DNMT1 Assay Kit

Catalog Number KA0682

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

The DNMT1 Assay Kit is very suitable for measuring Dnmt amounts quantitatively from fresh tissue and cultured cells of human and mouse.

Background

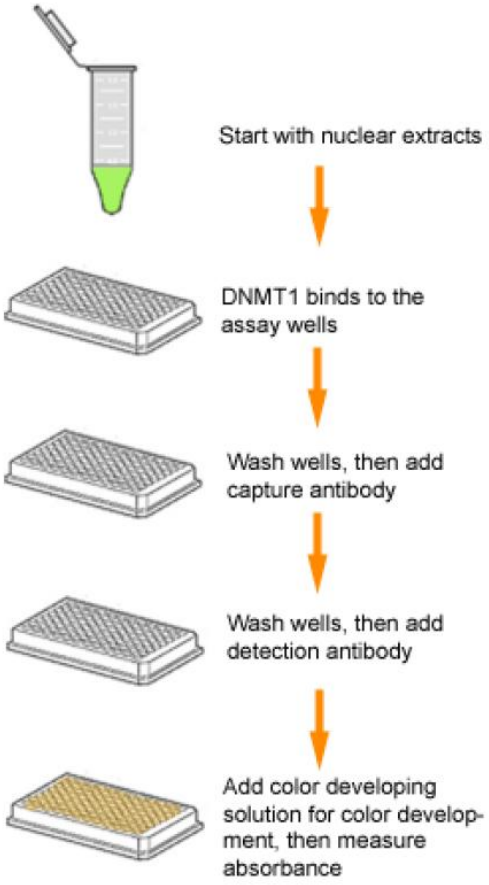
Epigenetic inactivation of genes play a critical role in many important human diseases, especially in cancer. A core mechanism for epigenetic inactivation of the genes is methylation of CpG islands in genome DNA. Methylation of CpG islands involves the course in which DNA methyltransferases (Dnmts) transfer a methyl group from S-adenosyl-L-methionine to the fifth carbon position of the cytosines. At least three families of Dnmts have been identified in mammals now: DNMT1, Dnmt2 and Dnmt3. DNMT1 prefers to methylate cytosine residues in hemimethylated DNA. Increased activation or amount of DNMT1 is believed to be involved in carcinogenesis, and other genetic and epigenetic diseases.

The major assay for measuring the expression or amount of DNMT1 protein currently is Western blot. This method requires electrophoresis and transfer process, which makes the assay inconvenient, time consuming, and has low throughput. The DNMT1 Assay Kit addresses these problems by using a unique procedure to measure the amount of DNMT1. The kit has the following features:

- ✓ Very rapid procedure, which can be finished within 3.5 hours.
- ✓ Innovative colorimetric assay to quantitatively measure the amount of DNMT1 without the need for electrophoresis.
- ✓ Strip microplate format makes the assay flexible: manual or high throughput analysis.
- ✓ Simple, reliable, and consistent assay conditions.

Principle of the Assay

The DNMT1 Assay Kit is designed for measuring total DNMT1 amount from tissues or cells. In an assay with this kit, the unique Dnmt affinity substrate is stably coated on the strip well. The sample is added into the well and DNMT1 contained in the sample binds to the substrate. The bound DNMT1 can be recognized with specific DNMT1 antibody and colorimetrically quantified through an ELISA-like reaction. The amount of DNMT1 is proportional to the intensity of color development.



DNMT1 Assay Kit

General Information

Materials Supplied

List of component

Component	Amount
D1 (10X Wash Buffer)	28 mL
D2 (Assay Buffer)	10 mL
D3 (DNMT1 Standard, 20 µg/mL)*	40 µL
D4 (Capture Antibody, 500 µg/mL)*	16 µL
D5 (Detection antibody 200 µg/mL)*	20 µL
D6 (Developing Solution)	12 mL
D7 (Stop Solution)	11 mL
Blocking Buffer	20 mL
8-Well Assay Strip (with Frame)	12 strips

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

Storage Instruction

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 D3 and D5 at -20°C away from light; (2) Store D1, D2, D4, D6 Blocking buffer 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: (1) Check if wash buffer, D1, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved. (2) Check if a blue color is present in D6 (Developing Solution), which would indicate contamination of the solution and should not be used. To avoid contamination, transfer the amount of D6 required into a secondary container (tube or vial) before adding D6 into the assay wells.

Component	Amount
D1 (10X Wash Buffer)	4°C
D2 (Assay Buffer)	4°C
D3 (DNMT1 Standard, 20 µg/mL)*	-20°C
D4 (Capture Antibody, 500 µg/mL)*	4°C
D5 (Detection antibody 200 µg/mL)*	-20°C
D6 (Developing Solution)	4°C
D7 (Stop Solution)	RT
Blocking Buffer	4°C
8-Well Assay Strip (with Frame)	4°C

Materials Required but Not Supplied

- ✓ Adjustable pipette or multiple-channel pipette
- ✓ Multiple-channel pipette reservoirs
- ✓ Aerosol resistant pipette tips
- ✓ Microplate reader capable of reading absorbance at 450 nm
- ✓ 1.5 mL Microcentrifuge tubes
- ✓ Incubator for 37°C incubation
- ✓ Distilled water
- ✓ Nuclear extracts
- ✓ Parafilm M or aluminum foil

Precautions for Use

- ✓ Quality Control: Abnova guarantees the performance of all products in the manner described in our product instructions.
- ✓ Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.
- ✓ Usage Limitation: The DNMT1 Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

Assay Protocol

Reagent Preparation

- ✓ Prepare Diluted D1 1X Wash Buffer:
  Add 26 mL of D1 10X Wash Buffer to 234 mL of distilled water and adjust pH to 7.2-7.5.
  This Diluted D1 1X Wash Buffer can now be stored at 4°C for up to six months.

- ✓ Prepare Diluted D4 Capture Antibody Solution:
  Dilute D4 Capture Antibody with Diluted D1 1X Wash Buffer at a ratio of 1:500 (i.e., add 1 µL of D4 to 500 µL of Diluted D1). 50 µL of Diluted D4 will be required for each assay well.

- ✓ Prepare Diluted D5 Detection Antibody Solution:
  Dilute D5 Detection Antibody with Diluted D1 1X Wash Buffer at a ratio of 1:2000 (i.e., add 1 µL of D5 to 2000 µL of Diluted D1). 50 µL of Diluted D5 will be required for each assay well.

- ✓ Prepare Diluted DNMT1 Standard:
  Suggested Standard Curve Preparation: First, dilute D3 DNMT1 Standard with D2 to the concentrations of 1, 2, 5, 10 and 20 ng/µL according to the following dilution chart:

Tube	D3 (20 ng/µL)	D2	Resulting D3 Concentration
1	1.0 µL	19.0 µL	1 ng/µL
2	1.0 µL	9.0 µL	2 ng/µL
3	1.0 µL	3.0 µL	5 ng/µL
4	2.0 µL	2.0 µL	10 ng/µL
5	4.0 µL	0.0 µL	20 ng/µL

Note: Keep each of the diluted solutions except D1 1X Wash Buffer on ice until use. Any remaining diluted solutions other than Diluted D1 should be discarded if not used within the same day.

Assay Procedure

For the best results, please read the protocol in its entirety prior to starting your experiment.
Input Amount: The amount of nuclear extracts for each assay can be between 1 µg and 20 µg with an optimal range of 5 to 10 µg.
Nuclear Extraction: You can use your method of choice for preparing nuclear extracts from the treated and untreated samples.
Nuclear extracts should be stored at –80°C in aliquots until use.

✓ Suggested Buffer and Solution Setup

Reagents	1 well	1 strip (8 well)	2 strips (16 wells)	6 strips (48 wells)	12 strips (96 wells)
Diluted D1	2.5 mL	20 mL	40 mL	120 mL	240 mL
D2	100 µL	800 µL	1600 µL	4900 µL	9600 µL
Blocking Buffer	0.15 mL	1.2 mL	2.5 mL	7.5 mL	14.5 mL
D3 Standard control	N/A	N/A	4 µL (optional)	8 µL	8 µL
Diluted D4	50 µL	400 µL	800 µL	2400 µL	4800 µL
Diluted D5	50 µL	400 µL	800 µL	2400 µL	4800 µL
D6	0.1 mL	0.8 mL	1.6 mL	4.8 mL	9.6 mL
D7	0.1 mL	0.8 mL	1.6 mL	4.8 mL	9.6 mL

✓ DNMT1 Binding

1. Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (include blank and positive controls) to ensure that the signal generated is validated. Carefully remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).
2. Blank Wells: Add 100 µL of D2 to each blank well.
3. Standard Wells: Add 98 µL of D2 and 2 µL of Diluted DNMT1 Standard to each standard well with a minimum of five wells, each at a different concentration between 2 and 40 ng/µL (based on the dilution chart in Prepare Diluted DNMT1 Standard in Reagent Preparation section; see Plate Layout as an example).
4. Sample Wells: Add 94 to 98 µL of D2 and 2 to 6 µL of your nuclear extracts to each sample well. Total volume should be 100 µL per wells.
   Note: (1) Follow the suggested well setup diagrams on page 12; (2) It is recommended to use 5 µg to 10 µg of nuclear extract per well.
5. Cover strip-well microplate with Parafilm M or aluminum foil to avoid evaporation and incubate at 37°C for 90 to 120 min.
6. Remove the reaction solution from each well. Add 150 µL of Blocking Buffer to each well, then cover with Parafilm M or aluminum foil and incubate 37°C for 30 min.
7. Remove the Blocking Buffer from each well. Wash each well three with 150 µL of the Diluted D1 1X Wash Buffer each time.

✓ Antibody Binding & Signal Enhancing

1. Add 50 µL of the Diluted D4 to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 60 min.
2. Remove the Diluted D4 solution from each well.
3. Wash each well three times with 150 µL of the Diluted D1 each time.
4. Add 50 µL of the Diluted D5 to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 30 min.

5. Remove the Diluted D5 solution from each well.
6. Wash each well four times with 150 µL of the Diluted D1 each time.

Note: Ensure any residual wash buffer in the wells is removed as much as possible at each wash step.

✓ Signal Detection

1. Add 100 µL of D6 to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color change in the sample wells and control wells. The D6 solution will turn blue in the presence of sufficient demethylated products.
2. Add 100 µL of D7 to each well to stop enzyme reaction when color in the positive control wells turns medium blue. Mix the solution by gently shaking the frame and wait 1-2 min to allow the color reaction to be completely stopped. The color will change to yellow after adding D7 and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

   Note: (1) Most microplate readers have the capability to carry out dual wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice, once at 450 nm and once at 655 nm. Then, manually subtract the 655 nm ODs from 450 nm ODs; (2) If the strip-well microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.

Data Analysis

Calculation of Results

✓ DNMT1 Calculation

- Calculate the average duplicate readings for the sample wells and blank wells.
- Calculate % DNMT1 change using the following formula:

$$\text{DNMT1 Change\%} = \frac{\text{Treated (Tested) Sample OD} - \text{Blank OD}}{\text{Untreated (Control) Sample OD} - \text{Blank OD}} \times 100\%$$

Example calculation:

Average OD450 of treated sample is 0.5

Average OD450 of untreated control is 0.9

Average OD450 of blank is 0.1

$$\text{DNMT1 change\%} = \frac{(0.5\text{-}0.1)}{0.9\text{-}0.1} \times 100\% = 50\%$$

✓ For Detailed Quantification:

- Generate a standard curve and plot OD value versus amount of D3 Standard at each concentration point.
- Determine the slope as OD/ng (you can use Microsoft Excel statistical functions for slope calculation), then calculate the amount of DNMT1 using the following formula:

$$\text{DNMT1 (ng/mg protien)} = \frac{\text{(Sample OD-Blank OD)}}{\text{Slope x Proein Amount (ug*)}} \times 1000$$

* Nuclear extract added into sample wells at DNMT1 Binding step 4.

Resources

Troubleshooting

No Signal for Both the Positive Control and the Samples

Problem	Possible Cause	Suggesting
No signal or weak signal in both the positive control and sample wells	Reagents are added incorrectly	Check if reagents are added in the proper order with the right amount, and if any steps in the protocol may have been omitted by mistake.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
	Incorrect absorbance reading.	Check if appropriate absorbance wavelength (450 nm) is used.
	Kit was not stored or handled properly.	Ensure all components of the kit were stored at the appropriate temperature and the cap is tightly capped after each opening or use.
No signal or weak signal in only the standard curve wells	The standard amount is insufficiently added to the well in Step 3 in DNMT1 Binding	Ensure a sufficient amount of standard is added.
	The standard is degraded due to improper storage conditions	Follow the Shipping & Storage guidance in this Protocol for storage of D3 DNMT1 Standard.
High background present in the blank wells	Insufficient washing of wells	Check if washing recommendations at each step is performed according to the protocol.
	Contaminated by sample or standard.	Ensure the well is not contaminated from adding sample or standard accidentally or from using contaminated tips.
	Incubation time with Diluted D5 is too long	The incubation time at Step 4 in Antibody Binding & Signal Enhancing
	Over-development of color	Decrease the development time in Step 1 before adding D6 Stop solution in Step2 in Signal Detection.

No signal or weak signal only in sample wells	Protein sample is not properly extracted or purified.	Ensure your protocol is suitable for histone protein extraction.
	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of nuclear extracts is used as indicated in Step 2. The sample can be titrated to determine the optimal amount to use in the assay.
	Sample was not stored properly or has been stored for too long.	Ensure sample is stored in aliquots at –80°C, with no more than 6 months nuclear extracts.
	Little or no DNMT1 in the sample.	This problem may be a result of many factors. If the affecting factors cannot be determined, use new or re-prepared nuclear extracts.
Uneven color development	Insufficient washing of the wells.	Ensure the wells are washed according to the guidance of washing and residue washing buffer is removed as much as possible.
	Delayed color development or delayed stopping of color development in the wells.	Ensure color development solution or stop solution is added sequentially and is consistent with the order you added the other reagents (e.g., from well A to well G or from well 1 to well 12).
Large variation between replicate wells	Color reaction is not evenly stopped due to an inconsistency in pipetting time.	Ensure D6 Developer Solution and D7 Stop Solution are added at the same time between replicates or otherwise maintain consistent timing between each addition of solutions.
	Color reaction is not evenly stopped due to an inconsistent order when adding solutions.	Ensure all solutions, particularly D6 Developer Solution and D7 Stop Solution, are added in the same order each time as all other solutions.
	The solutions are not evenly added due to an inconsistency in pipetting volume.	Ensure the solution in each pipette tip is equal in the multi-channel pipette. Equilibrate the pipette tip in any solutions before adding them. Ensure the solutions, especially those with small volumes (ex: 1 µL) are completely added into the wells.
	Solutions or antibodies were not actually added into the wells.	Do not allow pipette tip to touch the outer edges or inner sides of the wells in order to prevent solutions from sticking to the surface.
	Did not sufficiently shake the solutions in the wells evenly after add D7 Stop Solution in Step 2 in Signal Detection.	Gently shake the plate fame across a flat surface so that the solutions in the wells are better distributed. Do not stir.

	Did not use the same pipette device throughout the experiment.	Use the same multi-channel pipette device throughout the entire experiment, as different pipette devices may have slight variations in performance.

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H