EpiQuik™ Histone H3 Citrullination ELISA Kit (Colorimetric)

Base Catalog # P-3095

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ Histone H3 Citrullination ELISA Kit (Colorimetric) is suitable for specifically measuring global histone H3 citrullination from a broad range of species such as mammals, fungi, and bacteria, 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 should be histone extracts. The amount of histone extracts for each assay can be 50 ng to 200 ng with an optimal amount of 100 ng.

Internal Control: The citrullinated histone H3 (H3cit) assay control is provided in this kit for the quantification of global histone H3 citrullination. Because content of H3cit 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. As histone H3 content may vary in the histone extracts, we also strongly recommend to perform parallel measurement of total histone H3 for normalizing accuracy of the quantified H3cit %. EpigenTek offers a Total Histone H3 Quantification Kit (Cat. # P-3062) for this application.

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

Part 1 of 2

Component	48 Assays Cat. #P-3095-48	96 Assays Cat. #P-3095-96	Shipping Temperature	Storage Upon Receipt	Storage Checklist
WB (10X Wash Buffer)	14 ml	28 ml	Ambient	4°C	
HCB (Histone Coating Buffer)	4 ml	8 ml	Ambient	4°C	
BB (Blocking Buffer)	10 ml	20 ml	Ambient	4°C	
DS (Developer Solution)	5 ml	10 ml	Ambient	4°C	
SS (Stop Solution)	5 ml	10 ml	Ambient	RT	
8-Well Assay Strips (With Frame)	4	10	Ambient	4°C	
Control Strips (With Frame)[#]	2	2	Ambient	4°C	
Adhesive Covering Film	1	1	Ambient	RT	

Part 2 of 2

Component	48 Assays Cat. #P-3095-48	96 Assays Cat. #P-3095-96	Shipping Temperature	Storage Upon Receipt	Storage Checklist
CAb (Capture Antibody, 1000X)*	5 µl	10 µl	Ice Pack	4°C	
DAb (Detection Antibody, 1000X)*	6 µl	12 µl	Ice Pack	–20°C	
H3cit Control (50 µg/ml)	10 µl	20 µl	Ice Pack	–20°C	

**Spin the solution down to the bottom prior to use.*
[#]Control Assay Strips are green trimmed for distinguishing from 8-well Assay Strips (for samples). The Control Strips are only for control use and should not be used for sample assay.

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 **DAb** and **H3cit Control** at –20°C away from light; (2) Store **WB**, **HCB**, **BB**, **CAb**, **DS**, **8-Well Assay Strips** and **Control Strips** at 4°C away from light; and (3) Store remaining components (**SS** and **Adhesive Covering Film**) 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 **WB** (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 **DS** (Developer Solution), which would indicate contamination of the solution and should not be used. To avoid contamination, transfer the amount of **DS** required into a secondary container (tube or vial) before adding **DS** 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™ Histone H3 Citrullination ELISA 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™ Histone H3 Citrullination ELISA Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic application.

A BRIEF OVERVIEW

Arginine histone citrullination is one of the many important epigenetic marks, and is essential for the regulation of epigenetic chromatin remodeling and immune cell's extracellular trap processes. Citrullinated arginine of histone H3 (Arg2, 8, 17) regulates gene expression and is mainly catalyzed by peptidylarginine deiminase 4 (PAD4). Furthermore, citrullination of histones, in particular histone H3, was revealed as a convergence point for diverse inflammatory signals that trigger the neutrophil response to infections. It was reported that citrullinated histone H3 could be a potential serum biomarker for the early diagnosis of septic shock. More immunological diseases such as multiple sclerosis and rheumatoid arthritis seems to be also associated with change of histone H3 citrullination.

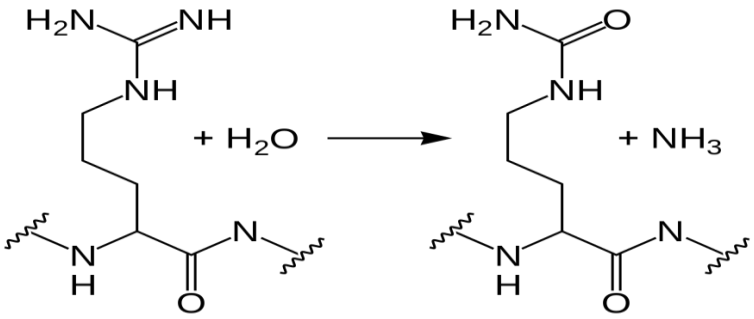

Fig. 1. Histone arginine citrullination reaction catalyzed by PAD4.

The global histone H3 citrullination can be changed by inhibition or activation of PADs. Therefore, quantitative detection of global histone H3 citrullination would provide useful information for better understanding epigenetic regulation of gene activation and silencing, as well as for developing PAD-targeted drugs.

The EpiQuik™ Histone H3 Citrullination ELISA Kit (Colorimetric) is designed to quantitatively detect global histone H3cit. This kit has the following advantages:

- Quick and efficient procedure, which can be finished within 3.5 hours.
- High sensitivity and specificity. The detection limit is as low as 0.1 ng/well with dynamic range of 0.5-10 ng/well within the indicated amount range of the histone extracts. Only recognizes H3cit with no cross-reactivity with unmodified H3 or other modifications at the same arginine site.
- The control is conveniently included for the quantification of H3cit.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The EpiQuik™ Histone H3 Citrullination ELISA Kit (Colorimetric) is designed for measuring global H3cit. In an assay with this kit, the histone proteins are stably spotted on the strip wells. The citrulline H3 can be recognized with a high-affinity antibody and detected with a detection antibody, followed by a color development reagent. The ratio of H3cit is proportional to the intensity of absorbance. The absolute amount of H3cit can be quantitated by comparing to the standard control.

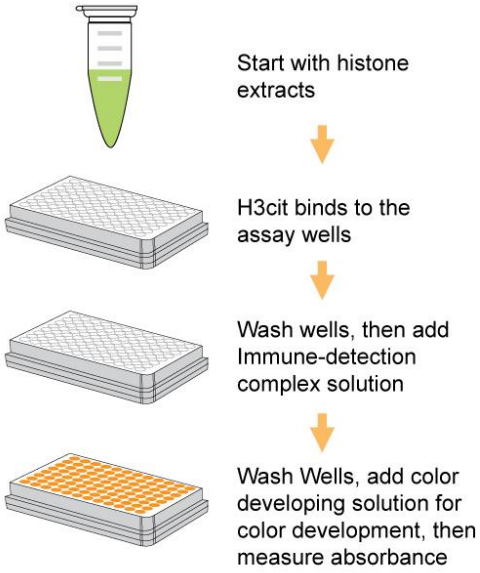

Fig 2. Schematic procedure of EpiQuik™ Histone H3 Citrullination ELISA Kit (Colorimetric).

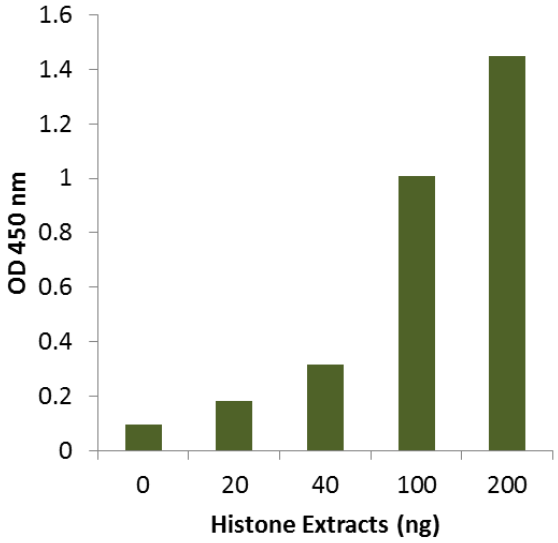

Fig 3. Histone extracts were prepared from HL-60 cells using the EpiQuik™ Total Histone Extraction Kit (Cat. # OP-0006) and the amount of H3cit was measured using the EpiQuik™ Histone H3 Citrullination ELISA Kit (Colorimetric).

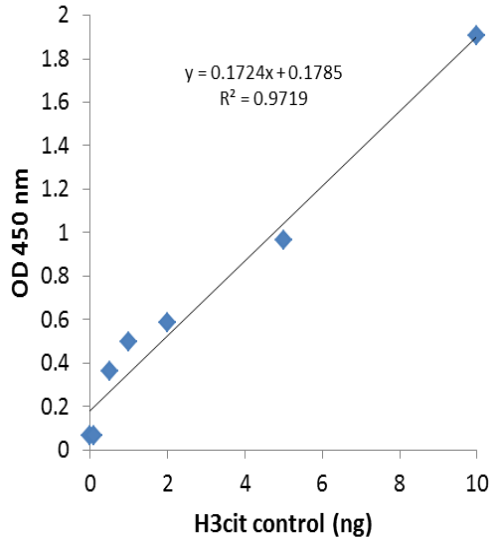

Fig 4. Illustrated standard curve generated. H3cit control.

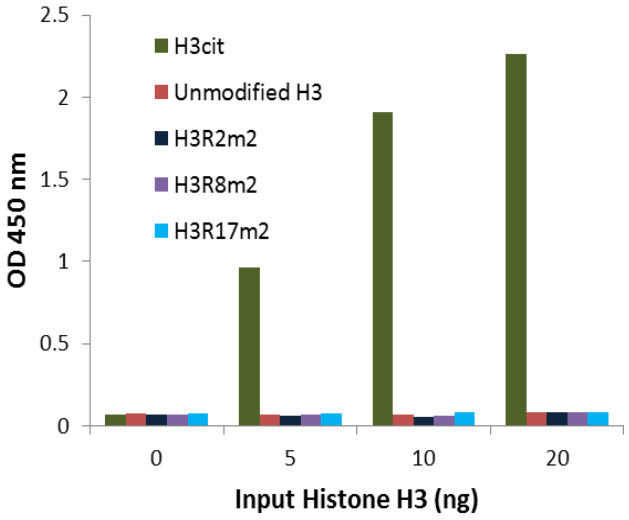

Fig 5. Demonstration of high sensitivity & specificity of H3cit detection: H3cit, unmodified H3, and methylated H3 at arginine site 2, 8, and 17, respectively were added to the assay wells at the different concentrations and then measured with the EpiQuik™ Histone H3 Citrullination ELISA Kit (Colorimetric).

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 200 ng with an optimal amount of 100 ng.

Histone Extraction: You can use your method of choice for preparing histone extracts from the treated and untreated samples. The isolated histone extracts should not contain detergents for the best binding of the histone protein to the wells. 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 WB** (1X Wash Buffer):

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

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

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

b. Prepare **Diluted H3cit Control** Standard

Suggested Standard Curve Preparation: First, dilute **H3cit Control** to 10 ng/µl by adding 2 µl of **H3cit Control** to 8 µl of **HCB** (Histone Coating Buffer) and to 2 ng/µl by adding 1 µl of **H3cit Control** to 24 µl of **HCB**. Then, further prepare seven concentrations by using the 2 ng/µl and 10 ng/µl of **Diluted H3cit Control** with **HCB** into final concentrations of 0.1, 0.2, 0.5, 1, 2, 5, and 10 ng/µl according to the following dilution chart:

Tube	H3cit (2 ng/µl)	H3cit (10 ng/µl)	HCB	Resulting H3cit Concentration
1	1.0 µl		19.0 µl	0.1 ng/µl
2	1.0 µl		9.0 µl	0.2 ng/µl
3		1.0 µl	19.0 µl	0.5 ng/µl
4		1.0 µl	9.0 µl	1 ng/µl
5		1.0 µl	4.0 µl	2 ng/µl
6		2.0 µl	2.0 µl	5 ng/µl
7		4.0 µl	0.0 µl	10 ng/µl

Note: *Keep each of the diluted solutions except* ***WB*** *(1X Wash Buffer) on ice until use. Any remaining diluted solutions other than* ***Diluted WB*** *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 unneeded 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 49 µl of **HCB** to each blank well.

c. Standard Wells: Add 49 µl of **HCB** and 1 µl of **Diluted H3cit Control** to each standard well with a minimum of five wells, each at a different concentration between 0.5 and 10 ng/µl (based on the dilution chart in Step 1b; see Table 2 under the "Suggested Strip Well Setup" section as an example).

d. Sample Wells: Add 46 - 49 µl of **HCB** and 1 - 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 100 ng of histone extract per well.*

e. Tightly cover strip-well microplate with **Adhesive Covering Film** to avoid evaporation and incubate at 37°C for 90 to 120 min.

Note: *The **Adhesive Covering Film** can be cut to the required size to cover the strips based on the number of strips to be used.*

f. Remove the reaction solution from each well. Add 150 µl of **BB** block buffer to each well, then cover with Parafilm M or aluminum foil and incubate at 37°C for 30 min.

g. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted WB** (1X Wash Buffer) each time.

3. Antibody Binding

a. Prepare the **Immuno-Detection Complex** solution: Add 1 ul of **CAb** and 1 ul of **DAb** per 1 ml of **Diluted WB** (1X Wash Buffer). Mix well.

b. Add 50 µl of the **Immuno-Detection Complex** to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 60 min.

c. Remove the **Immuno-Detection Complex** solution from each well.

d. Wash each well four times with 150 µl of the **Diluted WB** each time.

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

4. Signal Detection

a. Add 100 µl of **DS** 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 **DS** solution will turn blue in the presence of sufficient citrullinated products.

b. Add 100 µl of **SS** 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 **SS** 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. H3cit Calculation

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

b. Calculate % Hcit change using the following formula:

$$H3cit\% = \frac{Treated\ (Tested)\ Sample\ OD - Blank\ OD}{Untreated\ (Control)\ Sample\ OD - Blank\ OD} \times 100\%$$

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

$$H3cit\ \% = \frac{(0.5 - 0.1)}{0.9 - 0.1} \times 100\% = 50\%$$

For accurate calculation:

1. Generate a standard curve and plot OD value versus amount of **H3cit Control** at each concentration point.
2. Determine the slope as OD/ng (you can use Microsoft Excel statistical functions for slope calculation), then calculate the amount of H3cit using the following formulas:

$$H3cit\ (ng/mg\ protein) = \frac{(Sample\ OD - Blank\ OD)}{Slope \times Protein\ Amount\ (ug^*)} \times 1000$$

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

SUGGESTED BUFFER AND SOLUTION SETUP

Table 1. Approximate amount of required buffers & 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 WB	2.5 ml	20 ml	40 ml	120 ml	240 ml
HCB	50 µl	400 µl	800 µl	2400 µl	4800 µl
BB	0.15 ml	1.2 ml	2.5 ml	7.5 ml	14.5 ml
H3cit Control	N/A	N/A	4 µl (optional)	8 µl	8 µl
Immuno-Detection Complex	50 µl	400 µl	800 µl	2400 µl	4800 µl
Diluted ES	50 µl	400 µl	800 µl	2400 µl	4800 µl
Developer Solution	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml
Stop Solution	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml

SUGGESTED STRIP WELL SETUP

Table 2. The suggested strip-well plate setup for H3cit 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. Strip 1 and strip 2 are the control strips.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	H3cit 0.1 ng	H3cit 0.1 ng	Sample	Sample	Sample	Sample
C	H3cit 0.2 ng	H3cit 0.2 ng	Sample	Sample	Sample	Sample
D	H3cit 0.5 ng	H3cit 0.5 ng	Sample	Sample	Sample	Sample
E	H3cit 1 ng	H3cit 1 ng	Sample	Sample	Sample	Sample
F	H3cit 2 ng	H3cit 2 ng	Sample	Sample	Sample	Sample
G	H3cit 5 ng	H3cit 5 ng	Sample	Sample	Sample	Sample
H	H3cit 10 ng	H3cit 10 ng	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.	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 temperatures and the caps are tightly closed after each opening or use.

No signal or weak signal in only the standard curve wells	Not enough standard amount is 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 **H3cit 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 **Immuno-Detection Complex** is too long.	The incubation time at Step 3b should not exceed 90 min.
	Over-development of color.	Decrease the development time in Step 4a before adding **SS** Stop Solution in Step 4b.
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. # OP-0006).
	Sample amount added into the wells is insufficient.	Ensure the amount of histone extracts (Step 2). The sample can be titrated to determine the optimal amount for the assay.
	Sample was not stored properly or has been stored for too long.	Ensure histone extracts are stored in aliquots at –80°C, for no more than 6 months.
	Little or no H3cit in the sample.	This problem may be a result of many factors. If the affecting factors cannot be determined, use new or re-prepared 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).

RELATED PRODUCTS

Histone Extract Preparation
OP-0006 EpiQuik™ Total Histone Extraction Kit

Histone H3 Quantification
P-3062 EpiQuik™ Total Histone H3 Quantification Kit (Colorimetric)