



EpiQuik™ Tissue Methyl-Histone H3-K9 ChIP Kit

Base Catalog # P-2008

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The EpiQuik™ Tissue Methyl-Histone H3-K9 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.

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-2008-24 | 48 reactions P-2008-48 |
|-------------------------------------|---------------------------|---------------------------|
| 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 μ l | 40 μ l |
| Non-immune IgG (1 mg/ml)* | 10 μ l | 20 μ l |
| Proteinase K (10 mg/ml)* | 25 μ l | 50 μ l |
| Anti-Dimethyl-H3-K9 (1 mg/ml)* | 25 μ l | 50 μ l |
| 8-Well Assay Strips (with Frame) | 3 | 6 |
| 8-Well Strip Caps | 3 | 6 |
| F-Spin Column | 30 | 50 |
| F-Collection Tube | 30 | 50 |

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

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-Dimethyl-H3-K9, 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
- ☐ 37% formaldehyde
- ☐ Glycine solution
- ☐ 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 method of use contain proprietary technologies by EpigenTek. *EpiQuik*™ is a trademark of EpigenTek, Inc.

A BRIEF OVERVIEW

The position of the histone H3 at lysine 9 (H3-K9) is important for epigenetic regulation of the genes, as it can be either methylated or acetylated. It was well demonstrated that switching acetylation to methylation on H3-K9 leads to chromatin gene silencing in many organisms. Methylated H3-K9 has been involved in tumorigenesis through inactivation of tumor suppression genes. Thus, identification of genes silenced through H3-K9 methylation is of particular significance for understanding and analyzing biological processes under the normal and pathological conditions.

Chromatin Immunoprecipitation (ChIP) is a powerful technique for studying protein-DNA interaction *in vivo*. ChIP also offers an advantageous tool that allows identification of silenced

genes associated with methylated histone H3-K9. ChIP coupled with microarrays could be further used for profiling or mapping histone H3-K9 methylation patterns. Requirement for using ChIP to analyze histone-DNA interaction in solid tissues and tumors is rapidly increasing. There are several methods used for chromatin immunoprecipitation; however, most of these methods available so far are considerably time consuming, labor intensive, or have low throughput. Furthermore, these methods are not specifically designed for solid tissues.

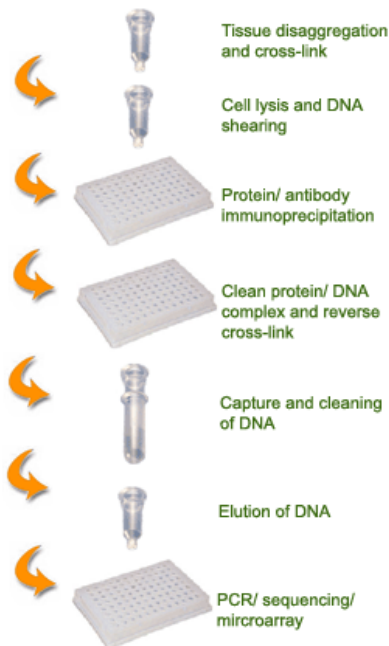
The *EpiQuik*[™] Tissue Methyl-Histone H3-K9 ChIP Kit uses a proprietary and unique procedure and composition to investigate interactions of histone H3-K9 methylation and DNA in a variety of mammalian tissues. The *EpiQuik*[™] Tissue Methyl-Histone H3-K9 ChIP Kit has the following features:

- 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 Methyl-Histone H3-K9 ChIP Kit contains all reagents necessary for carrying out a successful chromatin immunoprecipitation for methyl-histone H3-K9 from mammalian tissues. Particularly, this kit includes a ChIP-grade methyl-histone H3-K9 antibody and a negative control Non-immune IgG. Chromatin from the cells is extracted, sheared, and added into the micro-well immobilized with the antibody. DNA is released from the antibody-captured methyl-histone H3-K9 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 Methyl-Histone H3-K9 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; 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 strip wells once with 150 μ l of **CP1**.
2. Add 100 μ l of **CP2** to each well and then add the antibodies: 1 μ l of **Non-immune IgG** as the negative control, and 1 μ l of **Anti-Dimethyl-H3-K9** 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 the sample into small pieces (1-2 mm³) with a scalpel or scissors.
2. Transfer tissue pieces to a 15 ml conical tube. Prepare the **Cross-Link Solution** by adding formaldehyde to culture medium (final concentration is 1%. Ex: add 270 μ l 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 an orbital shaker (50-100 rpm).
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 **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 μ l of **PIC** to each 1 ml of **CP3**) to re-suspend the disaggregated tissue pellet (100 μ l/20 mg of tissue). Transfer cell suspension to a 1.5 ml vial (500 μ l 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 μ l 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 μ l of **CP4** to 100 μ l of supernatant).
2. Remove 5 μ l 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 μ l of **CP2** by pipetting in and out.
4. Transfer 100 μ l of the *diluted supernatant* to each strip well. Cover the strip wells with Parafilm M and incubate at room temperature ($22-25^{\circ}\text{C}$) for 1 hour on an orbital shaker (50-100 rpm).
5. Remove supernatant. Wash the wells six times with 150 μ l of **CP1**. Allow 2 minutes on an orbital shaker (100 rpm) for each wash. Wash the wells once (for 2 minutes) with 150 μ l of 1X TE Buffer.

Cross-Linked DNA Reversal/DNA Purification

1. Add 1 μ l of **Proteinase K** to each 40 μ l of **CP5** and mix. Add 40 μ l of **CP5** containing **Proteinase K** to the samples (including "input DNA" vial). Cover the sample wells with strip caps and incubate at 65°C in a waterbath for 15 minutes.
2. Add 40 μ l 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 μ l 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 μ l of **CP7** to the samples and transfer mixed solution to the column. Centrifuge at 12,000 rpm for 20 seconds.
4. Add 200 μ l 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 μ l 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 μ l of 90% ethanol at 12,000 rpm for 35 seconds.
7. Place the column in a new 1.5 ml vial. Add 10-20 μ l 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

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|---|--|
| 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 tissue lysis. | Follow the guidelines in the protocol. Check the tissue lysis by observing a 5 μ l portion of the tissue lysate under the microscope. |
| 4. Insufficient/too much sonication. | Follow the protocol instruction 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. Wrong 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. | 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 procedure. |
| 2. Antibody is added into the well for the negative control by mistake. | Ensure antibody is added into the correct well. |
| 3. No enrichment of methyl H3-K9 in target promoters. | N/A. |

4. 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.

RELATED PRODUCTS

| | |
|--------|--|
| P-2002 | EpiQuik™ Chromatin Immunoprecipitation (ChIP) Kit |
| P-2003 | EpiQuik™ Tissue Chromatin Immunoprecipitation Kit |
| P-2006 | EpiQuik™ Methyl-Histone H3-K9 ChIP Kit |
| P-2007 | EpiQuik™ Methyl-Histone H3-K4 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-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 |