EpiQuik™ Total Histone H4 Quantification Kit (Colorimetric)

Base Catalog # P-3072

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ Total Histone H4 Quantification Kit (Colorimetric) 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, EpigenTek offers a histone extraction kit (Cat. # OP-0006) optimized for use with this kit. Histone extracts can be used immediately or stored at –80°C for future use.

Input Material: Input materials can be histone extracts or nuclear extracts. The amount of histone extracts for each assay can be 50 ng to 1 µg with an optimal range of 0.1 to 0.2 µg.

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.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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KIT CONTENTS

Component	48 Assays Cat. #P-3072-48	96 Assays Cat. #P-3072-96	Storage Upon Receipt
C1 (10X Wash Buffer)	14 ml	28 ml	4°C
C2 (Histone Assay Buffer)	4 ml	8 ml	4°C
C3 (Capture Antibody, 1000X)*	5 µl	10 µl	4°C
C4 (Color Developer)	5 ml	10 ml	4°C
C5 (Stop Solution)	5 ml	10 ml	RT
Standard control (100 µg/ml)	10 µl	20 µl	–20°C
Signal Reporter (2000X)*	6 µl	12 µl	–20°C
Enhancer Solution	120 µl	240 µl	–20°C
8-Well Assay Strips (With Frame)	6	12	4°C

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

SHIPPING & STORAGE

The kit is shipped in two parts: the first part at ambient room temperature, and the second part on frozen ice packs at 4°C. Upon receipt: (1) Store **Standard Control**, **Signal Reporter**, and **Enhancer Solution** at –20°C away from light; (2) Store **C1**, **C2**, **C3**, **C4**, and **8-Well Assay Strips** at 4°C away from light; and (3) Store remaining components at room temperature away from light.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

Note: *(1) Check if **C1** (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; and (2) check if a blue color is present in **C4** (Color Developer), which would indicate a contamination of the solution and should not be used. To avoid contamination, transfer the amount of **C4** required into a secondary container (tube or vial) before adding **C4** into the assay wells.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ 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

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiQuik™ Total Histone H4 Quantification Kit (Colorimetric) is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The EpiQuik™ Total Histone H4 Quantification Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic application.

A BRIEF OVERVIEW

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 that 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.

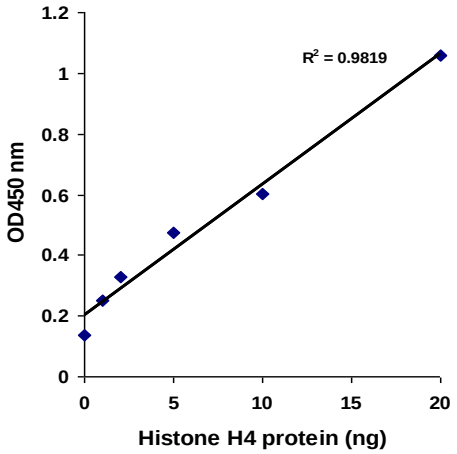
Epigentek provides a series of kits used for quantifying all sites/degrees of histone H4 modification. For added convenience and more quantitative interpretation of results, we provide here the *EpiQuik*™ Total Histone H4 Quantification Kit (Colorimetric). This kit is designed for quantifying levels 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 EpigenTek histone modification quantification kit series. 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 a detection limit as low as 20 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.

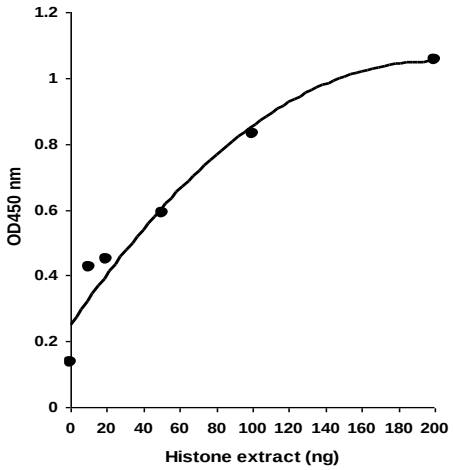
PRINCIPLE & PROCEDURE

The EpiQuik™ Total Histone H4 Quantification Kit (Colorimetric) is designed for measuring total histone H4 amount. 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 it to the standard control.

Schematic procedure of the EpiQuik™ Total Histone H4 Quantification Kit (Colorimetric)



Illustrated standard curve generated with H4 standard



Histone nuclear extracts were prepared from MDA-231 cells using EpiQuik Total Histone Extraction Kit (Cat. # OP-0006) and the ODs generated from histone H4 are measured.

PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Input Amount: The amount of histone extracts for each assay can be between 50 ng and 1 ug 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. EpigenTek also offers a histone extraction kit (Cat # OP-0006) optimized for use with this kit.

Histone extracts should be stored in aliquots at –80°C until use.

1. Working Buffer and Solution Preparation

a. Prepare **Diluted C1** 1X Wash Buffer:

48-Assay Kit: Add 13 ml of **C1**10X Wash Buffer to 117 ml of distilled water and adjust pH to 7.2-7.5.

96-Assay Kit: Add 26 ml of **C1** 10X Wash Buffer to 234 ml of distilled water and adjust pH to 7.2-7.5.

This **Diluted C1** 1X Wash Buffer can now be stored at 4°C for up to six months.

b. Prepare **Diluted Standard Control**:

Suggested Standard Curve Preparation: First, dilute **Standard Control** with **C2** Histone Binding Buffer to 50 ng/µl by adding 5 µl of **Standard Control** to 5 µl of **C2** Histone Binding Buffer. Then, further prepare five concentrations by combining the 50 ng/µl **Diluted Standard Control** with **C2** Histone Binding Buffer into final concentrations of 1, 2, 5, 10, 20, and 50 ng/µl according to the following dilution chart:

Tube	SC (50 ng/µl)	C2	Resulting SC Concentration
1	1.0 µl	49.0 µl	1 ng/µl
2	1.0 µl	24.0 µl	2 ng/µl
3	1.0 µl	9.0 µl	5 ng/µl
4	1.0 µl	4.0 µl	10 ng/µl
5	2.0 µl	3.0 µl	20 ng/µl
6	3.0 µl	0.0 µl	50 ng/µl

Note: *Keep each of the diluted solutions except* ***Diluted C1*** *1X Wash Buffer on ice until use. Any remaining diluted solutions other than* ***Diluted C1*** *should be discarded if not used within the same day.*

2. Histone Binding

a. 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).

b. Blank Wells: Add 50 µl of **C2** to each blank well.

c. Standard Wells: Add 49 µl of **C2** 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 Step 1e; see Table 2 under the "Suggested Strip Well Setup" section as an example).

d. Sample Wells: Add 46 to 49 µl of **C2** and 1 to 4 µl of your histone extracts. Total volume should be 50 µl per well.

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

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

Meanwhile, prepare the **Detection Solution**: for every 1 ml of **Detection Solution** to be prepared, add 1 µl of **C3,** 0.5 µl of the **Signal Reporter** and 20 µl of **Enhancer Solution** to 980 µl of the **Diluted C1** 1X Wash Buffer, then mix and incubate at room temperature for 10 minutes

f. Aspirate and wash each well with 150 µl of the **Diluted C1** 1X Wash Buffer three times.

g. Add 50 µl of the prepared **Detection Solution** to each well and incubate at room temperature for 60 minutes.

h. Remove the reaction solution from each well. Wash each well four times with 150 µl of the **Diluted C1** 1X Wash Buffer each time.

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

3. Signal Detection

a. Add 100 µl of **C4** to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color change in the sample wells and control wells. The **C4** solution will turn blue in the presence of sufficient histone H4.

b. Add 50 µl of **C5** to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding **C5** and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

Note: *(1) Most microplate readers have the 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 strip-well microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.*

5. Total Histone Calculation

a. Calculate the average duplicate readings for the sample wells and blank wells.

b. Calculate % histone H4 change using the following formula:

$$H4\% = \frac{\textit{Treated (Tested) Sample OD} - \textit{Blank OD}}{\textit{Untreated (Control) Sample OD} - \textit{Blank OD}} \times 100\%$$

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:

1. Generate a standard curve and plot OD value versus amount of **Standard Control** at each concentration point.

2. Determine the slope as OD/ng. You can use Microsoft Excel statistical functions for slope calculation. Use the most linear part of the standard curve (inculding at least 4 points), then calculate the amount of histone H4 using the following formulas:

$$H4\ (ng/mg\ protein) = \frac{(Sample\ OD - Blank\ OD)}{Slope \times Protein\ Amount\ (ug^*)} \times 1000$$

** Histone extract added into sample wells at step 2d.*

SUGGESTED BUFFER AND SOLUTION SETUP

Table 1. Approximate amount of required buffers and solutions for defined assay wells based on the protocol.

Reagents	1 well	1 strip (8 wells)	2 strips (16 wells)	6 strips (48 wells)	12 strips (96 wells)
Diluted C1	2.5 ml	20 ml	40 ml	120 ml	240 ml
C2	50 µl	400 µl	800 µl	2400 µl	4800 µl
Standard control	N/A	N/A	4 µL (optional)	8 µl	8 µl
Detection solution	50 µl	400 µl	800 µl	2400 µl	4800 µl
C4	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml
C5	0.05 ml	0.4 ml	0.8 ml	2.5 ml	5 ml

SUGGESTED STRIP WELL SETUP

Table 2. The suggested strip-well plate setup for H4 quantification in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can be measured in duplicate.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	SC 1 ng	SC 1 ng	Sample	Sample	Sample	Sample
C	SC 2 ng	SC 2 ng	Sample	Sample	Sample	Sample
D	SC 5 ng	SC 5 ng	Sample	Sample	Sample	Sample
E	SC 10 ng	SC 10 ng	Sample	Sample	Sample	Sample
F	SC 20 ng	SC 20 ng	Sample	Sample	Sample	Sample
G	SC 50 ng	SC 50 ng	Sample	Sample	Sample	Sample
H	Sample	Sample	Sample	Sample	Sample	Sample

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
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.	Use appropriate absorbance wavelength (450 nm).

	Kit was not stored or handled properly.	Ensure all components of the kit were stored at the appropriate temperature and the cap is tightly closed after each opening or use.
No signal or weak signal in only the standard curve wells	Insufficient standard amount added to the well in Step 2c.	Ensure a sufficient amount of standard is added.
	Degraded standard due to improper storage conditions.	Follow the Shipping & Storage 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 by adding sample or standard accidentally or from contaminated tips.
	Incubation with **Detection Solution** is too long.	Incubation time at Step 3d should not exceed 90 min.
	Over-development of color.	Decrease the development time in Step 3a before adding Stop Solution in Step 3b.
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 EpigenTek's histone extraction Kit (Cat. No. OP-0006).
	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of histone extracts is used as indicated in Step 2. The sample can be titrated to determine the optimal amount to use in the assay.
	Sample was not stored properly.	Ensure sample is stored in aliquots at –80°C, with no more than 6 months histone extracts.
Uneven color development	Insufficient well washing.	Ensure the wells are washed according to the guidance and residue washing buffer is removed.
	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).

RELATED PRODUCTS

Histone Extract Preparation

OP-0006 EpiQuik™ Total Histone Extraction Kit

Modified Histone H4 Assy

P-3062 EpiQuik™ Total Histone H3 Quantification Kit (Colorimetric)
P-3073 EpiQuik™ Total Histone H4 Quantification Kit (Fluorometric)
P-4023 EpiQuik™ Global Acetyl Histone H4-K5 Quantification Kit (Fluorometric)
P-4024 EpiQuik™ Global Acetyl Histone H4-K8 Quantification Kit (Colorimetric)
P-4025 EpiQuik™ Global Acetyl Histone H4-K8 Quantification Kit (Fluorometric)
P-4026 EpiQuik™ Global Acetyl Histone H4-K16 Quantification Kit (Colorimetric)
P-4027 EpiQuik™ Global Acetyl Histone H4-K16 Quantification Kit (Fluorometric)
P-4028 EpiQuik™ Global Acetyl Histone H4-K12 Quantification Kit (Colorimetric)
P-4029 EpiQuik™ Global Acetyl Histone H4-K12 Quantification Kit (Fluorometric)