EpiQuik™ DNA MethyltransferaseActivity/Inhibition Assay Kit(Fluorometric)

Base Catalog # P-3004

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ DNA MethyltransferaseActivity/Inhibition Assay Kit (Fluorometric) is suitable for measuring Dnmt activity/inhibition from a broad range of species including mammalian cells/tissues, plants, and bacteria.

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KIT CONTENTS

Components	48 assays P-3004-48	96 assays P-3004-96
MD1 (10X Wash Buffer)	11 ml	22 ml
MD2 (Dnmt Assay Buffer)	1.5 ml	3 ml
MD3 (Adomet, 8 mM)*	35 µl	70 µl
MD4 (Dnmt Positive Control)*	5 µl	10 µl
MD5 (Capture Antibody)*	5 µl	8 µl
MD6 (Detection Antibody 200 µg/ml)*	10 µl	20 µl
MD7 (Fluoro Developer)*	12 µl	24 µl
MD8 (Fluoro Enhancer)*	12 µl	24 µl
MD9 (Fluoro Dilution)	4 ml	8 ml
8-Well Substrate-Coated Strip (with frame)	6	12

** 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 MD3, MD4, MD6, and MD7 at –20°C away from light; (2) Store **all other components** at 4°C away from light. The kit is stable for up to 6 months from the shipment date, when stored properly.

Note: *Check if wash buffer, MD1, 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
- ☐ Fluorescence microplate reader
- ☐ 1.5 ml microcentrifuge tubes

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.

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

Intellectual Property: The *EpiQuik*™ DNA Methyltransferase Activity/Inhibition Assay Kit (Fluorometric) and methods of use contain proprietary technologies by Epigentek. *EpiQuik*™ is a trademark of EpigenTek Group Inc.

A BRIEF OVERVIEW

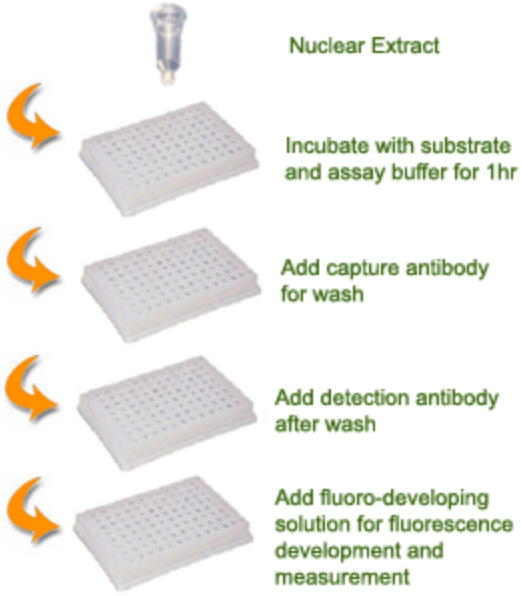
Epigenetic inactivation of genes plays a critical role in many important human diseases, especially in cancer. A core mechanism for epigenetic inactivation of the genes is methylation of CpG islands in genome DNA. Methylation of CpG islands involves the course in which DNA methyltransferases (Dnmts) 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, thus Dnmt inhibitors are currently being developed as potential anti-cancer 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 (Fluorometric) addresses these problems by using a unique procedure to measure Dnmt activity/inhibition. The kit has the following features:

- Extremely fast procedure, which can be completed within 3 hours.
- Innovative fluorometric assay with high sensitivity for detecting Dnmt activity generated from as low as 2 ng of enzyme.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*™ DNA Methyltransferase Activity/Inhibition Assay Kit (Fluorometric) 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 a methyl group to cytosine from Adomet to methylate the DNA substrate. The methylated DNA can be recognized with an anti-5-methylcytosine antibody. The ratio or amount of methylated DNA, which is proportional to enzyme activity, can then be fluorometrically quantified through an ELISA-like reaction.



Schematic Procedure for Using the *EpiQuik*™ DNA Methyltransferase Activity/Inhibition Assay Kit (Fluorometric)

PROTOCOL

1. Prepare nuclear extracts by using your own successful method. For your convenience and the best results, EpigenTek offers a nuclear extraction kit (Cat. No. 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 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 MD1 10X Wash Buffer with distilled water (pH 7.2-7.5) at a 1:10 ratio (e.g., 1 ml of MD1 + 9 ml of distilled water). Wash strip wells once with 150 µl of the diluted MD1.
3. Dilute MD3 with MD2 (at a 1:5 ratio) to 1.6 mM. Add 24 µl of MD2 and 3 µl of the diluted MD3 to each strip well. Then add 3 µl of nuclear extracts (4-10 µg) or purified Dnmt enzymes. Mix and cover the strip wells with Parafilm M and incubate at 37°C for 90 minutes. For the positive control, add 0.5-1 µl of MD4 and 2-2.5 µl of MD2 instead of nuclear extracts. For Dnmt inhibition, add 3 µl of tested inhibitors at different concentrations and reduce MD2 volume to 21 µl. For the blank, add 3 µl of MD2 instead of nuclear extracts.
4. Aspirate and wash each well with 150 µl of diluted MD1 three times.
5. Dilute MD5 (at a 1:1000 ratio) to 1 µg/ml with the diluted MD1. Add 50 µl of diluted MD5 to each strip well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).
6. Aspirate and wash each well with 150 µl of diluted MD1 four times.
7. Dilute MD6 (at a 1:1000 ratio) to 0.2 µg/ml with the diluted MD1. Add 50 µl of diluted MD6 to each strip well and incubate at room temperature for 30 minutes.
8. Aspirate and wash each well with 150 µl of diluted MD1 five times.
9. Prepare the Fluoro-Development Solution by adding 1 µl of MD7 and 1 µl of MD8 into every 400 µl of MD9. Add 50 µl of the Fluoro-Development Solution into the wells and incubate at room temperature for 5-10 minutes away from light. The color in the standard wells containing the

higher concentrations may turn slightly pink during this period. Measure and read fluorescence on a fluorescence microplate reader at $530_{EX}/590_{EM}$ nm.

Note: If the strip well frame does not fit the fluorescence reader, transfer the solution to a standard 96-well microplate and read fluorescence at $530_{EX}/590_{EM}$ nm.

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

$$\text{Dnmt activity (RFU/h/mg)} = \frac{\text{(No inhibitor RFU – blank RFU)}}{\text{Protein amount } (\mu\text{g})^{*} \text{ x hour}^{**}} \text{ x } 1000$$

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

* 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 the proper order and if any steps of the procedure may have been omitted by mistake.
Incubation time and temperature is incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.

No Signal or Very Weak Signal for Only the Positive Control

The positive control enzyme is insufficiently added to the well.	Ensure a 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 the 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 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 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 sufficiently.	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.

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)
P-3015	EpiQuik™ In Situ Histone H3-K4 Methylation Assay Kit
P-3016	EpiQuik™ In Situ Histone H3-K9 Methylation Assay Kit
P-3017	EpiQuik™ Global Histone H3-K4 Methylation Assay Kit
P-3018	EpiQuik™ Global Histone H3-K9 Methylation Assay Kit
P-3019	EpiQuik™ DNA Demethylase Activity/Inhibition Assay Kit
P-3020	EpiQuik™ Global Histone H3-K27 Methylation Assay Kit