DNA Methyltransferase 3B Activity/Inhibitor Screening Assay Core Kit

Catalog Number KA1547

48 assays

Version: 02

Intended for research use only

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Table of Contents

Introduction

Intended Use

The DNA Methyltransferase 3B Activity/Inhibitor Screening Assay Core kit is suitable for screening Dnmt3B inhibitors which directly interact with Dnmt3B. This “core” kit does not come with Dnmt3B enzymes.

Background

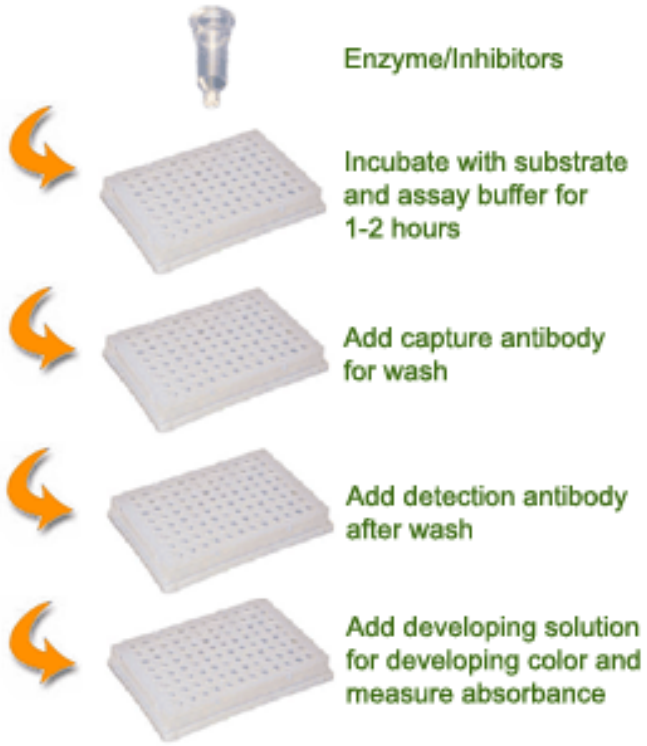
Epigenetic inactivation of genes play a critical role in many important human diseases, especially in cancer. A core mechanism for epigenetic inactivation of the genes is methylation of CpG islands in genome DNA. Methylation of CpG islands involves the course in which DNA methyltransferases (Dnmts) transfer 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. Dnmt3B has been demonstrated to methylate both unmethylated and hemimethylated DNA equally and is supposed to mediate de novo methylation together with Dnmt1. Increased activation or amount of Dnmt3B is believed to be involved in carcinogenesis, and other genetic and epigenetic diseases. The selective inhibition of Dnmt3B may lead to demethylation and expression of the silenced tumor suppressor genes. Thus, the selective Dnmt3B inhibitors could be a new addition to cancer therapeutic agents.

There are few methods used for selectively screening Dnmt 3B inhibitors. The DNA Methyltransferase 3B Activity/Inhibitor Screening Assay Core Kit addresses this problem by using a unique procedure to screen Dnmt 3B 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 of the Assay

The DNA Methyltransferase 3B Activity/Inhibitor Screening Assay Core Kit is designed for screening Dnmt3B 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 Dnmt3B enzyme (not included) transfers a methyl group to cytosine from Adomet to methylate the DNA substrate. The methylated DNA can then 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 the DNA Methyltransferase 3B Activity/Inhibitor Screening Assay Core Kit.

General Information

Materials Supplied

List of component

Component	Amount
MT1 (10X Wash Buffer)	11 mL
MT2 (Dnmt Assay Buffer)	2 mL
MT3 (Adomet, 8 mM)*	35 µL
MT5 (Capture Antibody)*	5 µL
MT6 (Detection Antibody)*	10 µL
MT7 (Developing Solution)*	6 mL
MT8 (Stop Solution)	3 mL
Enhancer Solution	6 µL
8-Well Substrate-Coated Strips (With Frame)	6

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

Storage Instruction

Upon receipt: (1) Store MT3, MT6 and Enhancer Solution at –20°C away from light; (2) Store MT1, MT2, MT5, MT7, and the 8-Well Substrate-Coated Strips at 4°C away from light; (3) Store MT8 at room temperature. The kit is stable for up to 6 months from the shipment date, when stored properly.

Note: Check if wash buffer, MT1, 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
- ✓ Plate seal or Parafilm M
- ✓ Purified Dnmt3B enzyme

Precautions for Use

Please read this entire user guide before use.

✓ General product information

- Quality Control: Abnova guarantees the performance of all products in the manner described in our product instructions.
- Product Updates: Abnova reserves the right to change or modify any product to enhance its performance and design.
- Usage Limitation: The DNA Methyltransferase 3B Activity/Inhibitor Screening Assay Core Kit is for research use only and is not intended for diagnostic or therapeutic application.

Assay Protocol

Assay Procedure

✓ Enzymatic Reaction

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 MT1 10X Wash Buffer with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (e.g., 1 mL of MT1 + 9 mL of distilled water).
2. Dilute MT3 with MT2 (at a 1:4 ratio, ex: 1 µL of MT3 + 4 µL of MT2) to 1.6 mM.
3. (a) For blank wells: Add 27 µL of MT2 and 3 µL of diluted MT3.
   (b) For the untreated control wells: Add 25 to 26 µL of MT2, 3 µL of diluted MT3, and 1 to 2 µL of purified Dnmt3B enzyme.
   (c) For inhibitor wells: Add 22 to 23 µL of MT2, 3 µL of diluted MT3, 1 to 2 µL of Dnmt3B enzyme and 3µL of tested compounds at desired concentration.
   Mix and cover the strip wells with Parafilm M and incubate at 37°C for 60-90 minutes.
   Note: The final concentration of inhibitors, before adding them to the wells, should be 1:10 with MT2 (ex: add 0.5 µL of inhibitor to 4.5 µL of MT2) 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 MT1 four times.
5. Dilute MT5 (at a 1:1000 ratio) with diluted MT1. Add 50 µL of diluted MT5 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 MT1 four times.
7. Dilute MT6 (at a 1:1000 ratio) with diluted MT1. Add 50 µL of diluted MT6 to each strip well and incubate at room temperature for 30 minutes.
8. Aspirate and wash each well with 150 µL of diluted MT1 four times.
9. Dilute Enhancer Solution (at a 1:5000 ratio) with diluted MT1. 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 MT1 four times.
11. Add 100 µL of MT7 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 MT8 to each well to stop enzyme reaction when the color in the no inhibitor 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.

Note: If the strip well frame does not fit the microplate reader, transfer the solution to a standard 96-well microplate and read absorbance at 450 nm.

Data Analysis

Calculation of Results

✓ Calculate Dnmt 3B activity or inhibition using the following formula:

$$\text{Dnmt activity (OD/h/}\mu\text{g)} = \frac{\text{(No inhibitor OD – blank OD)} \times 1000}{\text{Dnmt3B (ng) added in the reaction} \times \text{hour *}}$$

* Incubation time used at Step 3.

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

Resources

Troubleshooting

Problem	Possible Cause	Suggestion
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.
	The Dnmt3B enzyme is insufficiently added to the well.	Ensure a sufficient amount of enzyme is added.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
	The Dnmt3B enzyme has lost activity due to incorrect storage.	Follow the guidance in the protocol for storage of the 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 9.

Plate Layout

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