EpiQuik™ Tissue Acetyl-Histone H3 ChIP Kit

Base Catalog # P-2012

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ Tissue Acetyl-Histone H3 ChIP Kit is suitable for combining the specificity of immunoprecipitation with qualitative and quantitative PCR, MS-PCR, DNA sequencing, and southern blot, as well as DNA microarray.

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KIT CONTENTS

Important Information: The amount of components supplied in this kit is designed for reaction count, not sample count, such as negative IgG controls and input DNA. Thus, experiments with samples to be paired with both IgG and input may require additional columns or components to be purchased separately. Please calculate the necessary volumes based on the below kit contents and protocol prior to starting the experiment.

Components	24 reactions P-2012-24	48 reactions P-2012-48
CP1 (Wash Buffer)	28 ml	2 x 28 ml
CP2 (Antibody Buffer)	15 ml	30 ml
CP3 (Lysis Buffer)	4 ml	6 ml
CP4 (ChIP Dilution Buffer)	4 ml	6 ml
CP5 (DNA Release Buffer)	2 ml	2 x 2 ml
CP6 (Reverse Buffer)	2 ml	2 x 2 ml
CP7 (Binding Buffer)	5 ml	8 ml
CP8 (Elution Buffer)	0.6 ml	1.2 ml
Homogenizing Buffer	5 ml	10 ml
Protease Inhibitor Cocktail (100X)*	25 µl	40 µl
Non-immune IgG (1 mg/ml)*	10 µl	20 µl
Proteinase K (10 mg/ml)*	25 µl	50 µl
Anti-Acetyl Histone H3 (1 mg/ml)*	25 µl	50 µl
8-Well Assay Strips (with Frame)	3	6
8-Well Strip Caps	3	6
F-Spin Column	30	50
F-Collection Tube	30	50

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

SHIPPING & STORAGE

The kit is shipped in two parts: one part at ambient room temperature, and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store the following components at 4°C: **Protease Inhibitor Cocktail**, **Non-immune IgG**, **Anti-Acetyl Histone H3**, **Proteinase K** and **8-Well Assay Strips.** (2) Store **all other components** at room temperature.

The kit is stable for up to 6 months from the shipment date, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Variable temperature waterbath
- ☐ Vortex mixer
- ☐ Desktop centrifuge (up to 14,000 rpm)
- ☐ Dounce homogenizer
- ☐ Sonicator
- ☐ Orbital shaker
- ☐ Pipettes and pipette tips
- ☐ 1.5 ml microcentrifuge tubes
- ☐ 15 ml conical tube
- ☐ TE buffer (pH 8.0)
- ☐ Ethanol (96-100%)

GENERAL PRODUCT INFORMATION

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*™ ChIP kits are for research use only and are not intended for diagnostic or therapeutic application.

Intellectual Property: The *EpiQuik*™ ChIP kits and methods of use contain proprietary technologies by EpigenTek. *EpiQuik*™ is a trademark of EpigenTek, Inc.

A BRIEF OVERVIEW

Histone acetylation takes place at the α-amino groups of conserved lysine residues, where histone acetyltransferases (HATs) are responsible for catalyzing this modification. It has been demonstrated that histone H3 acetylation serves as an epigenetic marker of chromosomal domains. Histone H3 acetylation regulates various cellular physiological processes, including transcriptional activation of genes, chromatin assembly, cell proliferation, and some pathological processes such as tumorigenesis.

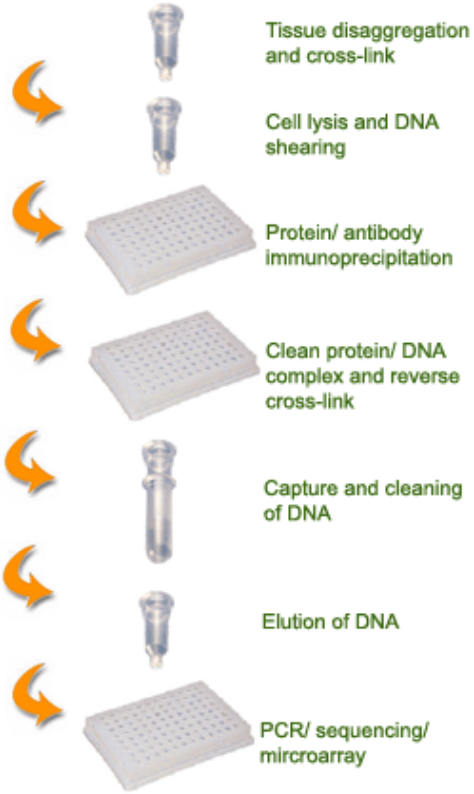
Chromatin Immunoprecipitation (ChIP) is a powerful technique for studying protein-DNA interaction *in vivo*. ChIP also offers an advantageous tool that allows identification of activated genes associated with acetylated histone H3. ChIP coupled with microarrays could be further used for profiling or mapping histone H3 acetylation patterns. There are several methods used for chromatin immunoprecipitation, however, most of these methods available so far are considerably time consuming and labor intensive, or have low throughput. Furthermore, these methods are not specifically designed for solid tissues.

The *EpiQuik*™ Tissue Acetyl-Histone H3 ChIP Kit uses a proprietary and unique procedure/composition to investigate interactions of histone H4 acetylation and DNA in variety of mammalian tissues. The *EpiQuik*™ Tissue Acetyl-Histone H3 ChIP Kit has the following features:

- The fastest procedure available, which can be finished within 5 hours.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Columns for DNA purification are included: save time and reduce labor.
- Compatible with all DNA amplification-based approaches.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*™ Tissue Acetyl-Histone H3 ChIP Kit contains all reagents required for carrying out a successful chromatin immunoprecipitation for acetyl-histone H3 from mammalian cells. Particularly, this kit includes a ChIP-grade acetyl-histone H3 antibody and a negative control Non-immune IgG. Chromatin from the cells is extracted, sheared, and added into the microwell immobilized with the antibody. DNA is released from the antibody-captured acetyl-histone H3 protein-DNA complex, reversed and purified through the specifically designed Fast-Spin Column. Eluted DNA can be used for various down-stream applications.



Schematic Procedure for Using the *EpiQuik*™ Tissue Acetyl-Histone H3 ChIP Kit

PROTOCOL

Note: Always cap spin columns before placing them in the microcentrifuge.

Before starting, perform the following:

1. Prepare the following required solutions (not included): 90% Ethanol; 70% Ethanol; 37% Formaldehyde; 1.25 M Glycine Solution; 1X TE Buffer (pH 8.0).
2. Ensure that all buffers are in clear solution. Shake or vortex if these buffers precipitate.

Antibody Binding to the Assay Plate

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). Wash the strip wells once with 150 µl of CP1.
2. Add 100 µl of CP2 to each well and then add the antibodies: 1 µl of Non-immune IgG as the negative control, and 1 µl of Anti-Acetyl Histone H3 for the samples.
3. Cover the strip wells with Parafilm M and incubate at room temperature for 60-90 minutes. Meanwhile, prepare the cell extracts as described in the next steps.

Tissue Disaggregation and *In Vivo* Cross-Link

1. Place the tissue sample into a 60 or 100 mm plate. Remove unwanted tissue such as fat and necrotic material from the sample. Weigh the sample and cut it into small pieces (1-2 mm^3) with a scalpel or scissors.
2. Transfer tissue pieces to a 15 ml conical tube. Prepare the Cross-Link Solution by adding *1% formaldehyde* to culture medium (final concentration is 1%. Ex: add 270 µl of *37% formaldehyde* to 10 ml of culture medium). Add 1 ml of the Cross-Link Solution per every 40 mg of tissue and incubate at room temperature for 15-20 minutes on a rocking platform.
3. Add 1 ml of *1.25 M Glycine solution* per every 9 ml of Cross-Link Solution; mix and centrifuge at 800 rpm for 5 minutes. Discard the supernatant. Wash cells with 10 ml of ice-cold PBS once by centrifugation at 800 rpm for 5 minutes. Discard the supernatant.
4. Transfer tissue pieces to a Dounce homogenizer. Add 1 ml of the Homogenizing Buffer per every 200 mg of tissue, and disaggregate tissue pieces by 10-30 strokes.
5. 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 5000 rpm for 5 minutes at 4°C. Remove supernatant.

Cell Lysis and DNA Shearing

1. Add CP3 containing Protease Inhibitor Cocktail (PIC) (Ex: 10 µl of PIC to each 1 ml of CP3) to re-suspend the disaggregated tissue pellet (100 µl/20 mg of tissue). Transfer cell suspension to a 1.5 ml vial (500 µl maximum for each vial). Incubate the sample on ice for 10 minutes and vortex occasionally.
2. Shear DNA by sonication. Usually, sonicate 4-5 pulses of 15-20 seconds each at level 2 using a Branson Microtip probe, followed by 30-40 seconds rest on ice between each pulse. (*The conditions of cross-linked DNA shearing can be optimized based on tissue type and sonicator*

equipment. If desired, remove 5 µl of sonicated cell lysate for agarose gel analysis. The length of sheared DNA should be between 200-1000 bp.)

3. Pellet cell debris by centrifuging at 14,000 rpm for 10 minutes.

Protein/DNA Immunoprecipitation

1. Transfer clear supernatant to a new 1.5 ml vial (supernatant can be stored at –80°C at this step). Dilute required volume of supernatant with **CP4** at a 1:1 ratio (ex: add 100 µl of **CP4** to 100 µl of supernatant).
2. Remove 5 µl of the *diluted supernatant* to a 0.5 ml vial. Label the vial as "input DNA" and place on ice.
3. Remove the incubated antibody solution and wash the strip wells three times with 150 µl of **CP2** by pipetting in and out.
4. Transfer 100 µl of the *diluted supernatant* to each strip well. Cover the strip wells with Parafilm M and incubate at room temperature (22-25°C) for 1 hour on a rocking platform (50-100 rpm)
5. Remove supernatant. Wash the wells six times with 150 µl of **CP1**. Allow 2 minutes on a rocking platform (100 rpm) for each wash. Wash the wells once (for 2 minutes) with 150 µl of *1X TE Buffer*.

Cross-Linked DNA Reversal/DNA Purification

1. Add 1 µl of **Proteinase K** to each 40 µl of **CP5** and mix. Add 40 µl of **CP5** containing **Proteinase K** to the samples (including the "input DNA" vial). Cover the sample wells with strip caps and incubate at 65°C in a waterbath for 15 minutes.
2. Add 40 µl of **CP6** to the samples, mix, and re-cover the wells with strip caps and incubate at 65°C in a waterbath for 90 minutes. Also add 40 µl of **CP6** to the vial containing *supernatant*, labeled as "input DNA." Mix and incubate at 65°C for 90 minutes.
3. Place a spin column into a 2 ml collection tube. Add 150 µl of **CP7** to the samples and transfer mixed solution to the column. Centrifuge at 12,000 rpm for 20 seconds.
4. Add 200 µl of *70% ethanol* to the column, centrifuge at 12,000 rpm for 15 seconds. Remove the column from the collection tube and discard the flowthrough.
5. Replace column to the collection tube. Add 200 µl of *90% ethanol* to the column and centrifuge at 12,000 rpm for 20 seconds.
6. Remove the column and discard the flowthrough. Replace column to the collection tube and wash the column again with 200 µl of *90% ethanol* at 12,000 rpm for 35 seconds.
7. Place the column in a new 1.5 ml vial. Add 10-20 µl of **CP8** directly to the filter in the column and centrifuge at 12,000 rpm for 20 seconds to elute purified DNA.

DNA is now ready for use or storage at –20°C.

Note: *For conventional PCR, the number of PCR cycles may need to be optimized for better PCR results.*

TROUBLESHOOTING

Little or No PCR Products

1. Insufficient tissues.	Increase tissue amount (ex: >10 mg of tissue/per reaction).
2. Insufficient or too much cross-linking.	Check if the appropriate cross-link step is carried out according to the protocol.
3. Insufficient cell lysis.	Follow the guidelines in the protocol. Check the cell lysis by observing a 5 μl portion of the tissue lysate under the microscope.
4. Insufficient/too much sonication.	Follow the protocol instructions for obtaining the appropriate sized DNA. Keep the sample on ice during the sonication.
5. Incorrect temperature/insufficient time for DNA release and reversal of cross-linking.	Follow the guidelines in the protocol for appropriate temperature and time.
6. Incorrect PCR conditions.	Check if all PCR components are added. Increase amount of DNA added to PCR reaction. Increase the number of cycles for PCR reaction.
7. Incorrect or bad primers.	Ensure the designed primers are specific to the target sequence.
8. The column is not washed with 90% ethanol.	Ensure that wash solution is 90% ethanol.
9. DNA is not completely passed through the filter.	Purify DNA before modification and increase centrifuge time to 1 minute at steps 3 to 7 of "Cross-Linked DNA Reversal/DNA Purification."

Little or No Amplification Difference Between the Sample and the Negative Control

1. Insufficient wash at each wash step.	Follow the protocol for appropriate wash.
2. Antibody is added into the well for the negative control by mistake.	Ensure antibody is added into the correct well.
3. Too many PCR cycles.	If using conventional PCR, decrease the cycles to appropriate cycle number. Differences between quantities of starting DNA can be measured

	generally within the linear PCR amplification phase.
4. Little or no enrichment of acetyl-histone H3 in target promoters.	N/A.

RELATED PRODUCTS

P-2002	EpiQuik™ Chromatin Immunoprecipitation (ChIP) Kit
P-2003	EpiQuik™ Tissue Chromatin Immunoprecipitation (ChIP) Kit
P-2006	EpiQuik™ Methyl-Histone H3-K9 ChIP Kit
P-2007	EpiQuik™ Methyl-Histone H3-K4 ChIP Kit
P-2008	EpiQuik™ Tissue Methyl-Histone H3-K9 ChIP Kit
P-2009	EpiQuik™ Tissue Methyl-Histone H3-K4 ChIP Kit
P-2010	EpiQuik™ Acetyl-Histone H3 ChIP Kit
P-2011	EpiQuik™ Acetyl-Histone H4 ChIP Kit
P-2013	EpiQuik™ Tissue Acetyl-Histone H4 ChIP Kit
P-2015	EpiQuik™ Methyl-Histone H3-K27 ChIP Kit
P-2016	EpiQuik™ Tissue Methyl-Histone H3-K27 ChIP Kit
P-2017	EpiQuik™ Methyl-CpG Binding Domain Protein 2 ChIP Kit
P-2018	EpiQuik™ Tissue Methyl-CpG Binding Domain Protein 2 ChIP Kit