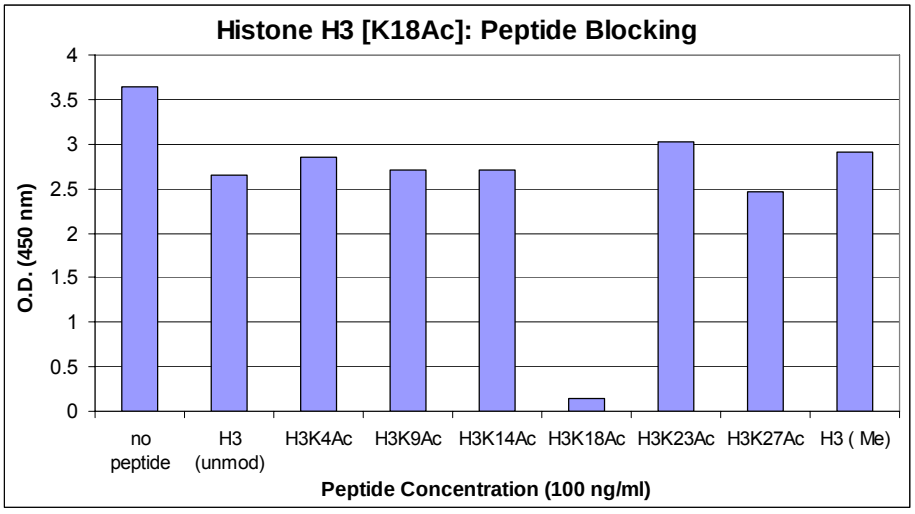
Linearity of Dilution

HeLa cells were treated with 3 µM Trichostatin A (TSA) for 4 hours and lysed with Cell Extraction Buffer and nucleus histone was extracted with Acid Extraction Buffer. This nuclear histone extract was diluted in *Standard Diluent Buffer* over the range of the assay and measured for histone H3 [K18Ac]. Linear regression analysis of sample values versus the expected concentrations yielded a correlation coefficient of 0.99.

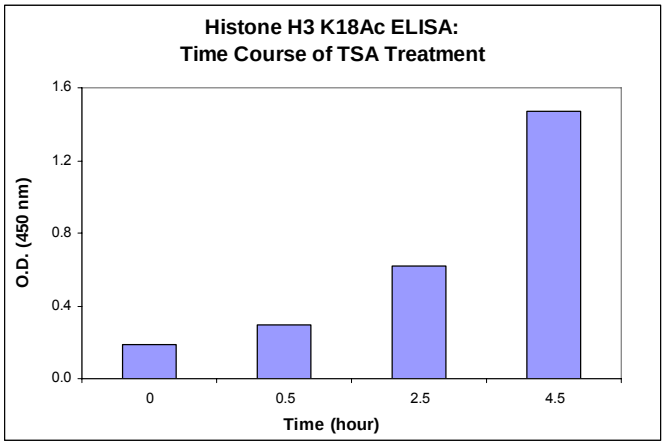
	Cell Lysate		
Dilution	**Measured (Units/mL)**	**Expected (Units/mL)**	**% Expected**
Neat	63.61	63.61	100
1/2	28.17	31.80	89
1/4	11.37	14.09	81
1/8	5.3	5.69	93
1/16	2.81	2.65	106

Specificity

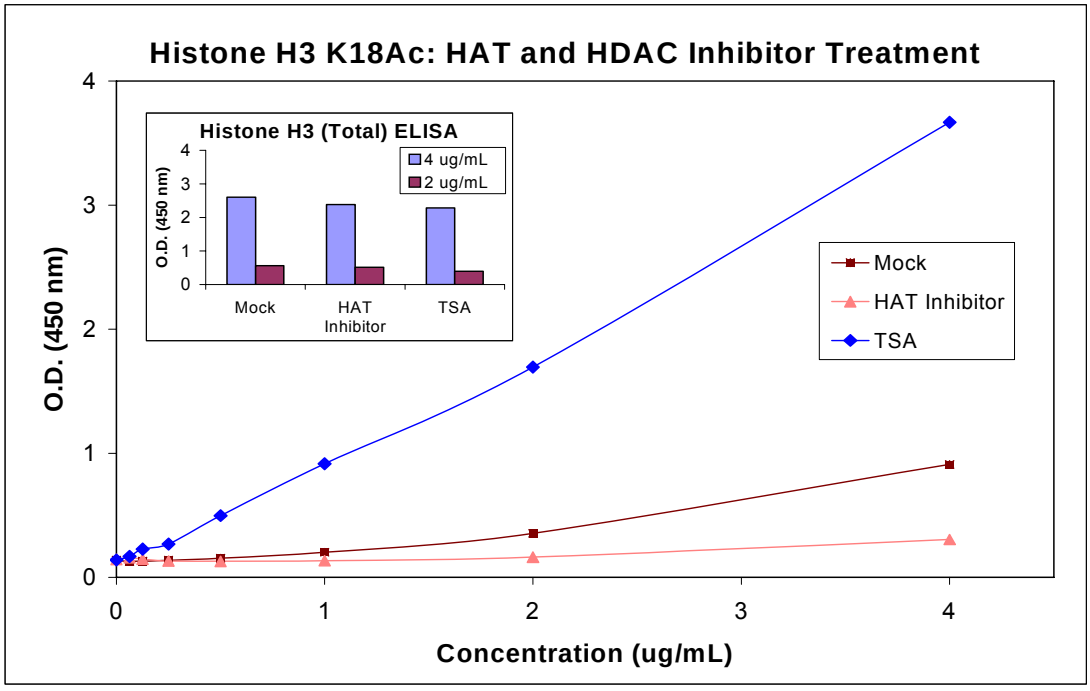
The specificity of this assay for acetylated histone H3 [K18Ac] was confirmed by peptide competition. The data below show that only the acetyl-peptide containing the acetylated lysine 18 blocks the ELISA signal. The non-acetylated peptide sequence did not block the signal, and the acetylated peptide at the other N-terminal H3 tail lysines (H3 K4Ac, K9Ac, K14Ac, K23Ac, and K27Ac) did not block the ELISA signal. Additionally, a peptide methylated on K4 and K9 did not compete for the antibody, therefore confirming the specificity of the antibody for acetylated K18 only.



HeLa cells were treated with 3 mM of Trichostatin A (TSA) for 0, 0.5, 2.5 and 4.5 hours. Cells were harvested and histones were extracted from each sample. The amount of histone H3 K18 acetylation was measured and analyzed with the histone H3 [K18Ac] ELISA (Cat. # KHO0831) using 50 ng of extracted histones. The results show that at 0.5 hour time point the amount of acetylation at H3 K18 is increased by 1.6 fold. At the 2.5 and 4.5 hour time point the acetylation increases by 3.3 and 7.9 fold, respectively.

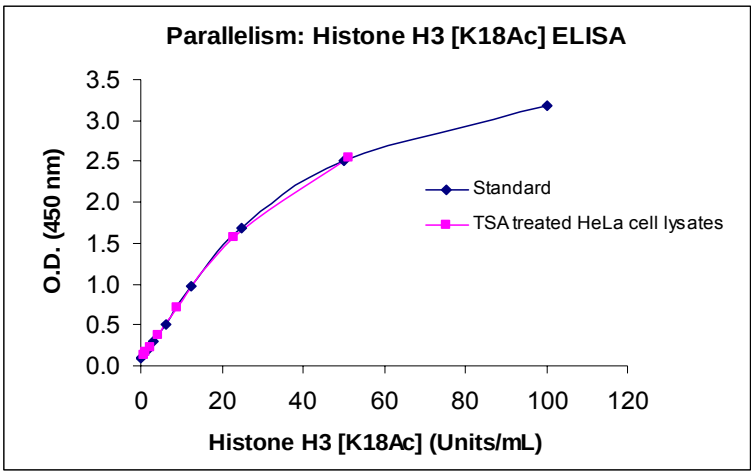


HeLa cells are treated with either 3 mM of TSA for 4 hours or HAT inhibitor for 24 hours or no treatment. Histones were extracted from harvested cells and levels of histone H3 K18 acetylation was analyzed by ELISA using between 0-4 mg/mL concentrations of nuclear extracted histones. The data show that the 2 mg/mL and 4 mg/mL histone loading results in an average decrease of 2.5 fold in acetylation levels in HAT inhibitor treated cells compared to untreated mock samples. The TSA treated samples result in an average of 4.3 fold increase compared to the mock samples. Overall loading of histones was normalized using the histone H3 (Total) ELISA (Cat. # KHO0661) and modifying the total detector concentration by diluting in PBS by 7 fold.



Parallelism

Natural histone H3 [K18Ac] from Trichostatin A (TSA) treated HeLa cell nucleus acid extract was serially diluted in *Standard Diluent Buffer.* The optical density of each dilution was plotted against the histone H3 [K18Ac] standard curve. Parallelism demonstrated by the figure below indicates that the standard accurately reflects natural histone H3 [K18Ac] content in samples.



Limitations of the Procedure

Do not extrapolate the standard curve beyond top standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute all samples above the highest standard point with *Standard Diluent Buffer*; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various extraction buffers has not been thoroughly investigated. The rate of degradation of native histone H3 or deacetylation of histone H3 [K18Ac] in various matrices has not been investigated. Although histone H3 degradation or deacetylation of histone H3 [K18Ac] in the Cell Extraction Buffer described in this protocol has not been seen to date, the possibility of this occurrence cannot be excluded.

Troubleshooting Guide

Standard curve wells develop, but sample wells produce weak or no signal.	*Cause:* Improper sample preparation. *Solution 1:* Make sure to prepare cell extracts in the protease inhibitor-supplemented Cell Extraction Buffer recommended in the protocol booklet. Other buffer formulations have not been evaluated. *Cause:* Samples contain materials that interfere with the assay. *Solution 1:* The Cell Extraction Buffer recommended in the protocol booklet contains SDS. This detergent can potentially interfere with the immunoassay. For this reason, we recommend that all samples be diluted by a factor of at least 1:10 using the Standard Diluent Buffer provided in the kit. This buffer also contains blocking proteins that will reduce background signal. *Cause:* The concentration of the target analyte is too dilute. *Solution 1:* When preparing the cell extracts, increase the number of cells per volume of protease inhibitor supplemented Cell Extraction Buffer. Ideally, the concentration of protein in each cell extract, as determined by the Quant-iT™ protein assay kit, will be between 1 and 10 mg/ml (Method 1) or 1 and 5 mg/ml (Method 2). *Solution 2:* Optimize the stimulation procedure and time. *Cause:* A sample treatment step was not performed. *Solution 1:* Certain analytes (e.g., ERK1/2 [pTpY185/187] and ERK1/2 Total) require a sample treatment step to improve performance with Invitrogen phosphoELISA™ kits. Please see the analyte-specific protocol booklet for information on sample treatment procedures. *Cause:* Samples deteriorated during storage. *Solution 1:* Make sure that the Cell Extraction Buffer is supplemented with phosphatase inhibitors and protease inhibitors just prior to use. *Solution 2:* All samples should be stored frozen at –80°C. *Solution 3:* Samples should be subjected to only one freeze-thaw cycle. *Solution 4:* Some proteins can be lost by absorption when stored in containers made of polystyrene or certain kinds of glass. Polypropylene tubes are best for storing samples.
Sample wells develop, but standard wells produce weak or no signal.	*Cause:* Improper dilution of standards. *Solution 1:* Check reconstitution volume of standard. *Solution 2:* Standard curves are generated by serially diluting the reconstituted standard. Check the serial dilution method. *Solution 3:* Standards should be used within 1 hr of reconstitution and serial dilution. *Cause:* Improper storage of standards. *Solution 1:* Standards are provided as lyophilized powders that should be stored at 2–8°C.

Neither the standard curve wells nor the sample wells develop.	*Cause:* Insufficient horseradish peroxidase (HRP)-conjugated secondary antibody activity. *Solution 1:* Check the dilution of the HRP secondary antibody. *Solution 2:* The HRP secondary antibody must be freshly diluted for each assay. *Solution 3:* The HRP secondary antibody must be stored at 2–8°C. *Solution 4:* Sodium azide is an irreversible inhibitor of horseradish peroxidase enzyme activity. Make sure to dilute the HRP secondary antibody in the correct buffer. A quick test can be performed to determine if the HRP secondary antibody is active. Into a clean test tube, dispense 200 µl of the TMB substrate solution provided in the kit. This TMB substrate solution should be clear to slightly blue-green tinted. Next, pipette 2 µl of the HRP secondary antibody. The color of the TMB will change to an intense aqua blue instantaneously if the HRP has retained its enzyme activity. *Cause:* Insufficient Detector Antibody. *Solution 1:* The Detector Antibody must be stored at 2–8°C. *Solution 2:* Improper dilution of Detector Antibody. *Cause:* TMB solution lost activity. *Solution 1:* The TMB solution should be clear or slightly blue-green tinted before it is dispensed into the wells of the microtiter plate. An intense aqua blue color indicates that the product is contaminated. Please contact our Technical Support Department if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Any TMB solution left in the trough should be discarded. *Solution 2:* Avoid contact of the TMB solution with items containing metal ions.
Standard curves are not developing consistently in between different runs.	*Cause:* Improper dilution of Secondary antibody. *Solution 1:* The HRP-labeled antibody 100x concentrate is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow HRP-labeled antibody concentrate to reach room temperature. Gently mix. Pipette concentrate slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

Technical Support

Contact Us

For more troubleshooting tips, information, or assistance, please call, email, or go online to www.invitrogen.com/ELISA.

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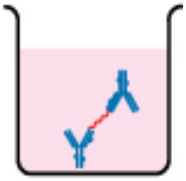
NOTES

Histone H3 [K18Ac] Assay Summary

Incubate 100 µl Standard or Cell Extract (>1:10) for 2 hours at RT

aspirate and wash 4x

Incubate 100 µl of Detection Antibody for 1 hour at RT

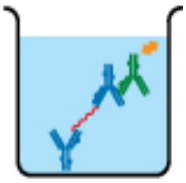


aspirate and wash 4x

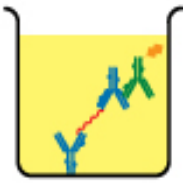
Incubate 100 µl of HRP Anti-Rabbit Antibody for 30 minutes at RT

aspirate and wash 4x

Incubate 100 µl of Stabilized Chromogen for 30 minutes at RT



Add 100 µl of Stop Solution and read at 450 nm



Total time: 4 hours