ab115092 – Histone H3 Total Quantification Kit (Fluorometric)

Instructions for Use

For the measurement of histone H3 proteins independent of its modified state

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

Version 3 Last Updated 8 September 2014

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1. BACKGROUND

Histone H3, along with H2A, H2B, and H4, is involved in the structure of chromatin in eukaryotic cells. Histone H3 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, phosphorylation, methylation, ubiquitination, and ADP-ribosylation, occur on the N-terminal tail domains of histone H3 through catalyzing of histone modifying enzymes, which result in remodeling of the nucleosome structure into an open conformation more accessible to transcription complexes. In most species, histone H3 is primarily acetylated at lysine 9, 14, 18, 23, and 56, and methylated at lysine 4, 9, 27, 36 and 79, and phosphorylated at ser10, ser28, Thr3, and Thr11, respectively. 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 provides a series of kits used for quantifying all sites/degrees of histone H3 modification. For added convenience and more quantitative interpretation of results, we provide the Histone H3 Total Quantification Kit (Fluorometric).

ab115092 is designed for quantifying levels of histone H3 proteins independent of its modified state and can also be used for normalizing the modified histone H3 content of samples when run in parallel with Abcam histone modification quantification kit series.

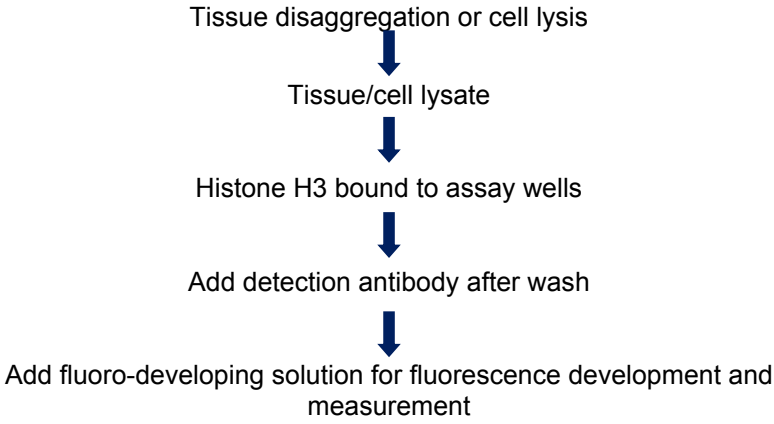
This kit has the following features:

- Quick and efficient procedure, which can be finished within 2.5 hours
- Innovative fluorometric assay without the need for radioactivity, electrophoresis, or chromatography
- Specifically capturing histone H3 with the detection limit as low as 2 ng/well and detection range from 10 ng-1 µg/well of histone extracts

- The unmodified Histone H3 control is conveniently included for the quantification of the amount of total histone H3
- Strip microplate format makes the assay flexible: manual or high throughput
- Simple, reliable, and consistent assay conditions

The Histone H3 Total Quantification Kit (Fluorometric) is designed for quantifying levels of histone H3 proteins independent of its modified state. In an assay with this kit, the histone H3 protein is captured to the strip wells coated with an anti-histone H3 antibody. The captured histone H3 can then be detected with a detection antibody, followed by a fluorescent development reagent. The ratio of histone H3 is proportional to the intensity of fluorescence. The absolute amount histone H3 can be quantified 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 upon receipt and away from light.

Observe the storage conditions for individual prepared components in sections 9 & 10. The components of the kit are stable for 6 months when stored properly.

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

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

5. MATERIALS SUPPLIED

Item	Quantity (48 tests)	Quantity (96 tests)	Storage Condition (Before Preparation)
10X Wash Buffer	10 mL	20 mL	4°C
Antibody Buffer	6 mL	12 mL	4°C
Detection Antibody, 1 mg/mL*	5 µL	10 µL	-20°C
Fluoro Developer*	12 µL	24 µL	-20°C
Fluoro Enhancer*	12 µL	24 µL	4°C
Fluoro Diluter	4 mL	8 mL	4°C
Standard Control (100 µg/mL)*	10 µL	20 µL	-20°C
8-Well Assay Strip (with Frame)	4	9	4°C
8-Well Standard Control Strips**	2	3	4°C

*Spin the solution down to the bottom prior to use.

** These have a Green Rim around the wells to help with identification.

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Orbital shaker
- Pipettes and pipette tips
- Reagent reservoir
- Fluorescence microplate reader
- 1.5mL conical tube
- 1.5mL microcentrifuge tubes

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**

Dilute 10X Wash Buffer with distilled water (pH 7.2-7.5) at a 1:10 ratio (1 mL of 10X Wash Buffer + 9 mL of water) to make 1X Wash Buffer.

9.2 **Detection Antibody**

Dilute Detection Antibody (at 1:1000 ratio) to 1 µg/mL with Antibody Buffer.

9.3 **Fluoro-Development Solution**

Add 1 µL of Fluoro Developer and 1 µL of Fluoro Enhancer into each 400 µL of Fluoro Dilution.

9.4 **Standard Control**

Dilute Standard Control with Antibody Buffer to 1-100 ng/µL at 5-7 points (e.g. 1.5, 3, 6, 12, 25, 50, and 100 ng/µL).

10. SAMPLE PREPARATION

You may prepare histone extracts from cells/tissues treated or untreated by using your own successful method (acid extraction or high salt extraction) For your convenience and the best results, Abcam offers the Histone Extraction Kit (ab113476) optimized for use with this kit. Preparation of histone extracts can also be performed using the following protocol.

10.1 **For tissues (treated and untreated):**weigh the sample and cut the sample into small pieces (1-2 mm^3) with a scalpel or scissors.

Transfer tissue pieces to a Dounce homogenizer.

Add TEB buffer (PBS containing 0.5% Triton X 100, 2 mM PMSF and 0.02% NaN_3) at 200 mg/mL, and disaggregate tissue pieces by 50-60 strokes.

Transfer homogenized mixture to a 15 mL conical tube and centrifuge at 3000 rpm for 5 minutes at 4°C. If total mixture volume is less than 2 mL, transfer mixture to a 2 mL vial and centrifuge at 10,000 rpm for 1 minute at 4°C.

Remove supernatant.

10.2 **For cells (treated and untreated):** harvest cells and pellet the cells by centrifugation at 1000 rpm for 5 minutes at 4°C.

Resuspend cells in TEB buffer at 10^7 cells/mL and lyse cells on ice for 10 minutes with gentle stirring.

Centrifuge at 3000 rpm for 5 minutes at 4°C. If total volume is less than 2 mL, transfer cell lysates to a 2 mL vial and centrifuge at 10,000 rpm for 1 minute at 4°C.

Remove supernatant.

10.3 **For both tissue and cells:** Resuspend cell/tissue pellet in 3 volumes (approx. 200 µL/10^7 cells or 200 mg tissues) of

extraction buffer (0.5N HCl + 10% glycerol) and incubate on ice for 30 minutes.

10.4 Centrifuge at 12,000 rpm for 5 minutes at 4°C and remove the supernatant fraction to a new vial.

10.5 Add 8 volumes (approx. 0.6 mL/10^7 cells or 200 mg tissues) of acetone and leave at –20°C overnight.

10.6 Centrifuge at 12,000 rpm for 5 minutes and air-dry the pellet. Dissolve the pellet in distilled water (30-50 µL/10^7 cells or 200 mg tissues).

10.7 Quantify the protein concentration. Aliquot the extract and store the extract at –20°C or –80°C.

Histone extracts can be used immediately or stored at–80°C for future use.

11. ASSAY PROCEDURE

11.1 Determine the number of strip wells required. Leave these strips in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at 4°C).

11.2 Add 50 µL of Antibody Buffer into each well. For the sample, add 1-2 µg of the histone extract into the sample wells. Add 1 µL of Standard Control at the different concentrations into the Standard Control Wells (marked with Green Rims). For the blank, do not add any nuclear extracts or standard control protein. Mix and cover the strip wells with Parafilm M and incubate at room temperature for 1-2 hours.

11.3 Aspirate and wash the wells with 150 µL of 1X Wash Buffer three times.

11.4 Add 50 µL of the diluted Detection Antibody to each well and incubate at room temperature for 60 minutes on an orbital shaker (100 rpm).

11.5 Aspirate and wash the wells with 150 µL of 1X Wash Buffer six times.

11.6 Prepare fluoro-development solution by adding 1 µL of Fluoro Developer and 1 µL of Fluoro Enhancer into each 400 µL of Fluoro Diluter. Add 50 µL of fluoro development solution into the wells and incubate at room temperature for 1-5 min away from light. The color in the standard wells containing the higher concentrations may turn slightly pink during this period. Measure and read fluorescence on fluorescence microplate reader at Ex/Em = 530/590.

Note: *If the strip well frame does not fit the fluorescence reader, transfer the solution to the standard 96-well microplate and read fluorescence at Ex/Em = 530/590.*

12. ANALYSIS

Calculate % Histone H3:

$$\text{Histone H3 \%} = \frac{\text{Treated (tested) sample RFU – blank RFU}}{\text{Untreated (control) sample RFU – blank RFU}} \times 100\%$$

For the amount quantification plot RFU versus amount of Standard Control and determine the slope as delta RFU/ng.

Calculate the amount of Histone H3 using the following formula:

$$\text{Amount (ng/mg protein)} = \frac{\text{Sample RFU – blank RFU}}{\text{Protein (µg)* x Slope}} \times 1000$$

*Histone extract amount added into the sample well at step 11.2.

13. TROUBLESHOOTING

Problem	Cause	Solution
No Signal for Both the Standard Control and the Samples	Reagents are added incorrectly.	Check if reagents are added in order and if some steps of the procedure are omitted by mistake.
	Incubation time and temperature is incorrect.	Ensure the incubation time and temperature described in the protocol is followed correctly.
No Signal or Very Weak Signal for Only the Standard Control	The amount of Standard control is not added into the “standard control wells or is added insufficiently.	Ensure a sufficient amount of control is added to the well.
No Signal for Only the Sample	The protein sample is not properly extracted.	Ensure the procedure and reagents are correct for the nuclear protein extraction.
	The protein amount is added into well insufficiently.	Ensure extract contains a sufficient amount of protein.
	Protein extracts are incorrectly stored.	Ensure the protein extracts are stored at –20°C or –80°C.
High Background Present for the Blank	The well is not washed sufficiently.	Check if wash at each step is performed according to the protocol.

Problem	Cause	Solution
	Contaminated by the Standard control	Ensure the well is not contaminated from adding the control protein or by using control protein contaminated tips.
	Overdevelopment	Decrease development time in step 11.6.

14. NOTES

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