DNA Methyltransferase 1 Activity/Inhibitor Screening Assay Core Kit

Catalog Number KA1604

48 assays

Version: 03

Intended for research use only

www.abnova.com

Table of Contents

Introduction

Intended Use

The DNA Methyltransferase 1 Activity/Inhibitor Screening Assay Core Kit is suitable for screening Dnmt 1 inhibitors which directly interact with Dnmt1. This “core” kit does not come with Dnmt1 enzymes.

Background

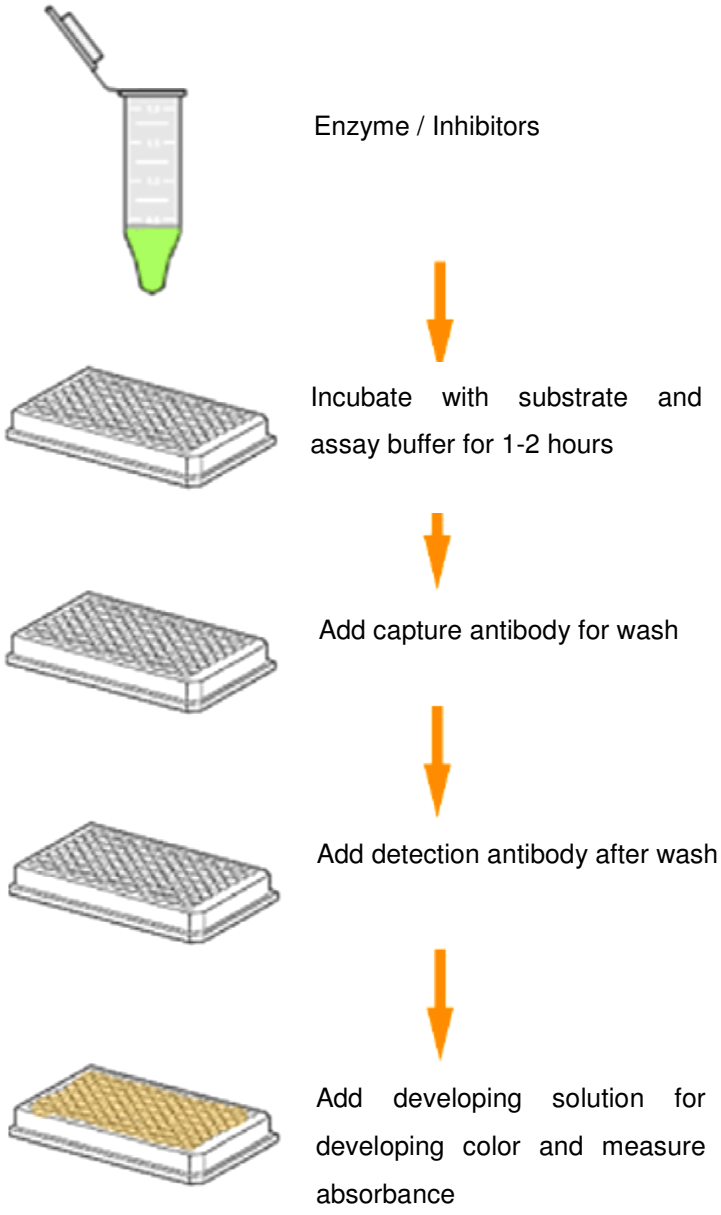
Epigenetic inactivation of genes plays a critical role in many important human diseases, especially in cancer. A core mechanism for epigenetic inactivation of the genes is methylation of CpG islands in genome DNA. Methylation of CpG islands involves the course in which DNA methyltransferases (Dnmts) 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. 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, selective Dnmt1 inhibitors could be a new addition to cancer therapeutic agents.

There are few methods used for selectively screening Dnmt1 inhibitors. The DNA Methyltransferase 1 Activity/Inhibitor Screening Assay Core 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 3.5 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 of the Assay

The DNA Methyltransferase 1 Activity/Inhibitor Screening Assay Core 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 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.



Schematic Procedure for using DNA Methyltransferase 1 Activity/Inhibitor Screening Assay Core Kit

General Information

Materials Supplied

List of component

Component	Amount
MO1 (10x Wash Buffer)	11 ml
MO2 (Dnmt Assay Buffer)	2 ml
MO3 (Adomet, 8 mM)*	35 µl
MO5 (Capture Antibody)*	5 µl
MO6 (Detection Antibody)*	10 µl
MO7 (Developing Solution)	6 ml
MO8 (Stop Soultionr)	3 ml
Enhancer Solution	6 µl
8-well Substrate-Coated Strips (with frame)	6

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

Storage Instruction

Upon receipt:

(1) Store MO3, MO6, and Enhancer Solution at -20℃ away from light;

(2) Store MO1, MO2, MO5, MO7, and 8-Well Substrate-Coated Strips at 4℃.

(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℃) 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
- ✓ Plate seal or Parafilm M
- ✓ Purified Dnmt1 enzyme

Precautions for Use

- ✓ Quality Control: Abnova guarantees the performance of all products in the manner described in our product instructions.
- ✓ Usage Limitation: The DNA Methyltransferase 1 Activity/Inhibitor Screening Assay Core Kit is for research use only.

Assay Protocol

Assay Procedure

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℃). 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.
3. a) For blank wells: Add 27 µl of MO2 and 3 µl of diluted MO3.
   b) For the untreated control wells: Add 25 to 26 µl of MO2, 3 µl of diluted MO3 and 1 to 2 µl of purified Dnmt1 enzyme.
   c) For inhibitor wells: Add 22 to 23 µl of MO2, 3 µl of diluted MO3, 1 to 2 µl of Dnmt1 enzyme and 3 µl of tested compounds at desired concentration.
   Mix and cover the strip wells with Parafilm M and incubate at 37℃ for 60-90 minutes.
   Note: The final concentration of the inhibitors before adding to the wells should be prepared with MO2 at 1:10 ratio (ex: add 0.5 µl of inhibitor to 4.5 µl of MO2) so that the original solvent of the inhibitor can be reduced to 1% of the reaction solution or less.
4. Aspirate and wash each well with 150 µl of diluted MO1 three times.
5. 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).
6. Aspirate and wash each well with 150 µl of diluted MO1 four times.
7. 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.
8. Aspirate and wash each well with 150 µl of diluted MO1 four times.
9. Dilute Enhancer Solution (at a 1:5000) with diluted MO1. Add 50 µl of diluted Enhancer Solution to each strip well and incubate at room temperature for 30 minutes.
10. Aspirate and wash each well with 150 µl of diluted MO1 four times.
11. 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).
12. 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.

Data Analysis

Calculation of Results

Calculate Dnmt 1 activity or inhibition using the following formula:

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

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

*Incubation time used at Step 3.

Resources

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 escribed 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.	Dnmt1 enzyme should be stored at -80℃ and avoid repeated freezing/thawing.

✓ 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 11.

Plate Layout

8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H