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Product Information

Imprint® Methylated DNA Quantification Kit

Catalog Number **MDQ1**
Storage Temperature: See Storage/Stability section for individual component storage conditions

TECHNICAL BULLETIN

Product Description

It has been well demonstrated that DNA methylation plays an important role in the regulation of gene expression. At any given time about 70% of all CpG dinucleotides in the mammalian genome are methylated.[1] Hypo- and hyper-methylation across the genome contribute to changes in gene expression. Determining the methylation status of DNA is an important tool in epigenetic studies.[2,3]

Shifts away from normal global DNA methylation levels have been observed in various cancers, neurological disorders, autoimmune diseases (such as lupus and cardiovascular diseases), and aging.[4]

The Imprint Methylated DNA Quantification Kit provides a high-throughput, non-radioactive means of measuring global DNA methylation shifts in about half of a day.

This kit contains all reagents required to detect relative levels of methylated DNA. Up to 200 ng of purified DNA is bound to the wells of the assay strip. The methylated DNA is detected using the Capture and Detection antibodies, then quantified colorimetrically. The amount of methylated DNA present in the sample is proportional to the absorbance measured.

A methylated DNA control is provided. A standard curve can be performed (range: 10–100 ng) or a single quantity of DNA can be used as a positive control. Because global methylation can vary from tissue to tissue, and from normal and diseased states, it is advised to run replicate samples at different DNA concentrations to ensure that the signal generated falls within the detection range of the plate reader used.

The kit will allow the user to determine relative methylation states of two different DNA samples.

Kit Components

Reagent	Catalog Number	48 Rxn	96 Rxn
10× Wash Buffer	W4267	11 mL	22 mL
DNA Binding Solution	D8568	1.5 mL	3 mL
Methylated Control DNA (50 ng/µL)	M8570	16 µL	32 µL
Block Solution	B6561	10 mL	20 mL
Capture Antibody	C3493	10 µL	20 µL
Detection Antibody	D8818	10 µL	25 µL
Developing Solution	D8693	6 mL	12 mL
Stop Solution	S3447	3 mL	6 mL
8 well Assay Strips	A4231	6 strips	12 strips

Materials and Reagents Required but Not Provided

- Water, Molecular Biology Reagent, Catalog Number W4502
- Isolated DNA of interest
- 1.5 mL microcentrifuge tubes
- Incubator for 37 °C incubation
- Adjustable Pipettes
- Aerosol Resistant Sterile Pipette tips
- Plate reader capable of reading absorbance at 450 nm
- Plate seals

Precautions and Disclaimer

The Imprint Methylated DNA Quantification Kit is for research use only. It is not intended and should not be used for any human or animal therapeutic or diagnostic purposes. Any such unauthorized use will be in violation of proprietary rights of Epigentek Group, Inc. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped on ice, but components should be stored as indicated in the table below.

Reagent	Catalog Number	Storage Temperature
10× Wash Buffer	W4267	2–8 °C
DNA Binding Solution	D8568	2–8 °C
Methylated Control DNA (50 ng/µL)	M8570	–20 °C
Block Solution	B6561	–20 °C
Capture Antibody	C3493	2–8 °C
Detection Antibody	D8818	2–8 °C
Developing Solution	D8693	2–8 °C
Stop Solution	S3447	2–8 °C
8 well Assay Strips	A4231	2–8 °C

Preparation Instructions

1× Wash Buffer

For the 48-reaction kit, add 11 mL of 10× Wash Buffer to 99 mL of Water, Molecular Biology Reagent. Store at room temperature for up to six months.

For the 96-reaction kit, add 22 mL of 10× Wash Buffer to 198 mL of Water, Molecular Biology Reagent. Store at room temperature for up to six months.

DNA Dilutions - Dilute sample or control DNA in DNA Binding Solution. Each well can bind a maximum of 200 ng of total DNA.

Procedure

DNA Binding

1. Add 30 µL of DNA diluted in DNA Binding Solution to each well. Ensure the solution coats the bottom of the well by gently tilting from side to side. The DNA Binding Solution alone can be used as a blank.
2. Cover and incubate at 37 °C for 60 minutes.
3. Add 150 µL of Block Solution directly to each well.
4. Cover and incubate at 37 °C for 30 minutes.
5. Remove DNA and Block Solutions from each well.
6. Wash three times with 150 µL of 1× Wash Buffer.

Methylated DNA Capture

7. Dilute Capture Antibody 1:1000 in 1× Wash Buffer.
8. Add 50 µL of diluted Capture Antibody to each well.
9. Cover and incubate at room temperature for 60 minutes.
10. Remove diluted Capture Antibody from well.
11. Wash four times with 150 µL of 1× Wash Buffer.
12. Dilute Detection Antibody 1:1000 in 1× Wash Buffer.
13. Add 50 µL of diluted Detection Antibody to each well.
14. Cover and incubate at room temperature for 30 minutes.
15. Remove diluted Detection Antibody from well.
16. Wash five times with 150 µL of 1× Wash Buffer.

Detection

17. Add 100 µL of Developing Solution to each well.
18. Cover and incubate at room temperature away from light for 1–10 minutes. Monitor reaction for color change. The solution will turn blue.
19. Add 50 µL of Stop Solution to each well. The reaction will turn yellow.
20. Read the absorbance at 450 nm on a plate reader.
21. Calculate relative global methylation levels.

Calculations
To calculate methylation levels relative to the Methylated Control DNA, use the following steps.

Single Point Method
Percent methylation relative to the Methylated Control DNA

1. Average the A_{450} replicates for the blank, sample(s) and Methylated Control DNA
2. Perform the following calculation to obtain the percent methylation of the sample(s) relative to the Methylated Control DNA

$[(A_{450\ av}\ \text{Sample} - A_{450\ av}\ \text{Blank})/(A_{450\ av}\ \text{Methylated Control DNA} - A_{450\ av}\ \text{Blank})] \times 100$

3. Sample calculation:

   Average A_{450} Blank = 0.090
   Average A_{450} for 60 ng Methylated Control DNA = 0.785
   Average A_{450} for 60 ng sample DNA = 0.654

   $[(0.654 - 0.090) / (0.785 - 0.090] \times 100 = 81.15\%$

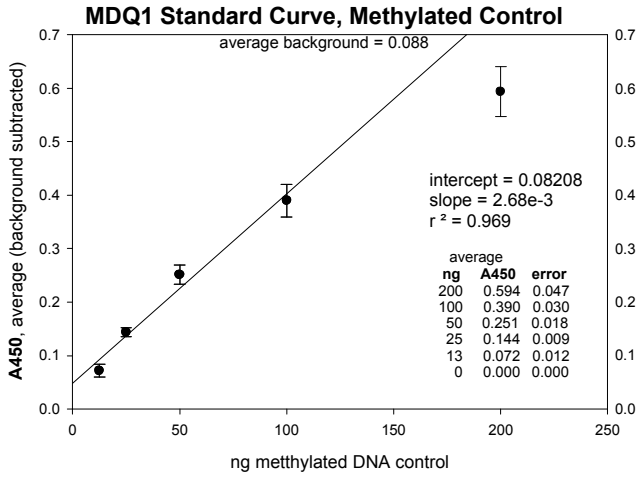
The sample DNA has a global methylation level that is 81.15% relative to the Methylated Control DNA

Standard Curve Method
ng Methylated DNA in sample(s) from standard curve of Methylated Control DNA

1. Create a standard curve by plotting ($A_{450\ av}$ Methylated Control DNA - $A_{450\ av}$ Blank) versus ng of the Methylated Control DNA. See sample curve.
2. Calculate the slope of the standard curve using linear regression
3. For each value of ($A_{450\ av}$ Sample - $A_{450\ av}$ Blank), calculate the ng methylated DNA as below:

ng methylated DNA in sample = $[(A_{450\ av}\ \text{Sample} - A_{450\ av}\ \text{Blank}) - \text{intercept}] / \text{slope}$

Note: in many, or most cases, the intercept will be zero.



Troubleshooting Guide

Observation	Cause	Recommended Solution
High Background Present in Blank (>0.2 OD)	Inappropriate/inconsistent washing	Perform all wash steps as indicated in the protocol. If necessary, one wash can be added at each of the wash steps.
	Contaminating DNA	Ensure a clean tip is used when applying DNA Binding Solution. When handling plate, ensure that no splashing occurs.
	Over-development	Monitor development of blue color once the Developing Solution has been added. Do not allow the reaction to incubate more than ten minutes.
No Signal for DNA-Containing Samples (<0.2 OD)	Addition of Reagents	Ensure reagents are added in the appropriate order and none of the procedure steps have been omitted.
	Washing	Ensure the wells of the assay strip are not washed prior to the application of the DNA sample.
	Incubation time and temperatures	Ensure incubation time and temperatures correspond to the written protocol.
	Addition of DNA to well	Ensure DNA is mixed thoroughly prior to application. The wells of the assay strip bind up to 200 ng of total DNA. The methylated control DNA (Catalog Number M8570) included in the kit can be detected at the 10 ng level.
	Degradation of DNA	Ensure DNA is stored at the appropriate temperature.

References

1. Shen, L., et al., Optimizing annealing temperature overcomes bias in bisulfite PCR methylation analysis. *Biotechniques,* **42,** 48-58 (2007).
2. Leone, G., et al., DNA methylation and demethylating drugs in myelodysplastic syndromes and secondary leukemias. *Haematologica*, **87,** 1324-1341 (2002).
3. Beier, V., et al., Monitoring methylation changes in cancer. *Adv Biochem Eng Biotechnol*;, **104,** 1-11 (2007).
4. Rodenhiser, D., and Mann, M., Epigenetics and human disease: translating basic biology into clinical applications. *CMAJ*, **174**, 341-348 (2006).

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