EpiQuik™ Total Histone H4 Quantification Kit (Fluorometric)

Base Catalog # P-3073

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ Total Histone H4 Quantification Kit (Fluorometric) 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 500 ng 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-3073-48	96 Assays Cat. #P-3073-96	Storage Upon Receipt
F1 (10X Wash Buffer)	14 ml	28 ml	4°C
F2 (Histone Assay Buffer)	4 ml	8 ml	4°C
F3 (Detection Antibody, 1000X)*	5 µl	10 µl	4°C
F4 (Fluoro Developer)*	12 µl	24 µl	–20°C
F5 (Fluoro Enhancer)*	12 µl	24 µl	–20°C
F6 (Fluoro Diluter)	4 ml	8 ml	4°C
Standard Control (50 µg/ml)*	10 µl	20 µl	–20°C
Signal Reporter (2000X)*	6 µl	12 µl	–20°C
Enhancer Solution*	6 µl	12 µl	–20°C
8-Well Assay Strips (With Frame)	6	12	4°C
User Guide	1	1	RT

** 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 parts on frozen ice packs at 4°C. Upon receipt: (1) Store **F4**, **F5**, **Standard Control**, **Signal Reporter**, and **Enhancer Solution** at –20°C away from light; (2) Store **F1**, **F2**, **F3**, **F6**, 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 **F1** (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.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Adjustable pipette or multiple-channel pipette
- ☐ Multiple-channel pipette reservoirs
- ☐ Aerosol resistant pipette tips
- ☐ Fluorescence microplate reader capable of reading fluorescence at 530ex/590em 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 (Fluorometric) 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 (Fluorometric) is for research use only and is not intended for diagnostic or therapeutic application.

A BRIEF OVERVIEW

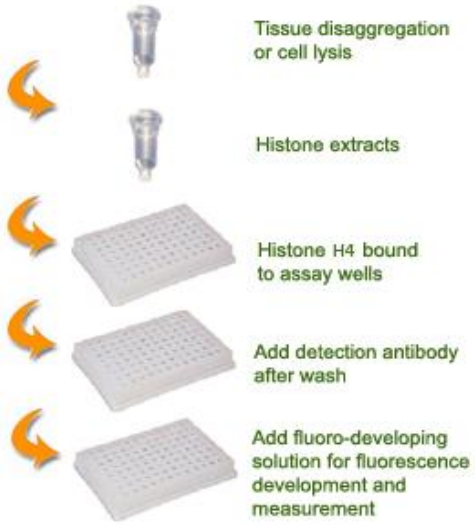
Histone H4, along with H2A, H2B, and H3, is involved in the structure of chromatin in eukaryotic cells. Histone H4 can undergo several different types of epigenetic modifications that influence cellular processes such as transcription activation/inactivation, chromosome packaging, and DNA damage/repair. These modifications, including acetylation and methylation, occur on the N-terminal tail domains of histone H4 through the 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.

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 (Fluorometric). 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:

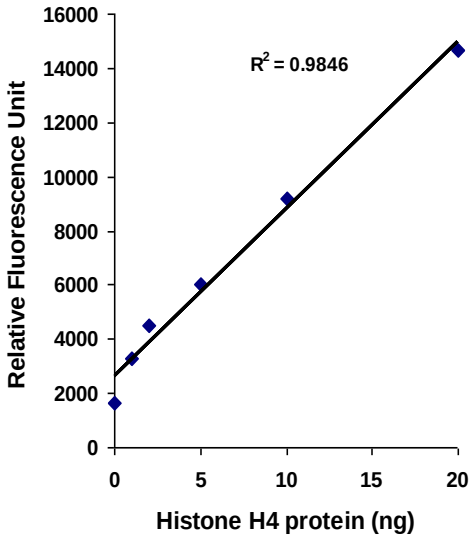
- Quick and efficient procedure, which can be finished within 3.5 hours.
- Innovative fluorometric assay without the need for radioactivity, electrophoresis, or chromatography.
- Specifically captures histone H4 with the detection limit as low as 20 ng/well and a detection range from 50 ng to 500 ng/well 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 (Fluorometric) 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 fluorescence development reagent. The ratio of histone H4 is proportional to the intensity of fluorescence. The absolute amount of histone H4 can be quantified by comparing to the standard control.



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



Illustrated standard curve generated with H4 standard control.

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 own method of choice when preparing histone extracts from 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 F1** 1X Wash Buffer:

48-Assay Kit: Add 13 ml of **F1** 10X Wash Buffer to 117 ml of distilled water and adjust pH to 7.2-7.5.

96-Assay Kit: Add 26 ml of **F1** 10X Wash Buffer to 234 ml of distilled water and adjust pH to 7.2-7.5.

This **Diluted F1** 1X Wash Buffer can now be stored at 4°C for up to six months.

b. Prepare **Diluted F3** Detection Antibody Solution:

Dilute **F3** Detection Antibody with **Diluted F1** 1X Wash Buffer at a ratio of 1:1000 (i.e., add 1 µl of **F3** to 1000 µl of **Diluted F1**). 50 µl of **Diluted F3** will be required for each assay well.

c. Prepare **Diluted Signal Reporter** Solution:

Dilute **Signal Reporter** with **Diluted F1** 1X Wash Buffer at a ratio of 1:2000 (i.e., add 1 µl of **Signal Reporter** to 2000 µl of **Diluted F1**). 50 µl of **Diluted Signal Reporter** will be required for each assay well.

d. Prepare **Diluted Enhancer** Solution:

Dilute **Enhancer Solution** with **Diluted F1** 1X Wash Buffer at a ratio of 1:5000 (i.e., add 1 µl of **Enhancer Solution** to 5000 µl of **F1**). About 50 µl of this **Diluted Enhancer** will be required for each assay well.

e. Prepare **Fluorescence Development Solution**:

Add 1 µl of **F4** Fluoro Developer and 1 µl of **F5** Fluoro Enhancer to every 500 µl of **F6** Fluoro Diluter. About 50 µl of **Fluorescence Development Solution** will be required for each well to be developed.

f. Prepare **Diluted Standard Control**:

Suggested Standard Curve Preparation: First, dilute **Standard Control** with **F2** Histone Assay Buffer to 50 ng/µl by adding 5 µl of **Standard Control** to 5 µl of **F2** Histone Assay Buffer. Then, further prepare five concentrations by combining the 50 ng/µl **Diluted Standard Control** with **F2** Histone Assay 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)	F2	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 F1*** *1X Wash Buffer on ice until use. Any remaining diluted solutions other than* ***Diluted F1*** *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 **F2** to each blank well.

c. Standard Wells: Add 49 µl of **F2** 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 **F2** and 1 µl 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 min to 120 min.

f. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted F1** 1X Wash Buffer each time.

3. Antibody Binding and Signal Enhancing

a. Add 50 µl of the **Diluted F3** to each well, then cover with Parafilm M or aluminium foil and incubate at room temperature for 60 min.

b. Remove the **Diluted F3** solution from each well.

c. Wash each well three times with 150 µl of the **Diluted F1** 1X Wash Buffer each time.

d. Add 50 µl of the **Diluted Signal Reporter** to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 30 min.

e. Remove the **Diluted Signal Reporter** solution from each well.

f. Wash each well four times with 150 µl of the **Diluted F1** 1X Wash Buffer each time.

g. Add 50 µl of the **Diluted Enhancer** to each well, then carefully cover with Parafilm M or aluminum foil and incubate at room temperature for 30 min.

h. Remove the **Diluted Enhancer** from each well.

i. Wash each well with 150 µl of the **Diluted F1** 1X Wash Buffer each time for five times.

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

4. Signal Detection

a. Add 50 µl of **Fluorescence Development Solution** to each well and incubate at room temperature. Continue to monitor the development (approximately 2-4 minutes). The color in the standard wells containing the higher concentrations may turn slightly pink during this period.

b. Read the fluorescence on a fluorescence microplate reader within 2 to 10 min at 530ex/590em nm.

Note: *If the stripwell 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:

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

Example calculation:

Average RFU of treated sample is 6000
Average RFU of untreated control is 11000
Average RFU of blank is 1000

$$H4\% = \frac{(6000 - 1000)}{11000 - 1000} \times 100\% = 50\%$$

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:

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

** 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 F1	2.5 ml	20 ml	40 ml	120 ml	240 ml
F2	50 µl	400 µl	800 µl	2400 µl	4800 µl
Standard Control	N/A	N/A	4 µL (optional)	8 µl	8 µl
Diluted F3	50 µl	400 µl	800 µl	2400 µl	4800 µl
Diluted Signal Reporter	50 µl	400 µl	800 µl	2400 µl	4800 µl
Diluted Enhancer	50 µl	400 µl	800 µl	2400 µl	4800 µl
Fluorescence Development Solution	0.05 ml	0.4ml	0.8 ml	2.4 ml	4.8 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 fluorescence reading.	Check if appropriate fluorescence wavelength (530ex/590em 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 2c.	Ensure a sufficient amount of standard is added.
	The standard is degraded 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 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 3d should not exceed 90 min.
	Over-development of fluorescence.	Decrease the development time in Step 4a.
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 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 fluorescence 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 fluorescence development n the wells.	Ensure fluorescence development 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-3072 EpiQuik™ Total Histone H4 Quantification Kit (Colorimetric)
P-4023 EpiQuik™ Global Acetyl Histone H4-K5Quantification Kit (Fluorometric)
P-4024 EpiQuik™ Global Acetyl Histone H4-K8Quantification Kit (Colorimetric)
P-4025 EpiQuik™ Global Acetyl Histone H4-K8Quantification 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)

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