DATA SHEET

EpiQuik™ DNA Methyltransferase 1 Activity/Inhibitor Screening Assay Core Kit Catalog No. P-3006A

INTRODUCTION

Epigenetic inactivation of genes play 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. Dnmt1 methylates cytosine residues, preferably in hemimethylated DNA. Mammalian Dnmt1 is believed to be involved in carcinogenesis, embryonic development, and several other biological functions. Hypermethylation by Dnmt1 is believed to inactivate the tumor suppressor genes leading to neoplastic transformation. The selective inhibition of Dnmt1 may lead to demethylation and expression of the silenced tumor suppressor genes. Thus, the selective Dnmt1 inhibitors could be a new addition to cancer therapeutic agents. There are few methods used for selectively screening Dnmt1 inhibitors. The EpiQuik™ DNA Methyltransferase 1 Activity/Inhibitor Screening Assay Kit addresses this problem by using a unique procedure to screen Dnmt 1 inhibitors. The kit has the 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 1 Activity/Inhibitor Screening Assay Kit is designed for screening Dnmt 1 inhibitors. 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. The Dnmt1 enzyme transfers a methyl group to cytosine from Adomet to methylate 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 colorimetrically quantified through an ELISA-like reaction.

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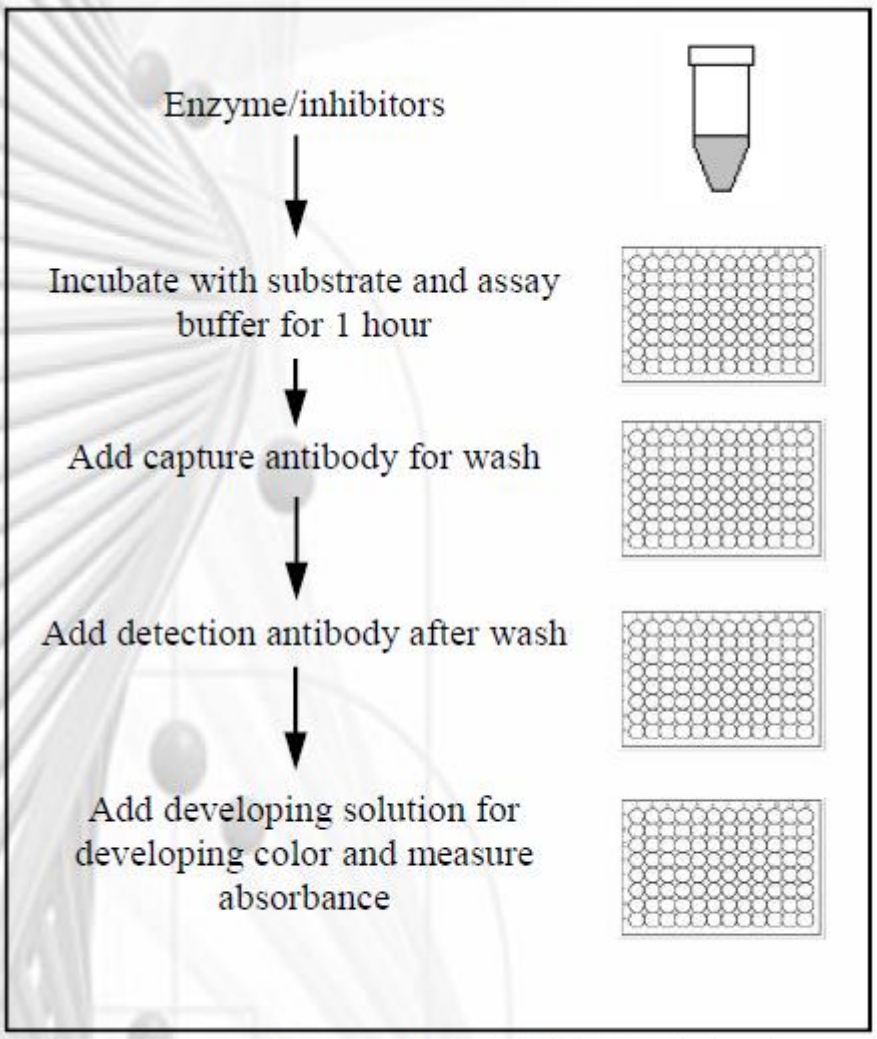

Schematic Procedure for Using the *EpiQuik*™ DNA Methyltransferase 1 Activity/Inhibitor Screening Assay Kit

PRODUCT USE INFORMATION

The EpiQuik™ DNA Methyltransferase 1 Activity/Inhibitor Screening Assay Kit is suitable for screening Dnmt 1 inhibitors which directly interact with Dnmt1. 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 1 Activity/Inhibitor Screening 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 1 Activity/Inhibitor Screening Assay Kit and methods of use are covered by a pending US patent.

KIT CONTENTS

Components	48 assays P-3006-48	96 assays P-3006-96
MO1 (10X Wash Buffer)	11 ml	22 ml
MO2 (Dnmt Assay Buffer)	2 ml	4 ml
MO3 (Adomet)*	35 µl	70 µl
MO5 (Capture Antibody)*	5 µl	8 µl
MO6 (Detection Antibody)*	10 µl	20 µl
MO7 (Developing Solution)	6 ml	12 ml
MO8 (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

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 MO3,and MO6, at –20°C away from light; (2)Store MO1, MO2, MO5, MO7, and 8-Well Substrate-Coated Strips at 4°C. (3) Store MO8 at room temperature. The kit is stable for up to 6 months from the shipment date, when stored properly.

Note: Check if wash buffer, MO1, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are redissolved.

MATERIALS REQUIRED BUT NOT SUPPLIED

Orbital shaker
Pipettes and pipette tips
Microplate reader
1.5 ml microcentrifuge tubes
Plate seal or Parafilm M
Purified Dnmt1 enzyme

PROTOCOL

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). Dilute MO1 10X Wash Buffer with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (ex: 1 ml of MO1 + 9 ml of distilled water).

2. Dilute MO3 with MO2 at a 1:4 ratio (ex: 1 µl of MO3 + 4 µl of MO2) to 1.6 mM.

5. For blank wells: Add 30 µl of DD2.

For the standard wells: Add 30 µl of DD2.

For the sample wells: Add 28 µl of DD2 to each well, followed by adding 2 µl of the protein extracts (5-20 µg) or purified demethylase.

For the no enzyme control wells: Add 28 µl of DD2 and 2 µl of your protein extraction buffer or enzyme buffer.

For inhibitor wells: Add 25 µl of DD2, 2 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 60-90 minutes.
3. Aspirate and wash each well with 150 µl of diluted MO1 three times.
4. Dilute MO5 (at a 1:1000 ratio) with diluted MO1. Add 50 µl of diluted MO5 to each strip well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).
5. Aspirate and wash each well with 150 µl of diluted MO1 four times.
6. Dilute MO6 (at a 1:1000 ratio) with diluted MO1. Add 50 µl of diluted MO6 to each strip well and incubate at room temperature for 30 minutes.
7. Aspirate and wash each well with 150 µl of diluted MO1 five times.
8. Add 100 µl of MO7 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).
9. Add 50 µl of MO8 to each well and transfer the mixed solution to a 96- well plate. Read absorbance on a microplate reader at 450 nm.
10. Calculate Dnmt 1 activity or inhibition using the following formula:

$$\text{Dnmt activity (OD/h/}\mu\text{g)} = \frac{(\text{No inhibitor OD} - \text{blank OD}) \times 1000}{\text{Dnmt1 amount (ng) added in the reaction} \times \text{h}}$$

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

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TROUBLESHOOTING

No Signal for the No Inhibitor Control	
Reagents are added incorrectly.	Check if reagents are added in 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.
The Dnmt1 enzyme is insufficiently added to the well.	Ensure a sufficient amount of enzyme is added.
The Dnmt1 enzyme has lost activity due to incorrect storage.	Follow the guidance in the protocol for storage of positive control.
No Inhibition by the Inhibitors	
The amount of the inhibitors added is insufficient.	Ensure the amount of inhibitors added into the reaction is sufficient.
The inhibitor does not interact directly with the enzyme.	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 8.

Product Overview

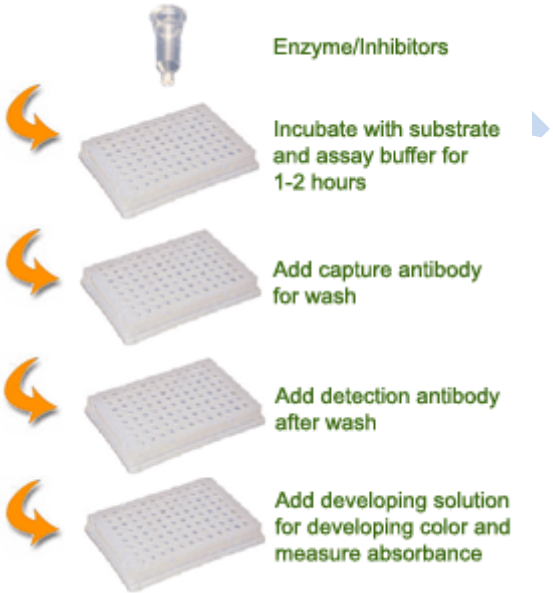
The *EpiQuik™ DNMT1 Activity/Inhibitor Screening Assay Core Kit* is a convenient set of tools that allows the experimenter to selectively screen DNA methyltransferase 1 activity/inhibitors in an ELISA-like format. The selective inhibition of DNMT1 may lead to demethylation and expression of silenced tumor suppressor genes. Thus, the selective

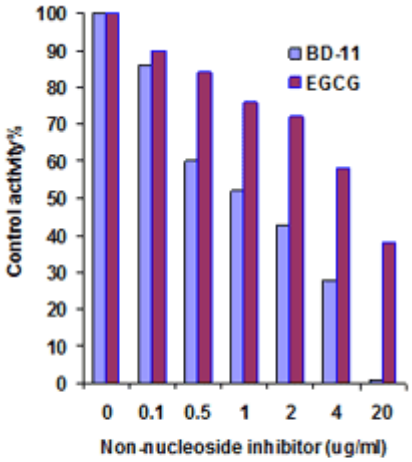
DNMT1 inhibitors could be a new addition to cancer therapeutic agents. The kit is ready-to-use and provides all the essential components needed to carry out a successful DNMT1 activity/inhibitor screening experiment without the need for radioactivity or any special equipment.

WHY CHOOSE THE EPIQUIK™ DNMT1 ACTIVITY/INHIBITOR SCREENING ASSAY CORE KIT?

- Very rapid procedure, which can be completed within 3 hours.
- Safe and innovative colorimetric assay without radioactivity, extraction, and chromatography.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Extremely simple, reliable, and consistent assay conditions.
- **Product Details**
-
- The EpiQuik™ DNMT1 Activity/Inhibitor Screening Assay Kit is designed for screening Dnmt 1 inhibitors. 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. A DNMT1 enzyme (not included) transfers 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 colorimetrically quantified through an ELISA-like reaction.

SCHEMATIC PROCEDURE:





▲ DNMT1 inhibition: Recombinant DNMT1 was incubated with substrate and inhibitors. DNMT1 activity was measured in the presence or absence of inhibitors.

Product Components

MO1	(10X	Wash		Buffer)
MO2	(Dnmt	Assay		Buffer)
MO3				(Adomet)*
MO5		(Capture		Antibody)*
MO6		(Detection		Antibody)*
MO7		(Developing		Solution)
MO8		(Stop		Solution)
8-Well	Substrate-Coated	Strip	(with	Frame)
User				guide

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