ab113469 – DNMT1 Assay Kit

Instructions for Use

For the measurement of DNMT1 amounts from fresh tissue and cultured cells of human and mouse

This product is for research use only and is not intended for diagnostic use.

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1. 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. 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.

ab113469 addresses these problems by using a unique procedure to measure the amount of DNMT1.

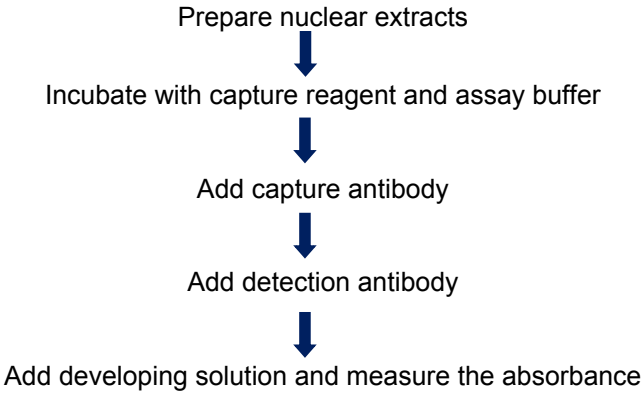
This kit has the following advantages and features:

- 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 through-put analysis
- Simple, reliable, and consistent assay conditions

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 a specific DNMT1 antibody and colorimetrically

quantified through an ELISA-like reaction. The amount of DNMT1 is proportional to the intensity of color development.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit as given in the table upon receipt away from light.

Observe the storage conditions for individual prepared components in sections 9 & 10.

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

Check if Wash Buffer contains salt precipitates before use. If so, warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.

Check if a blue color is present in Developing Solution, which would indicate contamination of the solution and should not be used. To avoid contamination, transfer the amount of Developing Solution required into a secondary container (tube or vial) before adding Developing Solution into the assay wells.

5. MATERIALS SUPPLIED

Item	48 Tests	96 Tests	Storage Condition (Before Preparation)
10X Wash Buffer	14 mL	28 mL	4°C
Assay Buffer	5 mL	10 mL	4°C
DNMT1 Standard, 20 µg/mL	20 µL	40 µL	-20°C
Capture Antibody, 500 µg/mL	8 µL	16 µL	4°C
Detection Antibody, 200 µg/mL	10 µL	20 µL	-20°C
Developing Solution	6 mL	12 mL	4°C
Stop Solution	6 mL	11 mL	4°C
Blocking Buffer	10 mL	20 mL	4°C
8-Well Assay Strips (with Frame)	6	12	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 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

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

9.1. **1X Wash Buffer**

48-Assay Kit:

Add 13 mL of 10X Wash Buffer to 117 mL of distilled water and adjust pH to 7.2-7.5.

96-Assay Kit:

Add 26 mL of 10X Wash Buffer to 234 mL of distilled water and adjust pH to 7.2-7.5.

This diluted 1X Wash Buffer can now be stored at 4°C for up to six months.

9.2. **Capture Antibody**

Dilute Capture Antibody with 1X Wash Buffer at a ratio of 1:500 (i.e., add 1 µL of Capture Antibody to 500 µL of 1X Wash Buffer). 50 µL of Diluted EDN4 will be required for each assay well.

9.3. **Detection Antibody**

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

9.4. **DNMT1 Standard**

Suggested Standard Curve Preparation: Dilute DNMT1 Standard with Assay Buffer to the concentrations of 1, 2, 5, 10 and 20 ng/µl according to the following dilution chart:

Tube	DNMT1 Standard (µL)	Assay Buffer (µL)	Final Conc (ng/µL)
1	1	19	1
2	1	9	2
3	1	3	5
4	2	2	10
5	4	0	20

Suggested Buffer and Solution Setup

Approximate amount of required buffers and solutions for defined assay wells based on the protocol:

Reagents	1 well	1 Strip (8 wells)	2 Strip (16 wells)	6 Strip (48 wells)	12 Strip (96 wells)
1X Wash Buffer	2.5 mL	20 mL	40 mL	120 mL	240 mL
Assay Buffer	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
DNMT1 Standard	NA	NA	4 µL (optional)	8 µL	8 µL
Capture Antibody	50 µL	400 µL	800 µL	2400 µL	4800 µL
Detection Antibody	50 µL	400 µL	800 µL	2400 µL	4800 µL
Developing Solution	0.1 mL	0.8 mL	1.6 mL	4.8 mL	9.6 mL
Stop Solution	0.1 mL	0.8 mL	1.6 mL	4.8 mL	9.6 mL

10. SAMPLE PREPARATION

10.1. **Prepare histone extracts.**

Use your preferred method to prepare nuclear extracts from treated or untreated cells or tissues. Alternatively, our Nuclear Extraction Kit (ab113474) enables preparation of a nuclear extract in 1 hour.

Nuclear extracts can be used immediately or stored at –80°C for future use.

11. PLATE PREPARATION

The suggested strip-well plate setup for the DNMT activity assay in a 48-assay format. The controls and samples can be measured in duplicates.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	DNMT1 Standard 2 ng	DNMT1 Standard 2 ng	Sample	Sample	Sample	Sample
C	DNMT1 Standard 4 ng	DNMT1 Standard 4 ng	Sample	Sample	Sample	Sample
D	DNMT1 Standard 10 ng	DNMT1 Standard 10 ng	Sample	Sample	Sample	Sample
E	DNMT1 Standard 20 ng	DNMT1 Standard 20 ng	Sample	Sample	Sample	Sample
F	DNMT1 Standard 40 ng	DNMT1 Standard 40 ng	Sample	Sample	Sample	Sample
G	Sample	Sample	Sample	Sample	Sample	Sample
H	Sample	Sample	Sample	Sample	Sample	Sample

12. ASSAY PROCEDURE

11.1 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).

11.2 Add 100 µL of Assay Buffer into each well. For the sample, add 5 - 10 µg of the nuclear extract into the sample wells. Add 2 µL of Standard Control at the different concentrations into the standard wells (ringed in green). The total volume of each well should be 100 µL. For the blank, do not add any nuclear extracts or standard control protein. Mix and cover the strip wells with Parafilm M and incubate at 37°C for 1.5 - 2 hours.

11.3 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 at 37°C for 30 min.

11.4 Remove the Blocking Buffer from each well. Wash each well three times with 150 µL of 1X Wash Buffer each time.

11.5 Add 50 µL of diluted Capture Antibody to each well and incubate at room temperature for 60 minutes on an orbital shaker (100 rpm).

11.6 Aspirate and wash the wells with 150 µL of diluted Wash Buffer three times.

11.7 Add 50 µL of diluted Detection Antibody to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 30 min.

11.8 Aspirate and wash the wells with 150 µL of diluted Wash Buffer four times.

11.9 Add 100 µL of Color Developer into the wells and incubate at room temperature for 1-10 minutes away from light. Monitor the color development in the sample and standard wells (blue).

11.10 Add 100 µL of Stop Solution to each well to stop enzyme reaction when the color in the standard wells containing the higher concentrations of standard control turns medium blue. The color should change to yellow and absorbance can be read on a microplate reader at 450 nm within 2-10 minutes.

13. ANALYSIS

12.1 **Simple Calculation % of DNMT1**

Calculate the average duplicate readings for the sample wells and blank wells.

Calculate % DNMT1 change using the following formula:

DNMT1 change % =

$$\frac{\text{Treated (Tested) Sample OD – Blank OD}}{\text{Untreated (Control) Sample OD – Blank OD}} \times 100\%$$

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

DNMT1 change % =

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

12.2 **Accurate Calculation of Histone H3K4 tri-methylation**

Generate a standard curve and plot OD value versus amount of DNMT1 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 formulas:

$$\text{DNMT1 (ng/mg protein)} = \frac{\text{Sample OD} - \text{Blank OD}}{\text{Slope x Protein Amount (µg*)}} \times 1000$$

*Nuclear extract amount added into the sample well at step 11.2

14. TROUBLESHOOTING

Problem	Cause	Solution
No Signal for Both the Positive Control and the Samples	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 are 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 standard protein is insufficiently added to the well	Ensure a sufficient amount of standard protein is added
	The DNMT1 standard has lost the binding activity due to incorrect storage.	Follow the guidance in the protocol for storage of DNMT1 Standard.
No Signal for Only the Sample	The protein amount is added into well insufficiently	Ensure extract contains a sufficient amount of protein
	Nuclear extracts are incorrectly stored	Ensure the nuclear extracts are stored at –80°C.
High Background Present for the Blank	The well is not washed sufficiently	Check if wash at each step is performed according to the protocol

High Background Present for the Blank	Contaminated by the DNMT1 standard.	Ensure the well is not contaminated from adding DNMT1 standard accidentally or from using DNMT1 contaminated tips.
	Overdevelopment	Decrease development time in step 11.6.
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)

15. NOTES

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