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ab156909 – Histone H4 Total Quantification Kit (Colormetric)

Instructions for Use

For specifically measuring total histone H4 from mammals, in a variety of forms including cultured cells and fresh tissues the extraction of histone proteins from mammalian cells and tissue

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Histone H4, along with H2A, H2B, and H3, is involved in the structure of chromatin in eukaryotic cells. Histone H4 can undergo several different types of epigenetic modifications that influence cellular processes such as transcription activation/inactivation, chromosome packaging, and DNA damage/repair. These modifications, including acetylation and methylation, occur on the N-terminal tail domains of histone H4 through catalyzation of histone modifying enzymes. This results in the remodeling of the nucleosome structure into an open conformation which is more accessible to transcription complexes. Thus, quantitative detection of various histone modifications would provide useful information for better understanding epigenetic regulation of cellular processes and for developing HMT-targeted drugs.

Abcam's Histone H4 Total Quantification Kit (Colorimetric) is designed for quantifying levels of histone H4 proteins independent of its modified state and can also be used for normalizing the modified histone H4 content of samples when run in parallel with other histone modification quantification kits.

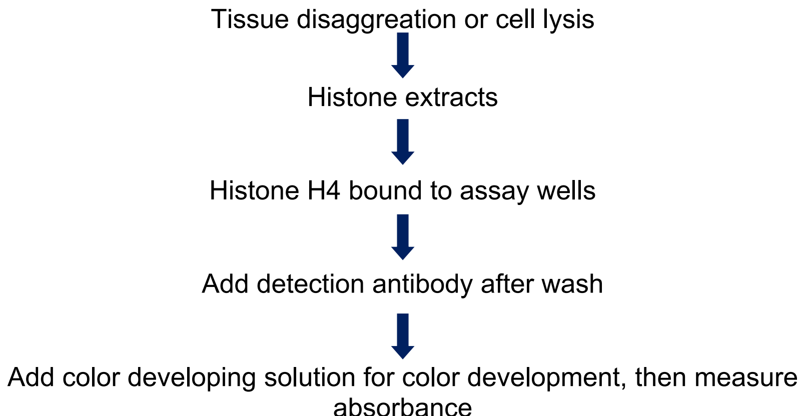
The kit has the following features:

- Quick and efficient procedure which can be finished within 3.5 hours
- Innovative colorimetric assay without the need for radioactivity, electrophoresis, or chromatography
- Specifically captures histone H4 with the detection limit as low as 0.5 ng/well and a detection range from 50 ng to 1 µg/well of histone extracts
- The control is conveniently included for the quantification of total histone H4
- Strip microplate format makes the assay flexible: manual or high throughput
- Simple, reliable, and consistent assay conditions

The kit is suitable for specifically measuring total histone H4 from mammals, in a variety of forms including cultured cells and fresh tissues. Histone extracts can be prepared by using your own successful method. For your convenience and the best results, Abcam offers a Histone Extraction Kit (ab113476) optimized for use with this kit. Histone extracts can be used immediately or can be stored at -80°C for future use.

In an assay with this kit, the histone proteins are stably spotted on the strip wells. The histone H4 can be recognized with a high-affinity antibody and detected with a signal reporter, followed by a color development reagent. The ratio of histone H4 is proportional to the intensity of absorbance. The absolute amount of histone H4 can be quantitated by comparing to the standard control.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit as given in the table and away from light upon receipt.

Observe the storage conditions for individual prepared components in sections 9, 10 & 11.

For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

Check if the 10X Wash Buffer contains salt precipitates before use. If so, warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.

Check if a blue color is present in the Color Developer, which would indicate contamination of the solution and should not be used. To avoid contamination, transfer the amount of the Color Developer required into a secondary container (tube or vial) before adding Color Developer into the assay wells.

5. MATERIALS SUPPLIED

Item	48 Tests	96 Tests	Storage Condition (Before Preparation)
10X Wash Buffer	14 mL	28 mL	4°C
Histone Assay Buffer	4 mL	8 mL	4°C
1000X Capture Antibody	5 µL	10 µL	4°C
Color Developer	5 mL	10 mL	4°C
Stop Solution	5 mL	10 mL	RT
Standard Control, 100 µg/mL	10 µL	20 µL	–20°C
2000X Signal Reporter	6 µL	12 µL	–20°C
Enhancer Solution	120 µL	240 µL	–20°C
8-Well Assay Strips (With Frame)	6	12	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Adjustable pipette or multiple-channel pipette
- Multiple-channel pipette reservoirs
- Aerosol resistant pipette tips
- Microplate reader capable of reading absorbance at 450 nm
- 1.5 mL microcentrifuge tubes
- Incubator for 37°C incubation
- Distilled water
- Histone extracts
- Parafilm M or aluminum foil

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Prepare fresh reagents immediately prior to use.

9.1 **1X Wash Buffer**

Add volume specified in the table below of 10X Wash Buffer to distilled water and adjust to pH 7.2-7.5.

	Volume to Dilute (mL)	Volume distilled water (mL)	Total Volume (mL)
48 Tests	**13**	**117**	**130**
96 Tests	**26**	**234**	**260**

This 1X Wash Buffer can now be stored at 4°C for up to six months.

9.2 **1X Capture Antibody**

Dilute 1000X Capture Antibody with 1X Wash Buffer at a ratio of 1:1000 (e.g. add 1 µL of 1000X Capture Antibody to 1000 µL of 1X Wash Buffer). 50 µL of 1X Capture Antibody will be required for each assay well.

9.3 **1X Signal Reporter**

Dilute 2000X Signal Reporter with 1X Wash Buffer at a ratio of 1:2000 (e.g. add 1 µL of 2000X Signal Reporter to 2000 µL of 1X Wash Buffer). 50 µL of 1X Signal Reporter will be required for each assay well.

9.4 **Diluted Enhancer Solution**

Dilute Enhancer Solution with 1X Wash Buffer at a ratio of 1:5000 (e.g. add 1 µL of Enhancer Solution to 5000 µL of 1X Wash Buffer). About 50 µL of Diluted Enhancer Solution will be required for each assay well.

Note: *Keep each of the diluted solutions except 1X Wash Buffer on ice until use. Any remaining diluted solutions other than 1X Wash Buffer should be discarded if not used within the same day.*

Approximate amount of required buffers and solutions for defined assay wells based on the protocol:

Reagents	1 Well	1 Strip	2 Strips	6 Strips	12 Strips
1X Wash Buffer	2.5 mL	20 mL	40 mL	120 mL	240 mL
Histone Assay Buffer	50 µL	400 µL	800 µL	2400 µL	4800 µL
Standard control (50 ng/µL)	N/A	N/A	4 µL (optional)	8 µL	8 µL
1X Capture Antibody	50 µL	400 µL	800 µL	2400 µL	4800 µL
1X Signal Reporter	50 µL	400 µL	800 µL	2400 µL	4800 µL
Diluted Enhancer Solution	50 µL	400 µL	800 µL	2400 µL	4800 µL
Color Developer	0.1 mL	0.8 mL	1.6 mL	4.8 mL	9.6 mL
Stop Solution	0.05 mL	0.4 mL	0.8 mL	2.5 mL	5 mL

10. SAMPLE PREPARATION

Input Amount: The amount of histone extracts for each assay can be between 50 ng and 1 µg with an optimal range of 0.1 to 0.2 µg.
Histone Extraction: You can use your method of choice for preparing histone extracts from the treated and untreated samples. Abcam provides a Histone Extraction Kit (ab113476) optimized for use with this kit. Histone extracts should be stored in aliquots at -80°C until use.

11. STANDARD PREPARATION

Suggested Standard Curve Preparation:

11.1. Dilute Standard Control with Histone Assay Buffer to 50 ng/µL by adding 5 µL of Standard Control to 5 µL of Histone Assay Buffer.

11.2. Then, further prepare five concentrations by combining the 50 ng/µL Standard Control with Histone Assay Buffer into final concentrations of 1, 2, 5, 10, 20, and 50 ng/µL according to the following dilution chart:

Tube	Standard Control (50 ng/µL) (µL)	Histone Assay Buffer (µL)	Resulting Standard Control Concentration (ng/µL)
1	1.0	49.0	1
2	1.0	24.0	2
3	1.0	9.0	5
4	1.0	4.0	10
5	2.0	3.0	20
6	3.0	0.0	50

Note: *Keep each of the diluted solutions except 1X Wash Buffer on ice until use. Any remaining diluted solutions other than 1X Wash Buffer should be discarded if not used within the same day.*

12. PLATE PREPARATION

The suggested strip-well plate setup for standard curve preparation in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can measured in duplicate.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	Standard Control 1 ng	Standard Control 1 ng	Sample	Sample	Sample	Sample
C	Standard Control 2 ng	Standard Control 2 ng	Sample	Sample	Sample	Sample
D	Standard Control 5 ng	Standard Control 5 ng	Sample	Sample	Sample	Sample
E	Standard Control 10 ng	Standard Control 10 ng	Sample	Sample	Sample	Sample
F	Standard Control 20 ng	Standard Control 20 ng	Sample	Sample	Sample	Sample
G	Sample	Sample	Sample	Sample	Sample	Sample
H	Sample	Sample	Sample	Sample	Sample	Sample

13. ASSAY PROCEDURE

- **Internal Control: The assay control (purified histone H4) is provided in this kit for the quantification of total histone H4. Because content of histone H4 can vary from tissue to tissue, and from normal and diseased states, it is advised to run replicate samples to ensure that the signal generated is validated**

13.1 Histone Binding

13.1.1 Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (include blank and positive controls) to ensure that the signal generated is validated. Carefully remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

13.1.2 Add 50 µL of Histone Assay Buffer to each blank well.

13.1.3 Add 49 µL of Histone Assay Buffer and 1 µL of Diluted Standard Control to each standard well with a minimum of six wells, each at a different concentration between 1 and 50 ng/µL (based on the dilution chart in Section 11 - Standard Preparation; see Section 12 – Plate Preparation for an example).

13.1.4 Add 46 to 49 µL of Histone Assay Buffer and 1 to 4 µL of your histone extracts to sample wells. Total volume should be 50 µL per well.

***Note**: (1) Follow the suggested well setup diagrams (Section 12); (2) It is recommended to use 0.2 µg of histone extract per well.*

13.1.5 Tightly cover strip-well microplate with Parafilm M to avoid evaporation and incubate at 37°C for 90 to 120 minutes.

13.1.6 Remove the reaction solution from each well. Wash each well three times with 150 µL of the 1X Wash Buffer each time.

13.2 Antibody Binding and Signal Enhancing

13.2.1 Add 50 µL of 1X Capture Antibody solution to each well, then cover Parafilm M or aluminum foil and incubate at room temperature for 60 minutes.

13.2.2 Remove the 1X Capture Antibody solution from each well.

13.2.3 Wash each well three times with 150 µL of 1X Wash Buffer each time.

13.2.4 Add 50 µL of 1X Signal Reporter to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 30 minutes.

13.2.5 Remove the 1X Signal Reporter solution from each well.

13.2.6 Wash each well four times with 150 µL of 1X Wash Buffer each time.

13.2.7 Add 50 µL of the Diluted Enhancer Solution to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 30 minutes.

13.2.8 Remove the Diluted Enhancer Solution from each well.

13.2.9 Wash each well five times with 150 µL of the 1X Wash Buffer each time.

***Note**: Ensure any residual wash buffer in the wells is thoroughly removed at each wash step.*

13.3 **Signal Detection**

13.3.1 Add 100 µL of Color Developer to each well and incubate at room temperature for 1-10 minutes away from light. Begin monitoring color changes in the sample wells and control wells. The Color Developer solution will turn blue in the presence of sufficient hydroxymethylated DNA.

13.3.2 Add 50 µL of Stop Solution to each well to stop enzyme reaction when the color in the positive control wells turns medium blue. The color will change to yellow after adding the Stop Solution and the absorbance should be read on a microplate reader within 2 to 10 minutes at 450 nm with an optional reference wavelength of 655 nm.

***Note:** (1) Most microplate readers have capability to carry out dual wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice – once at 450 nm and once at 655 nm. Then*

manually subtract the 655 nm ODs from 450 nm ODs; (2) If the stripwell microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.

13.3.3 Calculate % histone H4 using the formulae provided in Section 14 - Data Analysis.

14. ANALYSIS

Calculate the average duplicate readings for the sample wells and blank wells. Calculate % histone H4 change using the following formula:

$$H4\ (\%) = \frac{\text{Treated (tested) Sample OD} - \text{Blank OD}}{\text{Untreated (control) Sample OD} - \text{Blank OD}} \times 100\%$$

Example calculation:

Average OD450 of treated sample is 0.5
Average OD450 of untreated control is 0.9
Average OD450 of blank is 0.1

$$H4\ (\%) = \frac{(0.5 - 0.1)}{(0.9 - 0.1)} \times 100\% = 50\%$$

For accurate calculation, generate a standard curve and plot the OD values versus the amount of Standard Control at each concentration point. Determine the slope as delta OD/ng using the most linear part of the standard curve (including at least 4 points), then calculate the amount of histone H4 using the following formula:

$$H4\ (\text{ng/mg protein}) = \frac{\text{Sample OD} - \text{Blank OD}}{\text{Slope} \times \text{Protein amount* } (\mu g)} \times 1000$$

*Protein amount = Histone extract added into sample wells at step 13.1.4.

Typical Results

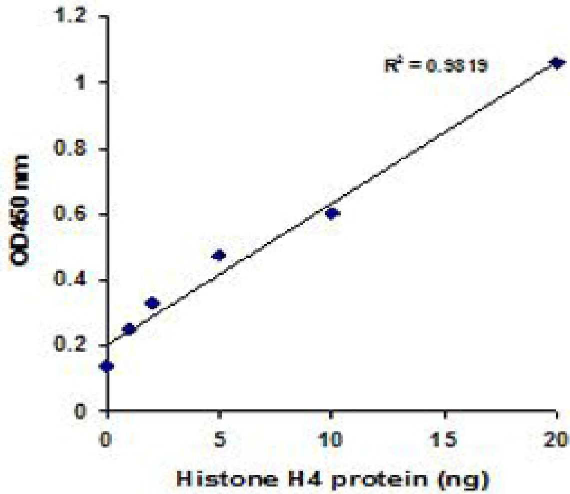


Figure 1. Illustrated standard curve generated with H4 standard provided in the kit.

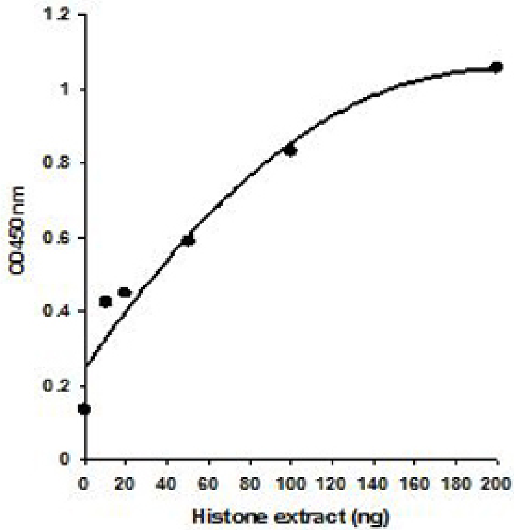


Figure 2. Histone nuclear extracts were prepared from MDA-231 cells using Abcam's Total Histone Extraction Kit (ab113476). Amount of Histone H4 present in the sample was determined using Abcam's Total Histone H4 and the ODs generated from histone H4 are measured.

15. TROUBLESHOOTING

Problem	Cause	Solution
No signal or weak signal in both the positive control and sample wells	Reagents are added incorrectly	Check if reagents are added in the proper order with the right amount, and if any steps in the protocol may have been omitted by mistake
	Incubation time and temperature are incorrect	Ensure the incubation time and temperature described in the protocol are followed correctly
	Incorrect absorbance reading	Check if appropriate absorbance wavelength (450 nm) is used
	Kit was not stored or handled properly	Ensure all components of the kit were stored at the appropriate temperature and the cap is tightly capped after each opening or use
No signal or weak signal in only the standard curve wells	The standard amount is insufficiently added to the well in step 13.1.2	Ensure a sufficient amount of standard is added
	The standard is degraded due to improper storage conditions	Follow the Storage and Stability guidance in this User Guide for storage of Standard Control
High background present in the blank wells	Insufficient washing of wells	Check if washing recommendations at each step is performed according to the protocol
	Contaminated by sample or standard	Ensure the well is not contaminated from adding sample or standard accidentally or from using contaminated tips
	Incubation time with Diluted Signal Reporter is too long	The incubation time at step 13.2.4 should not exceed 90 minutes

	Over-development of color	Decrease the development time in step 13.3.1 before adding Stop Solution in step 13.3.2
No signal or weak signal only in sample wells	Protein sample is not properly extracted or purified	Ensure your protocol is suitable for histone protein extraction. For the best results, it is advised to use Histone Extraction Kit (ab113476)
	Sample amount added into the wells is insufficient	Ensure a sufficient amount of histone extracts is used as indicated in step 13.1. The sample can be titrated to determine the optimal amount to use in the assay
	Sample was not stored properly or has been stored for too long	Ensure sample is stored in aliquots at -80°C, with no more than 6 months histone extracts
Uneven color development	Insufficient washing of the wells	Ensure the wells are washed according to the guidance of washing and residue washing buffer is removed as much as possible
	Delayed color development or delayed stopping of color development in the wells	Ensure color development solution or stop solution is added sequentially and is consistent with the order you added the other reagents (e.g. from well A to well G or from well 1 to well 12)

16. NOTES

Technical Support

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