EpiQuik™ Circulating Acetyl Histone H3K27 ELISA Kit (Colorimetric)

Base Catalog # P-3136

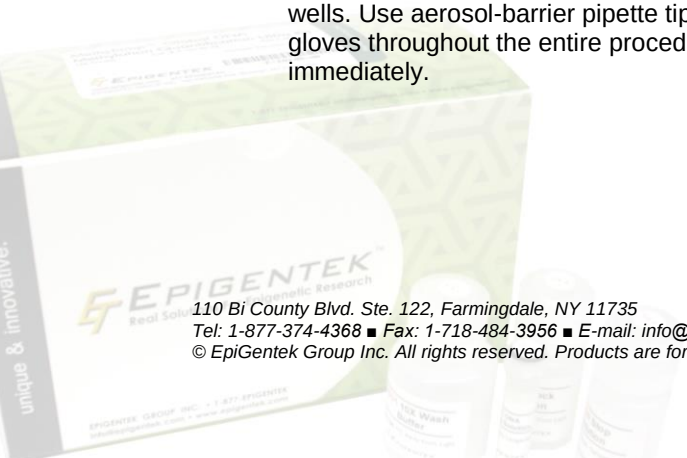
PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ Circulating Acetyl Histone H3K27 ELISA Kit (Colorimetric) is suitable for specifically measuring circulating acetyl histone H3K27 (H3K27ac) from biological fluid samples such as plasma and serum from human, mouse or rat.

Input Material: Input materials should be plasma or serum. The amount of plasma or serum for each assay can be 10 to 40 µl with an optimal amount of 30 µl.

Internal Control: The standard control is provided in this kit for the quantification of circulating histone H3K27ac. Because content of H3K27ac can vary from different individuals 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 plasma or serum, we also strongly recommend to perform parallel measurement of total histone H3 for normalizing accuracy of the quantified H3K27ac %. EpiGentek offers the EpiQuik™ Circulating Total Histone H3 Quantification Kit (Colorimetric) (Cat. # P-3091) 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-3136-48	96 Assays Cat. #P-3136-96	Shipping Temperature	Storage Upon Receipt	Storage Checklist
WB (10X Wash Buffer)	14 ml	28 ml	Ambient	4°C	
HAB (Histone Assay Buffer)	4 ml	8 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 Assay Strips (With Frame)[#]	2	2	Ambient	4°C	
Adhesive Covering Film	1	1	Ambient	RT	

Part 2 of 2

Component	48 Assays Cat. #P-3136-48	96 Assays Cat. #P-3136-96	Shipping Temperature	Storage Upon Receipt	Storage Checklist
DAb (Detection Antibody, 1000X)*	6 µl	12 µl	Ice Pack	–20°C	
Standard Control (100 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 Assay 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 **Standard Control** at –20°C away from light; (2) Store **WB**, **HAB**, **DS**, **8-Well Assay Strips** and **Control Assay 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
- ☐ Plasma or serum
- ☐ Parafilm M or aluminum foil

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiQuik™ Circulating Acetyl Histone H3K27 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. Thus, only use the User Guide that was supplied with the kit when using that kit.

Usage Limitation: The EpiQuik™ Circulating Acetyl Histone H3K27 ELISA Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic application.

A BRIEF OVERVIEW

Epigenetic activation or inactivation of genes plays a critical role in many important human diseases, especially in cancer. A major mechanism for epigenetic gene inactivation is methylation of CpG islands in genomic DNA caused by DNA methyltransferases. Histone acetyltransferases (HATs) and deacetylases (HDACs) control or regulate DNA methylation through chromatin-dependent transcriptional repression or activation. HATs transfer acetyl groups from acetyl CoA to the lysine residues of histone proteins. P300 is the major HAT that catalyzes acetylation of histone H3 at lysine 27 (H3K27) in mammalian cells. HDACs and SIRTs are the histone deacetylases that deacetylate H3K27. H3K27ac has been viewed as a signature mark of transcriptionally active genes, which is placed exclusively in the 5'- region downstream of the promoter. The H3K27ac can also be changed by inhibition or activation of HATs or HDAC/SIRTs. Circulating histone H3K27ac in plasma or serum

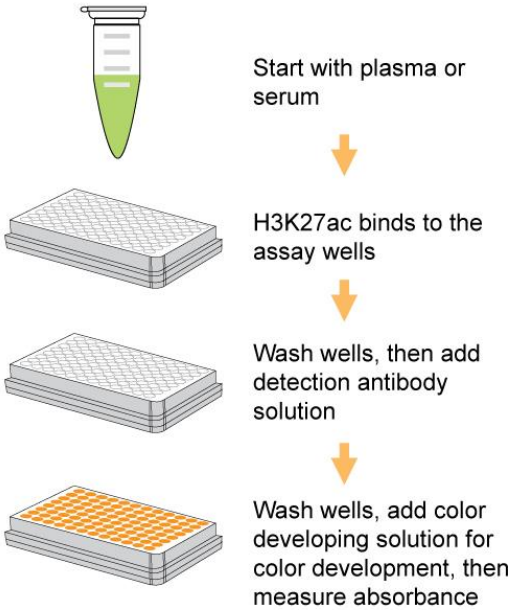
has been observed and demonstrated as the marker for many different diseases or pathological change such as cancer progression. Therefore, detection of circulating H3K27ac would provide useful information for a better understanding of epigenetic regulation of gene activation and silencing, histone modification-associated pathological processes, screening of disease-related biomarkers, as well as for developing histone modification-targeted drugs.

The EpiQuik™ Circulating Acetyl Histone H3K27 ELISA Kit (Colorimetric) is designed to quantitatively detect circulating histone H3K27ac from plasma and serum. This kit has the following advantages:

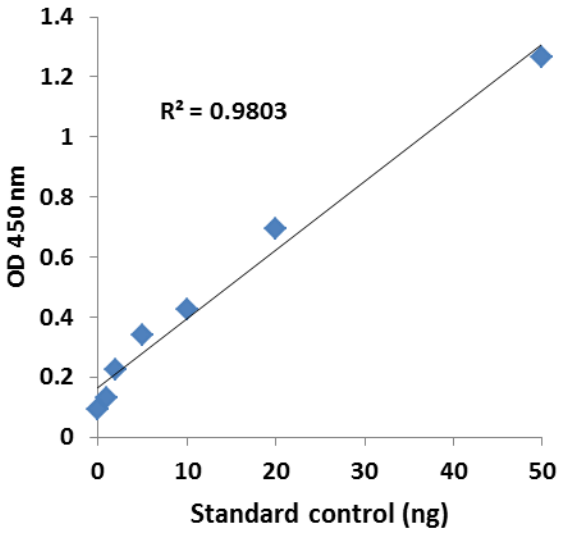
- Quick and efficient procedure, which can be finished within 2.5 hours.
- High sensitivity and specificity. The detection limit is as low as 0.5 ng/well with dynamic range of 1-20 ng/well within the indicated amount range of the plasma/serum. Only recognizes H3K27ac with no cross-reactivity with unmodified H3 or other modifications at the same lysine site.
- The control is conveniently included for the quantification of H3K27ac.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

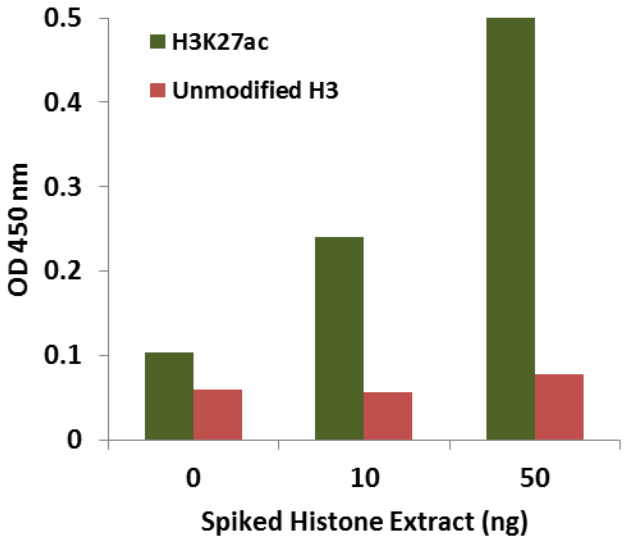
The EpiQuik™ Circulating Acetyl Histone H3K27 ELISA Kit (Colorimetric) is designed for measuring total H3K27acin plasma or serum. In an assay with this kit, the Histone H3 proteins acetylated at K27 in the plasma/serum sample are captured on the strip wells coated with anti-H3K27ac antibody. The captured H3K27ac proteins can be then recognized with detection antibody followed by a color development reagent. The ratio of H3K27ac is proportional to the intensity of absorbance. The absolute amount of H3K27ac can be quantitated by comparing to the standard control.



Schematic procedure of the EpiQuik™ Circulating Acetyl Histone H3K27 ELISA Kit (Colorimetric).



Illustrated standard curve.



Histone extracts were prepared from HL-60 cells using the EpiQuik™ Total Histone Extraction Kit (Cat. #OP-0006) and spiked into bovine plasma at different concentrations. The amount of H3K27ac was measured using the EpiQuik™ Circulating Acetyl Histone H3K27 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 plasma or serum for each assay can be 10 to 40 µl with an optimal amount of 30 µl.

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 DAb** Solution:

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

c. Prepare **Diluted Standard Control**

Suggested Standard Curve Preparation: First, dilute **Standard Control** to 50 ng/µl by adding 5 µl of **Standard Control** to 5 µl of **HAB** (Histone Assay Buffer) and to 5 ng/µl by adding 1 µl of **standard Control** to 19 µl of **HAB**. Then, further prepare seven concentrations by using the 5 ng/µl and 50 ng/µl of **Diluted Standard Control** with **HAB** into final concentrations of 0.5, 1, 2, 5, 10, 20, and 50 ng according to the following dilution chart:

Tube	Standard Control (5 ng/µl)	Standard Control (50ng/µl)	HAB	Resulting Concentration
1	1.0 µl		9.0 µl	0.5 ng/µl
2	1.0 µl		4.0 µl	1 ng/µl
3	2.0 µl		3.0 µl	2 ng/µl
4	4.0 µl		0 µl	5 ng/µl
5		1.0 µl	4.0 µl	10 ng/µl
6		2.0 µl	3.0 µl	20 ng/µl
7		4.0 µl	0.0 µl	50 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 50 µl of **HAB** to each blank well.

c. Standard Wells: Add 50 µl of **HAB** and 1 µl of **Diluted Standard Control** to each standard well, each at a different concentration between 0.5 and 50 ng/µl (based on the dilution chart in Step 1c; see Table 2 under the "Suggested Strip Well Setup" section as an example).

d. Sample Wells: Add 50 µl of **HAB** and 30 µl of your plasma or serum sample.

Note: *Follow the suggested well setup diagrams.*

e. Tightly cover strip-well microplate with **Adhesive Covering Film** to avoid evaporation and incubate at 37°C for 60 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. Wash each well three times with 150 µl of the **Diluted WB** (1X Wash Buffer) each time.

3. Detection Antibody Binding

a. Prepare **Diluted DAb** with **Diluted WB** 1X Wash Buffer at a ratio of 1:1000 (i.e., add 1 µl of **DAb** to 1000 µl of **Diluted WB**). Add 50 µl of the **Diluted DAb** to each well, then cover with Parafilm M or aluminium foil and incubate at room temperature for 60 min.

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

c. 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 H3K27ac product.

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. H3K27ac Calculation

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

b. Calculate % H3K27ac change using the following formula if the samples are from treated and un-treated control tests:

$$H3K27ac\% = \frac{Treated\ (Tested)\ Sample\ OD - Blank\ OD}{Untreated\ (Control)\ Sample\ OD - Blank\ OD} \times 100\%$$

Example calculation:

Average OD450 of treated sample is 0.3
Average OD450 of untreated control is 0.4
Average OD450 of blank is 0.1

$$H3K27ac\% = \frac{0.3 - 0.1}{0.4 - 0.1} \times 100\% = 66.7\%$$

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), then calculate the amount of H3K27ac using the following formulas:

$$H3K27ac\ (ng/ml) = \frac{(Sample\ OD - Blank\ OD)}{Slope\ x\ sample\ amount\ (ul^*)} \times 1000$$

** Plasma or serum added into sample wells at Step 2d.*

Note: *To measure the content of H3K27ac in total histone H3 for normalizing accuracy of the quantified H3K27ac %, total histone H3 amount in the samples should be quantified. The EpiQuik™ Circulating Total Histone H3 Quantification Kit (Colorimetric) (Cat # P-3091) can be used for this application.*

SUGGESTED BUFFER AND SOLUTION SETUP

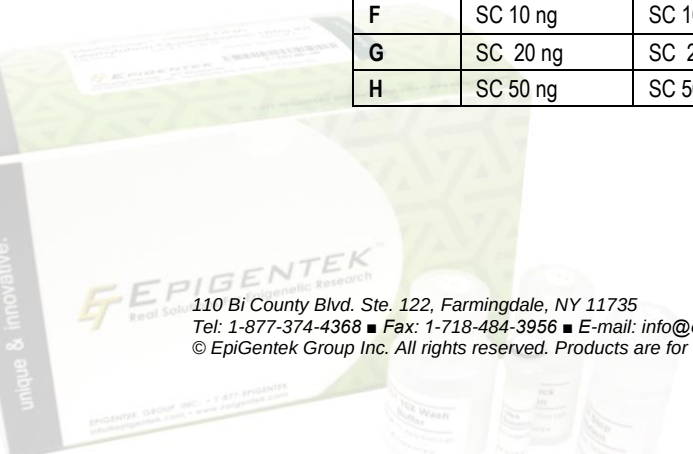
Table 1. Approximate amount of required buffers and solutions for defined assay wells based on the protocol.

Reagents	1 well	1 strip (8 wells)	2 strips (16 wells)	6 strips (48 wells)	12 strips (96 wells)
Diluted WB	2.5 ml	20 ml	40 ml	120 ml	240 ml
HAB	50 µl	400 µl	800 µl	2400 µl	4800 µl
Standard Control	N/A	N/A	4 µl (optional)	8 µl	8 µl
Diluted DAb	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

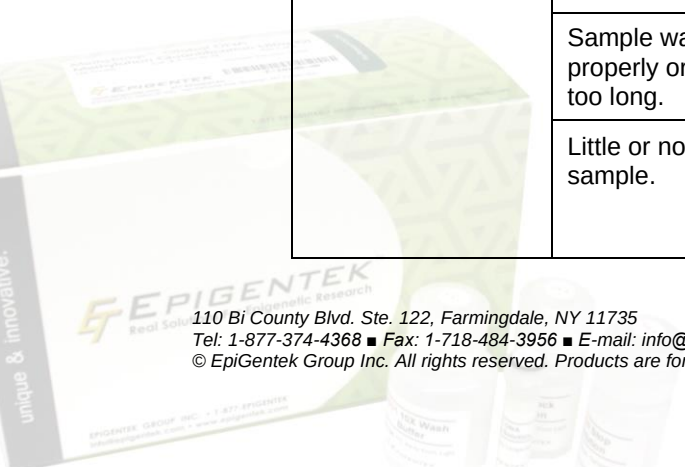
Table 2. The suggested strip-well plate setup for H3K27ac quantification in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can be measured in duplicate. Strip 1 and Strip 2 are the green trimmed control strips.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	SC 0.5 ng	SC 0.5 ng	Sample	Sample	Sample	Sample
C	SC 1 ng	SC 1 ng	Sample	Sample	Sample	Sample
D	SC 2 ng	SC 2 ng	Sample	Sample	Sample	Sample
E	SC 5 ng	SC 5 ng	Sample	Sample	Sample	Sample
F	SC 10 ng	SC 10 ng	Sample	Sample	Sample	Sample
G	SC 20 ng	SC 20 ng	Sample	Sample	Sample	Sample
H	SC 50 ng	SC 50 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 fastened 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 are 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 **DAb** is too long.	The incubation time at Step 3a 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	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of plasma or serum is used as indicated in Step 2d.
	Sample was not stored properly or has been stored for too long.	Ensure plasma or serum is stored in aliquots at proper temperature, for no more than 6 months.
	Little or no H3K27ac in the sample.	This problem may be a result of many factors. If the affecting factors cannot be determined, use new or re-prepared samples.



Uneven color development	Insufficient washing of the wells.	Ensure the wells are washed according to the guidance of washing and that 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 H or from well 1 to well 12).

RELATED PRODUCTS

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

Histone H3 Quantification
P-3091 EpiQuik™ Circulating Total Histone H3 Quantification Kit (Colorimetric)