EpiQuik™ Methylated DNA Immunoprecipitation Kit

Base Catalog # P-2019

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ MeDIP Kit can be used for immunoprecipitating the methylated DNA from a broad range of species including human, rat, and mouse.

The *EpiQuik*™ MeDIP Kit is suitable for combining the specificity of methylated DNA immunoprecipitation with qualitative and quantitative PCR, 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-2019-24	48 reactions P-2019-48
CP1 (Wash Buffer)	28 ml	2 x 28 ml
CP2 (Antibody Buffer)	15 ml	30 ml
CP3A (Pre-Lysis Buffer)	4 ml	8 ml
CP3B (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
Non-immune IgG (1 mg/ml)*	10 µl	15 µl
Proteinase K (10 mg/ml)*	25 µl	50 µl
Anti-5-Methylcytosine (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 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: Non-immune IgG, Proteinase K, Anti-5 Methylcytosine and 8-Well Assay Strips. (2) Store all other components at room temperature.

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

Note: *Avoid repeated thawing and re-freezing of temperature sensitive components. It is recommended that you aliquot accordingly ahead of time.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Variable temperature waterbath
- ☐ Vortex mixer
- ☐ Desktop centrifuge (up to 14,000 rpm)
- ☐ 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

Quality Control: EpigenTek guarantees the performance of all products in the manner described in our product instructions.

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*™ MeDIP Kit is for research use only and is not intended for diagnostic or therapeutic application.

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

A BRIEF OVERVIEW

Core mechanism for epigenetic alterations of genomic DNA is hypermethylation of CpG islands in specific genes and global DNA hypomethylation. Region-specific DNA methylation is mainly found in 5′-CpG-3′dinucleotides within the promoters or in the first exon of genes, which plays an important role in the repression of gene transcription. Global DNA hypomethylation is likely caused by methyl-deficiency due to a variety of environmental influences. It has been demonstrated that alterations in DNA methylation are associated with many diseases, and especially with cancer.

Highly specific isolation of methylated DNA should provide an advantage for convenient and comprehensive identification of methylation status of normal and diseased cells, such as cancer cells, which may lead to the development of new diagnostic and therapeutic methods of cancer. Several methods have been used for enriching methylated DNA, including methyl-CpG binding domain (MBD) based methylated DNA affinity column and methylated DNA immunoprecipitation.

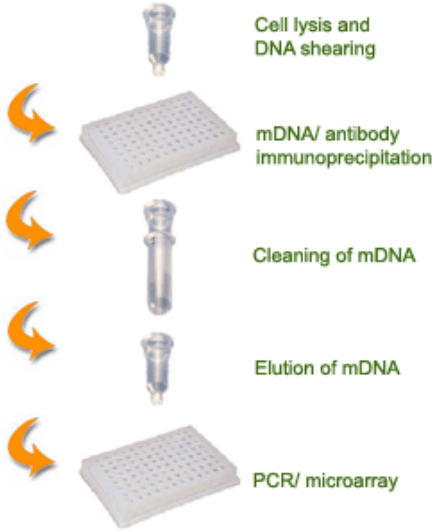
However, these methods so far are considerably time consuming, labor intensive, have low throughput, and particularly, need purified DNA as starting material.

The *EpiQuik*™ Methylated DNA Immunoprecipitation (MeDIP) Kit uses a proprietary and unique procedure and composition to enrich methylated DNA. In the assay, an antibody specific to methyl cytosine is used to immunoprecipitate methylated genomic DNA. The immunoprecipitated methylated fractions can then be used for a standard DNA detection. The *EpiQuik*™ Methylated DNA Immunoprecipitation Kit has the following features:

- Directly immunoprecipitate the methylated fractions of DNA from cell lysates.
- Highly efficient enrichment of methylated DNA: > 95%.
- The fastest procedure available, which can be finished within 4 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*™ Methylated DNA Immunoprecipitation (MeDIP) Kit contains all reagents required for carrying out a successful methylated DNA immunoprecipitation directly from mammalian cells. Particularly, this kit includes a ChIP-grade 5-methylcytosine antibody and a negative control Non-immune IgG. DNA in the cells is extracted, sheared, and added into the microwell immobilized with the antibody. DNA is released from the antibody-DNA complex 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*™ Methylated DNA Immunoprecipitation 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; 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-5-Methylcytosine for the sample.
3. Cover the strip wells with Parafilm M and incubate at room temperature for 60 minutes. Meanwhile, prepare the cell extracts as described in the next steps.

Cell Collection

For Monolayer or Adherent Cells:

1. Grow cells (treated or untreated) to 80%-90% confluency. Remove culture medium and wash cells with PBS once. (At least 1 x 10^5 cells are required for each reaction.)
2. Add 20 µl of CP3B Lysis Buffer to each well for the 96-well plate; or 100 µl of CP3B for the 24-well plate; or 200 µl of CP3B for the 12-well plate; or 400 µl of CP3B for the 6-well plate; or 1.5 ml of CP3B for a 100 mm plate. Incubate for 5 minutes at room temperature and pipette up and down several times to break cells.

Container	Cell Number (x 10^5)
96-well plate	0.3-0.6/well
24-well plate	1-3/well
12-well plate	3-6/well
6-well plate	5-10/well
100 mm plate	50-100

For Suspension Cells:

1. Collect cells (treated or untreated) into a 15 ml conical tube. (2-5 x 10^5 cells are required for each reaction). Centrifuge the cells at 1000 rpm for 5 minutes and discard the supernatant. Wash cells with 10 ml of PBS once by centrifugation at 1000 rpm for 5 minutes. Discard the supernatant.
2. Add CP3A to re-suspend the cell pellet (20 µl/1 x 10^5 cells). Transfer cell suspension to a 1.5 ml vial and incubate on ice for 10 minutes. Vortex vigorously for 10 seconds and centrifuge at 5000 rpm for 5 minutes. Discard the supernatant.
3. Add 10 µl of CP3B buffer to each 1 x 10^5 cells.

DNA Shearing

1. Transfer cell solution to a 1.5 ml vial (*500 µl maximum for each vial*) and incubate at room temperature for 10 minutes. Vortex vigorously for 10 seconds.
2. Shear DNA by sonication. Usually, sonicate 4 to 5 pulses of 10 to 12 seconds each at level 2 using a Branson Microtip probe, followed by 30 to 40 seconds rest on ice between each pulse. (*The conditions of DNA shearing can be optimized based on cells and sonicator equipment. If desired, remove 5 µl of the 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.

Methylated 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 60 to 90 minutes 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*.

Methylated DNA Isolation/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 30 minutes. Also add 40 µl of **CP6** to the vial containing *supernatant*, labeled as "input DNA." Mix and incubate at 65°C for 45 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 20 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.

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

Note: *For PCR positive control (methylation) and negative control (unmethylation), the primers for highly methylated sequences of H19ICR, LAP or XIST and the primer for unmethylated β-actin or GAPDH sequence could be used, respectively. For conventional PCR, the number of PCR cycles may need to be optimized for the better PCR results.*

References: Weber M et al: Nature Genetics, 37: 853-862, 2005.

TROUBLESHOOTING

Little or No PCR Products

1. Insufficient cells.	Increase tissue amount (ex: >1 million cells/per reaction).
2. 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.
3. Insufficient or too much sonication.	Follow the protocol instructions for obtaining the appropriate sized DNA. Keep the sample on ice during the sonication.
4. Incorrect temperature/insufficient time for DNA release.	Follow the guidelines in the protocol for appropriate temperature and time.
5. 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.
6. Incorrect or bad primers.	Ensure the designed primers are specific to the target sequence.
7. The column is not washed with 90% ethanol.	Ensure that wash solution is 90% ethanol.
8. DNA is not completely passed through the filter.	Increase centrifuge time to 1 minute at steps 3 to 7 of "Methylated DNA Isolation/Purification."

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

1. Insufficient wash at each wash step.	Check if washing recommendations at each step is performed according to the protocol.
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.

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-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-2012	EpiQuik™ Tissue Acetyl-Histone H3 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-2020	EpiQuik™ Tissue Methylated DNA Immunoprecipitation Kit

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