



Version 2.0902

**EpiQuik™ DNA Methyltransferase Activity/
Inhibition Assay Kit**

Catalog No. P-3001

User Guide*

***Always use the most updated User
Guide included in your current order.**

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FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

TABLE OF CONTENTS

Introduction	3
Principle and Procedure	4
Product Use Information	5
Kit Contents	6
Shipping and Storage	6
Materials Required But Not Supplied	6
Protocol	7
Troubleshooting	9
Ordering Information	11

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INTRODUCTION

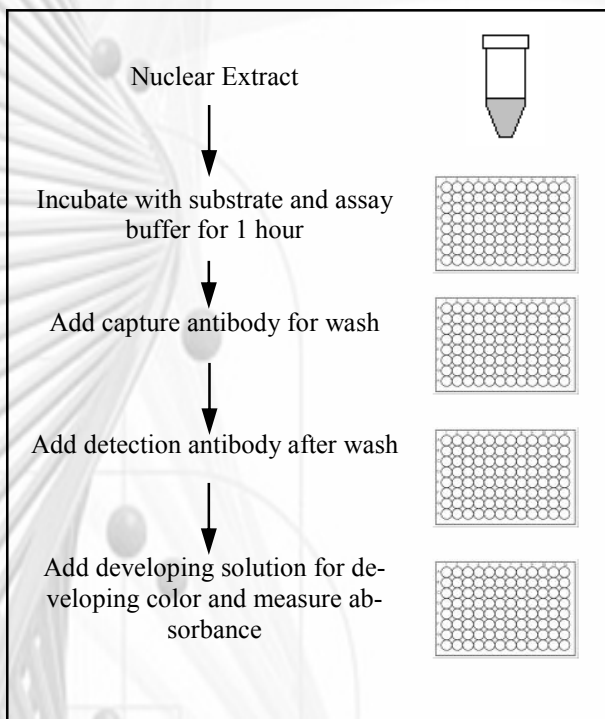
Epigenetic inactivation of genes plays a critical role in many important human diseases, especially in cancer. Core mechanism for epigenetic inactivation of the genes is methylation of CpG islands in genome DNA. Methylation of CpG islands involves the course in which DNA methyltransferases (Dnmts) transfer a methyl group from S-adenosyl-L-methionine to the fifth carbon position of the cytosines. Four active Dnmts have been identified in mammals. They are named DNMT1, DNMT2, DNMT3A, and DNMT3B. Inhibition of Dnmts may lead to demethylation and expression of the silenced genes. Dnmt inhibitors are currently being developed as potential anticancer agents.

There are only a couple of methods used for measuring Dnmt activity/inhibition. These methods available so far are time consuming, labor-intensive, have low throughput, or produce radioactive waste. The *EpiQuik*[™] DNA Methyltransferase Activity/Inhibition Assay Kit addresses these problems by using a unique procedure to measure Dnmt activity/inhibition. The kit has following features:

- Extremely fast procedure, which can be completed within 3 hours.
- Innovative colorimetric assay without radioactivity, extraction and chromatography.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE AND PROCEDURE

The *EpiQuik*[™] DNA Methyltransferase Activity/Inhibition Assay Kit is designed for measuring total Dnmt activity (de novo, maintenance). In an assay with this kit, the unique cytosine-rich DNA substrate is stably coated on the strip wells. These wells are specifically treated to have a high DNA absorption ability. Dnmt enzymes transfer methyl group to cytosine from Adomet to methylate DNA substrate. The methylated DNA can be recognized with anti-5-methylcytosine antibody. The ratio or amount of methylated DNA, which is proportional to enzyme activity, can then be colorimetrically quantified through an ELISA-like reaction.



Schematic Procedure for Using the *EpiQuik*[™] DNA Methyltransferase Activity/Inhibition Assay Kit

PRODUCT USE INFORMATION

The *EpiQuik*[™] DNA Methyltransferase Activity/Inhibition Assay Kit is suitable for measuring Dnmt activity/inhibition from a broad range of species including mammalian cells/tissues, plants, and bacteria.

Epigentek guarantees the performance of all products in the manner described in our product instructions.

Epigentek reserves the right to change or modify any product to enhance its performance and design.

The *EpiQuik*[™] DNA Methyltransferase Activity/Inhibition Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

EpiQuik[™] is a trademark of Epigentek Group Inc.

The *EpiQuik*[™] DNA Methyltransferase Activity/Inhibition Assay Kit and methods of use are covered by a pending US patent.

KIT CONTENTS

Components	48 assays P-3001-1	96 assays P-3001-2
M1 (10X wash buffer)	11 ml	22 ml
M2 (Dnmt assay buffer)	1.5 ml	3 ml
M3 (Adomet, 8 mM)*	35 μ l	70 μ l
M4 (Dnmt positive control)*	5 μ l	10 μ l
M5 (capture antibody)*	5 μ l	8 μ l
M6 (detection antibody 200 μ g/ml)*	10 μ l	20 μ l
M7 (developing solution)	6 ml	12 ml
M8 (stop solution)	3 ml	6 ml
8 well substrate-coated strip (with frame)	6	12
User guide	1	1

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

SHIPPING AND STORAGE

Store **M3**, **M4** and **M6**, at -20°C away from light. Store other components at 4°C away from light. All components are stable for 6 months from date of shipment in proper storage.

MATERIALS REQUIRED BUT NOT SUPPLIED

Orbital shaker
Pipettes and pipette tips
Microplate reader
1.5 ml microcentrifuge tubes

PROTOCOL

1. Prepare nuclear extracts by using your own successful method. For your convenience and best results, Epigentek offers a nuclear extraction kit (Cat # OP-0002-1) optimized for use in the *EpiQuik*[™] series. Nuclear extracts can be used immediately or stored at -80°C for future use.
2. Determine number of the strip wells required. Leave these strips in the plate frame (remaining unused strips can be put back in the bag. Seal the bag tightly and store at 4°C). Dilute **10 X M1** with distilled water (pH 7.2-7.5) to **1 X M1**. Wash strip wells once with $150\ \mu\text{l}$ of **1 X M1**.
3. Dilute **M3** with **M2** (at the 1:5 ratios) to 1.6 mM. Add $24\ \mu\text{l}$ of **M2** and $3\ \mu\text{l}$ of the diluted **M3** to each strip well. Then add $3\ \mu\text{l}$ of nuclear extracts (4-20 μg) or purified Dnmt enzymes, mix and cover the strip wells with Parafilm M and incubate at 37°C for 60-90 min. For the positive control, add 0.5-1 μl of **M4** and 2-2.5 μl of **M2** instead of nuclear extracts. For Dnmt inhibition, add $3\ \mu\text{l}$ of tested inhibitors at different concentrations and reduce **M2** volume to 21 μl . For blank, add $3\ \mu\text{l}$ of **M2** instead of nuclear extracts.
4. Aspirate and wash each well with $150\ \mu\text{l}$ of **1 X M1** 3 times.
5. Dilute the **M5** (at the 1:1000 ratios) to 1 $\mu\text{g}/\text{ml}$ with **1 X M1**. Add $50\ \mu\text{l}$ of diluted **M5** to each strip well and incubate at room temperature for 60 min on an orbital shaker (50-100 rpm).
6. Aspirate and wash each well with $150\ \mu\text{l}$ of **1 X M1** 4 times.
7. Dilute the **M6** (at the 1:1000 ratios) to 0.2 $\mu\text{g}/\text{ml}$ with **1 X M1**. Add $50\ \mu\text{l}$ of diluted **M6** to each strip well and incubate at room temperature for 30 min.
8. Aspirate and wash each well with $150\ \mu\text{l}$ of **1 X M1** 5 times.
9. Add $100\ \mu\text{l}$ of **M7** into the wells and incubate at room temperature for 2-10 min away from light. Monitor color development in the sample and standard well (blue).
10. Add $50\ \mu\text{l}$ of **M8** to each well to stop enzyme reaction when color in the standard wells containing the higher concentrations of standard control turns medium blue. The color should change to yellow and absorbance can be read on a microplate reader at 450 nm within 2-15 min.

11. Calculate Dnmt activity or inhibition using the following formula:

$$\text{Dnmt activity (OD/h/mg)} = \frac{(\text{No inhibitor OD} - \text{blank OD})}{\text{protein amount } (\mu\text{g})^* \times \text{hour}^{**}} \times 1000$$

$$\text{Inhibition \%} = \left(1 - \frac{\text{OD (inhibitor sample - blank)}}{\text{OD (no inhibitor control - blank)}}\right) \times 100\%$$

* Protein amount added into the reaction at step 3.

** incubation time at step 3.

TROUBLESHOOTING

No Signal for Both the Positive Control and the Samples

Reagents are added incorrectly. Check if reagents are added in order and if some steps of the procedure are omitted by mistake.

Incubation time and temperature is incorrect. Ensure the incubation time and temperature described in the protocol are correctly followed.

No Signal or Very Weak Signal for Only the Positive Control

The positive control enzyme is insufficiently added to the well. Ensure sufficient amount of control enzyme is added.

The positive control enzyme has lost activity due to incorrect storage. Follow the guidance in the protocol for storage of positive control.

No Signal for Only the Sample

The protein sample is not properly extracted. Ensure the nuclear protein extraction protocol is suitable for Dnmt protein extraction. Sodium chloride concentration of the extraction buffer should not be more than 100 mM.

The protein amount is added into well insufficiently. Ensure extract contains enough amount of proteins.

The sample is not prepared from fresh cells or tissues. The nuclear extracts from frozen cells or tissues significantly loses enzyme activity. The fresh sample should be used.

Nuclear extracts are incorrectly stored or have stored for a long period. Ensure the nuclear extracts are stored at -80°C for no more than 6 weeks.

Absence of Dnmt activity in the sample due to treatment. N/A

High Background Present for the Blank

The well is not washed enough. Check if wash at each step is performed according to the protocol.

Contaminated by the positive control. Ensure the well is not contaminated from adding enzyme accidentally or from using enzyme contaminated tips.

Over-development. Decrease development time in step 9.

ORDERING INFORMATION

Products	Size	Cat. No.
<i>EpiQuik</i> [™] DNA Methyltransferase Activity/ Inhibition Assay Kit	48 assays 96 assays	P-3001-1 P-3001-2

Available Related Products

	Cat. No.
<i>EpiQuik</i> [™] Histone Methyltransferase Activity/ Inhibition Assay Kit (H3-K4)	P-3002
<i>EpiQuik</i> [™] Histone Methyltransferase Activity/ Inhibition Assay Kit (H3-K9)	P-3003
<i>EpiQuik</i> [™] <i>In Situ</i> Histone H3-K4 Methylation Assay Kit	P-3015
<i>EpiQuik</i> [™] <i>In Situ</i> Histone H3-K9 Methylation Assay Kit	P-3016
<i>EpiQuik</i> [™] Global Histone H3-K4 Methylation Assay Kit	P-3017
<i>EpiQuik</i> [™] Global Histone H3-K9 Methylation Assay Kit	P-3018
<i>EpiQuik</i> [™] DNA Demethylase Activity/ Inhibition Assay Kit	P-3019
<i>EpiQuik</i> [™] Global Histone H3-K27 Methylation Assay Kit	P-3020

Need more components? You can also order parts separately by calling 1-877-374-4368 or e-mailing sales@epigentek.com.

