EpiQuik™ DNMT3B Assay Kit

Base Catalog # P-3013

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ DNMT3B Assay Kits are very suitable for measuring Dnmt3B amounts quantitatively from fresh tissue and cultured cells of human and mouse.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

KIT CONTENTS

Component	48 Assays Cat. #P-3013-2	96 Assays Cat. #P-3013-3	Storage Upon Receipt
DB1 (10X Wash Buffer)	12 ml	25 ml	4°C
DB2 (Assay Buffer)	5 ml	10 ml	4°C
DB3 (DNMT3B Standard, 20 µg/ml)*	16 µl	30 µl	–20°C
DB4 (Capture Antibody, 500 µg/ml)*	8 µl	16 µl	4°C
DB5 (Detection Antibody, 200 µg/ml)*	10 µl	20 µl	–20°C
DB6 (Developing Solution)	6 ml	12 ml	4°C
DB7 (Stop Solution)	6 ml	11 ml	RT
BB (Blocking Buffer)	10 ml	20 ml	4°C
8-Well Assay Strips (With Frame)	6	12	4°C

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

SHIPPING & STORAGE

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 store: (1) **DB3** and **DB5** at –20°C away from light; (2) **DB1**, **DB2**, **DB4**, **DB6**, **Blocking Buffer** and the **8-Well Assay Strips** at 4°C away from light; (3) **all other components** can be stored at room temperature. The kit is stable for 6 months from the shipment date, when stored properly.

***Note**: (1) Check if wash buffer, **DB1**, 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 **DB6** (Developing Solution), which would indicate contamination of the solution and should not be used. To avoid contamination, transfer the amount of **DB6** required into a secondary container (tube or vial) before adding **DB6** into the assay wells.*

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

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiQuik™ DNMT3B Assay Kit is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The EpiQuik™ DNMT3B Assay Kit is for research use only and is not intended for diagnostic or therapeutic applications.

Intellectual Property: The EpiQuik™ DNMT3B Assay Kit and methods of use contain proprietary technologies by Epigentek.

A BRIEF OVERVIEW

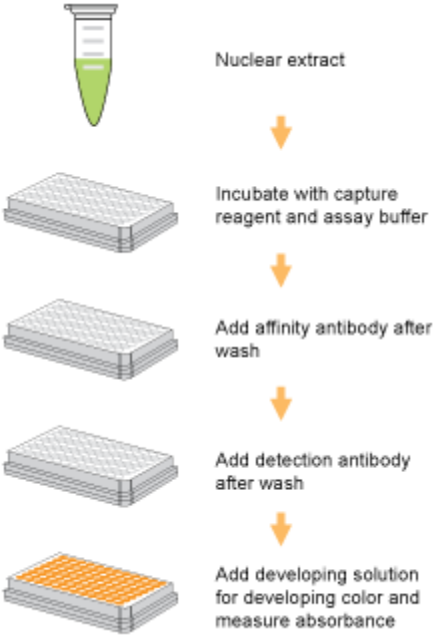
Epigenetic inactivation of genes plays a critical role in many important human diseases, especially in cancer. A core mechanism for epigenetic inactivation of the genes is methylation of CpG islands in genome DNA. Methylation of CpG islands involves the course in which DNA methyltransferases (Dnmts) 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 so far identified in mammals: DNMT1, DNMT2, and DNMT3. The DNMT3 family comprises of three different proteins: DNMT 3A, DNMT3B, and DNMT3L. DNMT3A and DNMT3B have been demonstrated to methylate both unmethylated and hemimethylated DNA equally and is supposed to mediate *de novo* methylation together with DNMT1. Increased activation or amounts of DNMT3 is believed to be involved in carcinogenesis, and other genetic and epigenetic diseases.

Several methods, such as Western blot, are used for measuring levels of Dnmt3B. However, these methods available so far are inconvenient, considerably time consuming, labor intensive, or have low throughput. The EpiQuik™ Dnmt3B Assay Kit addresses these problems by using a unique procedure to measure the amount of Dnmt3B. The kit has the following features:

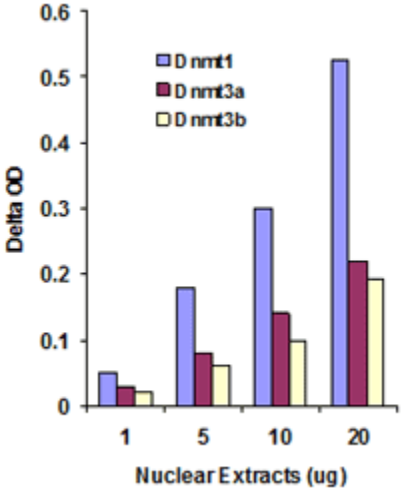
- Very rapid procedure, which can be finished within 3.5 hours.
- Innovative colorimetric assay to quantitatively measure the amount of DNMT3B without the need for electrophoresis.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The EpiQuik™ DNMT3B Assay Kit is designed for measuring total DNMT3B 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 DNMT3B contained in the sample binds to the substrate. The bound DNMT3B can be recognized with a specific DNMT3B antibody and colorimetrically quantified through an ELISA-like reaction. The amount of DNMT3B is proportional to the intensity of color development.



Schematic procedure of the EpiQuik™ DNMT3B Assay Kit



Nuclear extracts were prepared from MCF-7 cells using the EpiQuik Nuclear Extraction Kit and total amount of DNMT was measured.

PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Input Amount: The amount of nuclear extracts for each assay can be between 1 µg and 20 ug 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. EpigenTek also offers a nuclear extraction kit (Cat # OP-0002) optimized for use with this kit. (Nuclear extracts should be stored at –80°C in aliquots until use.)

1. Working Buffer & Solution Preparation

a. Prepare **Diluted DB1** 1X Wash Buffer:

48-Assay Kit: Add 13 ml of **DB1** 10X Wash Buffer to 117 ml of distilled water and adjust pH to 7.2-7.5.

96-Assay Kit: Add 26 ml of **DB1** 10X Wash Buffer to 234 ml of distilled water and adjust pH to 7.2-7.5.

This **Diluted DB1** 1X Wash Buffer can now be stored at 4°C for up to six months.

b. Prepare **Diluted DB4** Capture Antibody Solution:

Dilute **DB4** Capture Antibody with **Diluted DB1** 1X Wash Buffer at a ratio of 1:500 (i.e., add 1 µl of **DB4** to 500 µl of **Diluted DB1**). 50 µl of **Diluted DB4** will be required for each assay well.

c. Prepare **Diluted DB5** Detection Antibody Solution:

Dilute **DB5** Detection Antibody with **Diluted DB1** 1X Wash Buffer at a ratio of 1:2000 (i.e., add 1 µl of **DB5** to 2000 µl of **Diluted DB1**). 50 µl of **Diluted DB5** will be required for each assay well.

d. Prepare **Diluted DNMT3B Standard**:

Suggested Standard Curve Preparation: Dilute **DB3** DNMT3B Standard with **DB2** to the concentrations of 1, 2, 5, 10 and 20 ng/µl according to the following dilution chart:

Tube	DB3 (20 ng/µl)	DB2	Resulting DB3 Concentration
1	0.5 µl	9.5 µl	1 ng/µl
2	0.5 µl	4.5 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* ***DB1*** *1X Wash Buffer on ice until use. Any remaining diluted solutions other than* ***Diluted DB1*** *should be discarded if not used within the same day.*

2. DNMT3B Binding

a. 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).

b. Blank Wells: Add 100 µl of **DB2** to each blank well.

c. Standard Wells: Add 98 µl of **DB2** and 2 µl of **Diluted DNMT3B 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 Step 1e; see Table 2 as an example).

d. Sample Wells: Add 94 to 98 µl of **DB2** and 2 to 6 µl of your nuclear extracts to each sample well. Total volume should be 100 µl per well.

Note: *(1) Follow the suggested well setup diagrams; (2) It is recommended to use 5 µg to 10 µg of nuclear extract per well.*

e. Cover strip-well microplate with Parafilm M or aluminum foil to avoid evaporation and incubate at 37°C for 90 to 120 min.

f. 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.

g. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted DB1** 1X Wash Buffer each time.

3. Antibody Binding & Signal Enhancing

a. Add 50 µl of the **Diluted DB4** to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 60 min.

b. Remove the **Diluted DB4** solution from each well.

c. Wash each well three times with 150 µl of the **Diluted DB1** each time.

d. Add 50 µl of the **Diluted DB5** to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 30 min.

e. Remove the **Diluted DB5** solution from each well.

f. Wash each well four times with 150 µl of the **Diluted DB1** each time.

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

4. Signal Detection

a. Add 100 µl of **DB6** 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 **DB6** solution will turn blue in the presence of sufficient demethylated products.

b. Add 100 µl of **DB7** to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding **DB7** 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.*

5. DNMT3B Calculation

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

b. Calculate % DNMT3B change using the following formula:

$$DNMT3B\ change\ \% = \frac{Treated\ (Tested)\ Sample\ OD - Blank\ OD}{Untreated\ (Control)\ Sample\ OD - Blank\ OD} \times 100\%$$

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

$$DNMT3B\ change\ \% = \frac{(0.5 - 0.1)}{0.9 - 0.1} \times 100\% = 50\%$$

For Detailed Quantification:

1. Generate a standard curve and plot OD value versus amount of **DB3 Standard** at each concentration point.
2. Determine the slope as OD/ng (you can use Microsoft Excel statistical functions for slope calculation), then calculate the amount of DNMT3B using the following formulas:

$$DNMT3B\ (ng/mg\ protein) = \frac{(Sample\ OD - Blank\ OD)}{Slope \times Protein\ Amount\ (ug)^*} \times 1000$$

** Nuclear extract added into sample wells at Step 2d.*

SUGGESTED BUFFER AND SOLUTION SETUP

Table 1. Approximate amount of required buffers and solutions for defined assay wells based on the protocol.

Reagents	1 well	1 strip (8 wells)	2 strips (16 wells)	6 strips (48 wells)	12 strips (96 wells)
Diluted DB1	2.5 ml	20 ml	40 ml	120 ml	240 ml
DB2	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
DB3 Standard control	N/A	N/A	4 µL (optional)	8 µl	8 µl
Diluted DB4	50 µl	400 µl	800 µl	2400 µl	4800 µl
Diluted DB5	50 µl	400 µl	800 µl	2400 µl	4800 µl
DB6	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml
DB7	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml

SUGGESTED STRIP WELL SETUP

Table 2. The suggested strip-well plate setup for Dnmt1 quantification in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can be measured in duplicate.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	DB3 2 ng	DB3 2 ng	Sample	Sample	Sample	Sample
C	DB3 4ng	DB3 4ng	Sample	Sample	Sample	Sample
D	DB3 10 ng	DB3 10 ng	Sample	Sample	Sample	Sample
E	DB3 20 ng	DB3 20 ng	Sample	Sample	Sample	Sample
F	DB3 40 ng	DB3 40 ng	Sample	Sample	Sample	Sample
G	Sample	Sample	Sample	Sample	Sample	Sample
H	Sample	Sample	Sample	Sample	Sample	Sample

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
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 have been omitted.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
	Incorrect absorbance reading.	Check absorbance wavelength (450 nm).
	Kit was not stored or handled properly.	Ensure components were stored at the appropriate temperature and the cap is tightly closed after use.
No signal or weak signal in only the standard curve wells	Insufficient standard amount added to the well in Step 2c.	Ensure a sufficient amount of standard is added.
	Degraded standard due to improper storage conditions.	Follow the Shipping & Storage guidance in this User Guide for storage of **DB3** DNMT3B 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 or from using contaminated tips.
	Incubation with **Diluted DB5** is too long.	Incubation time at Step 3d should not exceed 90 min.
	Over-development of color.	Decrease the development time in Step 4a before adding **DB6** Stop Solution in Step 4b.
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. For the best results, o use EpigenTek's Nuclear extraction Kit (Cat. No. OP-0002).
	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of nuclear extracts is used 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 for nuclear extracts.
	Little or no DNMT3B 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 and residue washing buffer is completely removed
	Delayed color development or delayed stopping of color development in the wells.	Ensure color development or stop solution is added sequentially and consistently in the same order as adding the other reagents (e.g., from well A to G or from well 1 to well 12).

RELATED PRODUCTS

Nuclear Extract Preparation

OP-0002 EpiQuik™ Nuclear Extraction Kit

DNA Methyltransferase Activity/Inhibition Assay

P-3009 EpiQuik™ DNMT Activity/Inhibition Assay Ultra Kit (Colorimetric)
P-3010 EpiQuik™ DNMT Activity/Inhibition Assay Ultra Kit (Fluorometric)
P-3011 EpiQuik™ DNMT1 Assay Kit
P-3012 EpiQuik™ DNMT3A Assay Kit