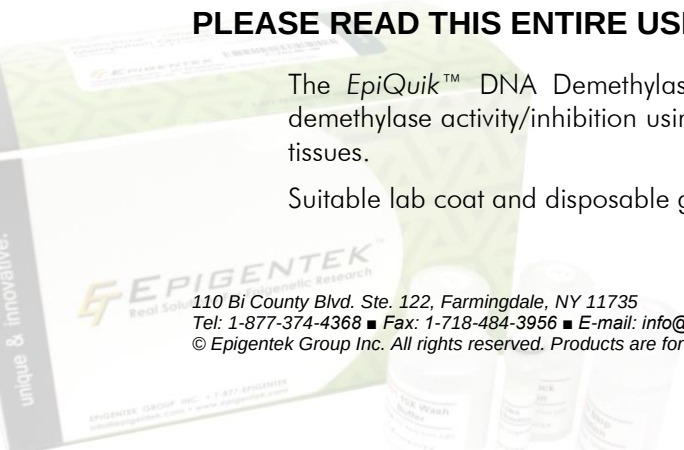
EpiQuik™ DNA Demethylase Activity/ Inhibition Assay Kit

Base Catalog # P-3019

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ DNA Demethylase Activity/Inhibition Assay Kit is suitable for measuring DNA demethylase activity/inhibition using purified enzyme or protein extracts from the cultured cells and tissues.

Suitable lab coat and disposable gloves are required when working with the kit.



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KIT CONTENTS

Components	48 assays P-3019-48	96 assays P-3019-96
DD1 (10X Wash Buffer)	11 ml	22 ml
DD2 (Demethylase Assay Buffer)	3 ml	6 ml
DD3 (Demethylase Substrate)*	0.1 ml	0.2 ml
DD4 (Demethylation Standard, 20 µg/ml)*	10 µl	20 µl
DD5 (Capture Antibody, 1000 µg/ml)*	5 µl	8 µl
DD6 (Detecting Antibody, 200 µg/ml)*	10 µl	20 µl
DD7 (Developing Solution)	5 ml	10 ml
DD8 (Stop Solution)	5 ml	10 ml
Substrate Binding Solution	5 ml	10 ml
8-Well Assay Strips (with Frame)	6	12
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** 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 **DD3**, **DD4**, and **DD6** at –20°C; (2) Store **DD1**, **DD2, DD5**, **DD7**, and **8-Well Assay Strips** at 4°C away from light; (3) Store **all other components** at room temperature. The kit is stable for up to 6 months from the shipment date, when stored properly.

Note: Check if wash buffer, **DD1**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.

MATERIALS REQUIRED BUT NOT SUPPLIED

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

GENERAL PRODUCT INFORMATION

Usage Limitation: The EpiQuik™ DNA Demethylase Activity/Inhibition Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

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.

Intellectual Property: EpiQuik™ is a trademark of Epigentek Group Inc.

A BRIEF OVERVIEW

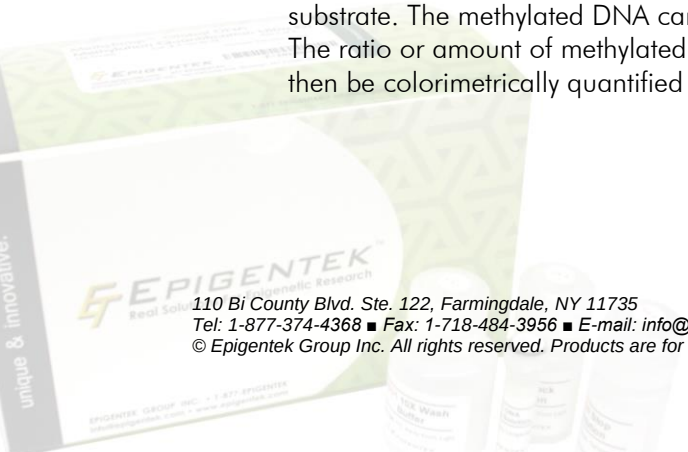
DNA methylation is known to play an essential role in all biological processes through the repression of transcription and development. Hypermethylation of CpGs in the promoters of tumor suppression gene has been demonstrated to cause the epigenetic silence of these genes and constitutes a common feature of many cancers. In contrast, DNA demethylation is necessary for the epigenetic reprogramming of the genes and involves the processes of many important diseases such as tumor progression. Demethylation of DNA can either be passive or active, or a combination of both. Active demethylation of DNA requires specific demethylase participation such as MBD2.

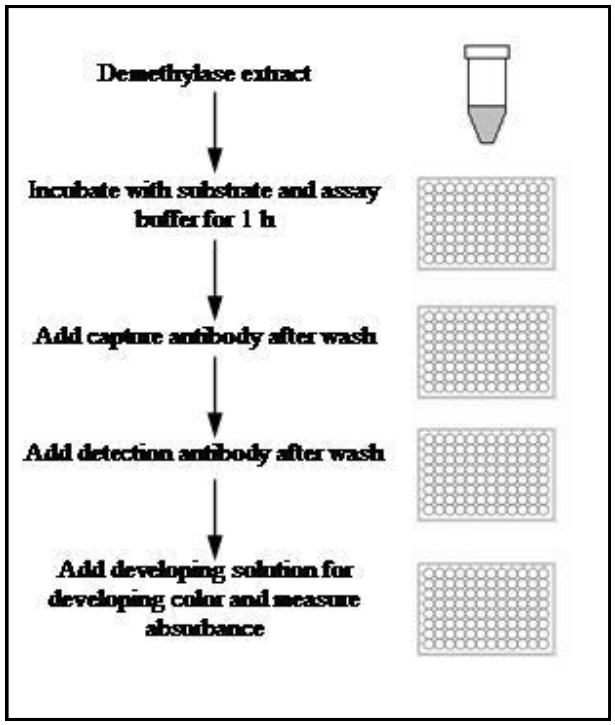
There is only the radioisotopic method currently available for measuring DNA demethylase activity/inhibition, which is time consuming, labor-intensive, and has low throughput and produces radioactive waste. The *EpiQuik*™ DNA Demethylase Activity/Inhibition Assay Kit addresses these problems by using a unique procedure to measure DNA demethylase. The principle of the assay is based on increased DNA demethylase activity causing the reduction of methylated DNA, which can be detected immunologically, and is proportional to the colorimetric intensity. The kit has the following features:

- Quick and efficient procedure, which can be finished within 4 hours.
- Innovative colorimetric assay without the need for radioactivity, electrophoresis, or chromatography.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The EpiQuik™ DNA Demethylase Activity/Inhibition Assay Kit is designed for measuring total DNA demethylase activity/inhibition. In an assay with this kit, the unique methylated DNA substrate is stably captured on the strip wells. Active DNA demethylases bind to and demethylate DNA substrate. The methylated DNA can be recognized with a high affinity 5-methylcytosine antibody. The ratio or amount of methylated DNA, which is inversely proportional to enzyme activity, can then be colorimetrically quantified through an ELISA-like reaction.





Schematic Procedure for Using the EpiQuik™ DNA Demethylase Activity/Inhibition Assay Kit

PROTOCOL

1. Prepare protein extracts by using your own successful method. For your convenience and the best results, Epigentek offers a series of protein extraction kits, which is optimized for extracting protein from cultured cells and tissues.

2. 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). Dilute **DD1** with distilled water (pH 7.2-7.5) at a 1:10 ratio (ex: 1 ml of **DD1** + 9 ml distilled water).

3. Dilute **DD3** at a 1:80 ratio with **Substrate Binding Solution**, and add 80 µl of the **diluted DD3** into each well, except the wells for the blank and standard curve. For Blank, add 80 µl of the **Substrate Binding Solution**. For preparation of the standard curve, add 80 µl of **Substrate Binding Solution** into the wells (without **DD3** added), followed by adding 1 µl of **DD4** at different amounts (0. 05 – 5 ng). Cover the wells with Parafilm M and incubate at room temperature for 75 minutes.

4. Aspirate and wash each well with 150 µl of **diluted DD1** two times.

5. For blank wells: Add 50 µl of **DD2.**
   For the standard wells: Add 50 µl of **DD2.**
   For the sample wells: Add 45-48 µl of **DD2** to each well, followed by adding 2-5 µl of the protein extracts (5-10 µg) or purified demethylase.

For the no enzyme control wells: Add 45-48 µl of **DD2** and 2-5 µl of your protein extraction buffer or enzyme buffer.
For inhibitor wells: Add 42-45 µl of **DD2,** 2-5 µl of protein extracts or enzyme and 3 µl of tested compounds at desired concentration.
Mix and cover the strip wells with Parafilm M and incubate at 37°C for 1 hour.

6. Aspirate and wash each well with 150 µl of **diluted DD1** two times.

7. Dilute **DD5** (at a 1:1000 ratio) to 1 µg/ml with **diluted DD1**. Add 50 µl of the **diluted DD5** to each well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).

8. Aspirate and wash each well with 150 µl of **diluted DD1** three times.

9. Dilute **DD6** (at a 1:2000 ratio) with **diluted DD1**. Add 50 µl of the **diluted DD6** to each well and incubate at room temperature for 30 min.

10. Aspirate and wash each well with 150 µl of **diluted DD1** five times.

11. Add 100 µl of **DD7** to each well and incubate at room temperature for 2-10 minutes away from light. Monitor the color development in the sample and control wells (blue).

12. Add 100 µl of **DD8** to each well to stop enzyme reaction when the color in the control well turns medium blue. The color should change to yellow and absorbance can be read on a microplate reader at 450 nm within 2-15 minutes.

13. Calculate DNA demethylase activity and inhibition. For simple calculation, use the following formula:

$$\text{Demethylase activity (OD/h/mg)} = \frac{[\text{OD (control – blank)} - \text{OD (sample – blank)}]}{[\text{Protein Amount (ug)}/1000\,]^{**} \times \text{Hour}^{*}}$$

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

Calculate DNA demethylase activity using the following formula:

$$\text{Activity (ng/h/mg)} = \frac{[\text{OD (control – blank)} - \text{OD (sample – blank)}]}{\text{Slope} \times \text{Protein Amount (ug)}^{**} \times \text{Hour}^{*}} \times 1000$$

* Protein amount (µg) added into the reaction at Step 5.
** Incubation time (Hour) at Step 5.

TROUBLESHOOTING

No Signal for the Sample

The protein sample is not properly extracted.	Ensure the nuclear protein extraction protocol is suitable for DNA demethylase protein extraction.
The protein amount is added into well insufficiently.	Ensure extract contains a sufficient amount of protein.
The sample is not prepared from fresh cells or tissues.	The nuclear extracts from frozen cells or tissues significantly lose enzyme activity. A fresh sample should be used.
Nuclear extracts are incorrectly stored or have been stored for a long time.	Ensure the nuclear extracts are stored at −80°C for no more than 6 weeks.
Absence of DNA demethylase activity in the sample due to treatment.	N/A.

High Background Present for the Blank

The well is not washed sufficiently.	Check if wash at each step is performed according to the protocol.
Overdevelopment.	Decrease development time at step 9.

RELATED PRODUCTS

P-3001	*EpiQuik*™ DNA Methyltransferase Activity/Inhibition Assay Kit
P-3002	*EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K4)
P-3003	*EpiQuik*™ Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K9)