

EpiQuik™ DNMT Activity/Inhibition ELISA Easy Kit (Colorimetric)

Base Catalog # P-3139

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ DNMT Activity/Inhibition ELISA Easy Kit (Colorimetric) is suitable for measuring total DNMT activity or inhibition using nuclear extracts or purified enzymes from a broad range of species in an easy and fast manner. The species include mammals, plants, fungi, bacteria, and viruses in a variety of forms including, but not limited to, cultured cells and fresh/frozen tissues. Nuclear extracts can be prepared by using your own successful method. For your convenience and the best results, EpigenTek also offers a nuclear extraction kit (Cat. No. OP-0002) optimized for use with this kit. Nuclear extracts can be used immediately or stored at -80°C for future use. Purified enzymes can be active DNMTs from recombinant proteins or isolated from cell/tissues.

Input Material: Input materials can be nuclear extracts or purified DNMT enzymes. The amount of nuclear extracts for each assay can be between $0.5\text{ }\mu\text{g}$ to $20\text{ }\mu\text{g}$ with an optimal range of $5\text{--}10\text{ }\mu\text{g}$. The amount of purified enzymes can be 0.2 ng to 100 ng , depending on the purity and catalytic activity of the enzymes.

Internal Control: A positive enzyme control is provided in this kit. Because DNMT activity can vary from tissue to tissue, and from normal and diseased states, it is advised to run replicate samples to ensure that the signal generated is validated.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

KIT CONTENTS

Component	48 Assays Cat. #P-3139-48	96 Assays Cat. #P-3139-96	Storage Upon Receipt
WB (10X Wash Buffer)	14 ml	28 ml	4°C
DAB (DNMT Assay Buffer)	4 ml	8 ml	RT
SAM (S-adenosylmethionine, 50X)*	60 µl	120 µl	-20°C
DEC (DNMT Enzyme Control, 50 µg/ml)*	6 µl	12 µl	-20°C
CA (Capture Antibody, 1000X*)	5 µl	10 µl	4°C
SI (Signal Indicator, 1000X)*	6 µl	12 µl	-20°C
ES (Enhancer Solution, 2000X)*	6 µl	12 µl	-20°C
DS (Developer Solution)	5 ml	10 ml	4°C
SS (Stop Solution)	5 ml	10 ml	RT
8-Well Assay Strips (With Frame)	6	12	4°C
Adhesive Covering Film	1	1	RT

**Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

The kit is shipped in two parts: the first part at ambient room temperature and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store **SAM**, **DEC**, **SI**, and **ES** at -20°C away from light; (2) Store **WB**, **CA**, **DS**, and the **8-Well Assay Strips** at 4°C away from light; (3) Store all remaining components (**DAB**, **SS**, and the **Adhesive Covering Film**) at room temperature away from light.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.



Take Note! (1) Check if **WB** (10X Wash Buffer) contains salt precipitates before use briefly warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.; (2) transfer the amount of **DS** required into a secondary container (tube or vial) before adding **DS** into the assay wells in order to avoid contamination. Check if a blue color is present in **DS** (Developer Solution) before each use, as this would indicate contamination of the solution and should not be used.

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 extract or purified enzyme samples containing DNMT activity
- ☐ DNMT inhibitors (optional)
- ☐ Parafilm M or aluminum foil

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of this product 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.epigenetek.com/datasheet.

Usage Limitation: This product is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: This product and methods of use contain proprietary technologies by EpigenTek.

A BRIEF OVERVIEW

DNA methylation occurs by a covalent addition of a methyl group at the 5-carbon of the cytosine ring, resulting in 5-methylcytosine. These methyl groups project into the major grooves of DNA and inhibit transcription. In human DNA, 5-methylcytosine is found in approximately 1.5% of genomic DNA, primarily at CpG sites. There are clusters of CpG sites at 0.3 to 2 kb stretches of DNA known as CpG islands that are typically found in or near promoter regions of genes, where transcription is initiated. In the bulk of genomic DNA, most CpG sites are heavily methylated. However, CpG islands in germ-line tissue and promoters of normal somatic cells remain unmethylated, allowing gene expression to occur. When a CpG island in the promoter region of a gene is methylated, the expression of the gene is repressed. The repression can be caused by directly inhibiting the binding of specific transcription factors, and indirectly by recruiting methyl-CpG-binding proteins and their associated repressive chromatin remodeling activity. In addition to the effect on gene transcription, DNA methylation is also involved in genomic imprinting, which refers to a parental origin specific expression of a gene, and the formation of a chromatin domain.

DNA methylation is controlled at several different levels in normal and diseased cells. The addition of methyl groups is carried out by a family of enzymes, DNA methyltransferases (DNMTs). Chromatin structure in the vicinity of gene promoters also affects DNA methylation and transcriptional activity. Three DNMTs (DNMT1, DNMT3A, and DNMT3B) are required for the establishment and maintenance of DNA methylation patterns. Two additional enzymes (DNMT2 and DNMT3L) may also have more

specialized but related functions. DNMT1 appears to be responsible for the maintenance of established patterns of DNA methylation, while DNMT3A and DNMT3B seem to mediate the establishment of new or *de novo* DNA methylation patterns. DNMT3L is found to be a catalytically inactive regulatory factor of DNA methyltransferases, which is essential for the function of DNMT3A and DNMT3B. Diseased cells such as cancer cells may be different in that DNMT1 alone is not responsible for maintaining abnormal gene hypermethylation and both DNMT1 and DNMT3B may be cooperative for this function. The local chromatin structure also contributes to the control of DNA methylation.

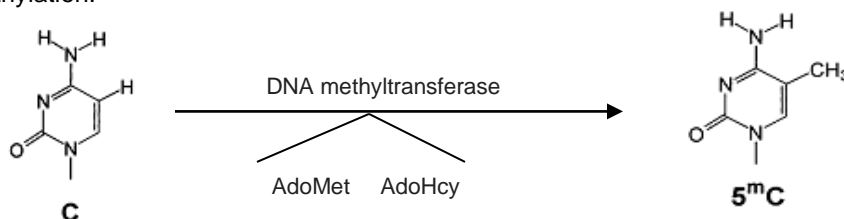


Fig 1. Methylation of cytosine in DNA via DNA methyltransferase and S-adenosylmethionine

The importance of DNA methylation is emphasized by the growing number of human diseases that are known to occur when DNA methylation information is not properly established and/or maintained. Abnormal DNA methylation associated with increased expression or the activity of DNMTs has been found in many different diseases, especially in cancer. Inhibition of DNMTs may lead to demethylation and expression of silenced genes. DNMT inhibitors are currently being developed as potential anticancer agents.

Conventional DNMT activity/inhibition assay methods are time consuming, labor-intensive, have low throughput, and/or produce radioactive waste. The original EpiQuik™ DNMT Activity/Inhibition Assay Kit addressed this issue by introducing a simple method with an ELISA-like 96-well plate format. The **EpiQuik™ DNMT Activity/Inhibition ELISA Easy Kit (Colorimetric)** is a further refinement of its predecessor kit by simplifying the steps, significantly decreasing assay time, and increasing assay sensitivity.

- **Fast** - Reduced steps so that the entire procedure only needs 2 hours and 10 minutes.
- **Sensitive** - Detection limit can be as low as 0.2 ng of purified enzymes, which is ten times better than the predecessor kit.
- **Convenient** - Ready to use with all essential components. No need for radioactivity or any special equipment.
- **Robust** - Improved kit composition allows the assay to have a greater "signal window" with reduced variation between replicates.
- **Flexible** - 96 strip-well microplate format makes the assay available for manual or high throughput analysis.

PRINCIPLE & PROCEDURE

The **EpiQuik™ DNMT Activity/Inhibition ELISA Easy Kit (Colorimetric)** contains all reagents necessary for the measurement of DNMT activity or inhibition. In this assay, a universal DNMT substrate is stably coated onto microplate wells. DNMT enzymes transfer a methyl group to cytosine from AdoMet to methylate DNA substrate and the methylated DNA can be recognized with an anti-5-methylcytosine antibody. The ratio or amount of methylated DNA, which is proportional to enzyme activity, can then be measured through an ELISA-like reaction by reading the absorbance in a microplate spectrophotometer at a wavelength of 450 nm. The activity of DNMT enzymes is proportional to the optical density intensity measured.

Prepare nuclear extracts
or purified enzyme



Incubate with
substrate for 90 min

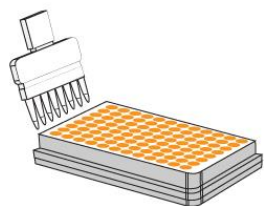
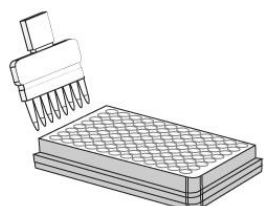
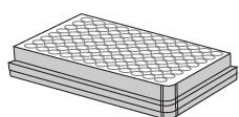


Wash wells, then
add detection
complex solution

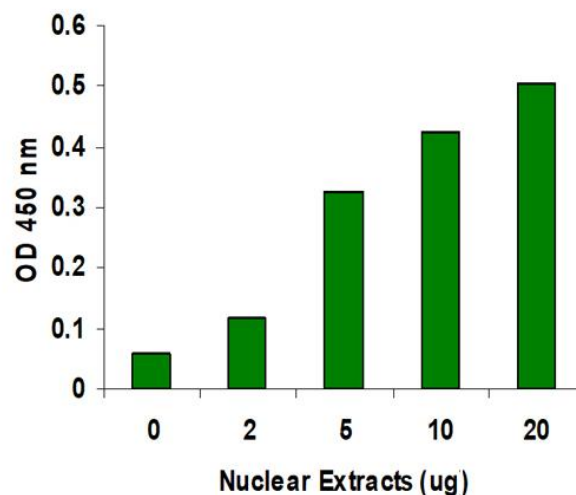
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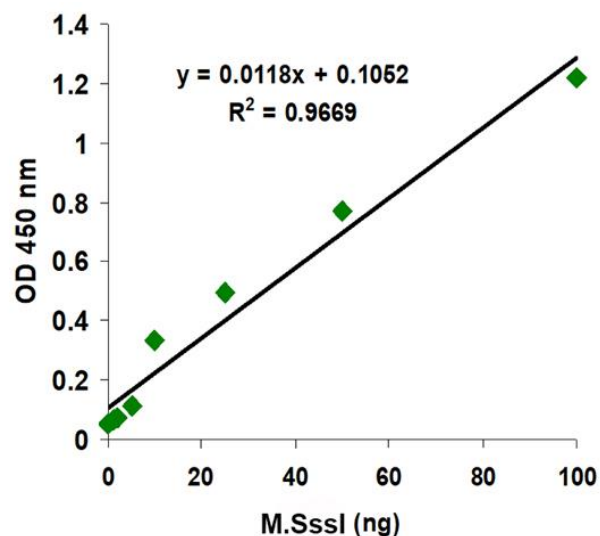
Add color developing
solution for color
development, then
measure absorbance



▲ Schematic procedure of the EpiQuik™ DNMT
Activity/Inhibition ELISA Easy Kit (Colorimetric).



▲ Demonstration of high sensitivity and specificity of
DNMT activity/inhibition achieved by using nuclear
extracts with the EpiQuik™ DNMT Activity/Inhibition
ELISA Easy Kit (Colorimetric). Nuclear extracts were
prepared from Hela cell line.



▲ Demonstration of high sensitivity and specificity of
DNMT activity/inhibition achieved by using DNA
methylase M.Sssl with the EpiQuik™ DNMT
Activity/Inhibition ELISA Easy Kit (Colorimetric).

ASSAY PROTOCOL

Starting Materials

Input Amount: The amount of nuclear extracts for each assay can be between 0.5 µg and 20 µg with an optimal range of 5 µg to 10 µg. The amount of purified enzymes can be 0.2 ng to 100 ng, depending on the purity and catalytic activity of the enzymes.

Nuclear Extraction: You can use your own method of choice for preparing nuclear extracts. EpigenTek also offers a nuclear extraction kit (Cat. No. OP-0002) optimized for use with this kit.

Nuclear Extract or Purified DNMT Storage: Nuclear extract or purified DNMT enzymes should be stored at –80°C until use

1. Working Buffer and Solution Preparation

- a. Prepare **Diluted WB** (1X Wash Buffer):

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

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

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

- b. Prepare **Working DAB** (DNMT Assay Buffer):

Freshly prepare the **Working DAB** Buffer required for the assay by adding 2 µl of **SAM** into 98 µl of **DAB**. About 50 µl of this **Working DAB** Buffer will be required for each assay well.

- c. About the **DEC** (DNMT Enzyme Control):

The **DEC** is an enzyme with activity of both maintenance and *de novo* DNMTs and is used as the positive control of this assay. We do not recommend using this enzyme control to generate a standard curve for quantifying the activity of your samples, as the amount of the enzyme is limited and catalytic activity/unit is different.



Take Note! Keep all diluted solutions (except **Diluted WB**) on ice until use. Any remaining diluted solutions, other than **Diluted WB**, should be discarded if not used within the same day.

The anticipated approximate volumes of reagents needed are reflected below for this assay.

Reagents	1 well	8 wells (1 strip)	16 wells (2 strips)	48 wells (6 strips)	96 wells (12 strips)
Diluted WB	2.5 ml	20 ml	40 ml	120 ml	240 ml
Working DAB	50 μ l	400 μ l	800 μ l	2400 μ l	4800 μ l
Detection Complex Solution	50 μ l	400 μ l	800 μ l	2400 μ l	4800 μ l
DS (Developer Solution)	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml
SS (Stop Solution)	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml
DNMT Enzyme Control	N/A	0.25 μ l – 1 μ l	0.5 μ l – 2 μ l	1 μ l – 4 μ l	2 μ l – 8 μ l

2. Enzymatic Reaction

Review the configuration of the strip-well plate setup a 48-assays format below (for a 96-reaction format, strips 7 through 12 can be configured as *Sample*). The controls and samples can be measured in duplicate, loaded vertically instead of horizontally.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Sample 2	Sample 6	Sample 10	Sample 14	Sample 18
B	Blank	Sample 2	Sample 6	Sample 10	Sample 14	Sample 18
C	DEC 0.5 μ l	Sample 3	Sample 7	Sample 11	Sample 15	Sample 19
D	DEC 0.5 μ l	Sample 3	Sample 7	Sample 11	Sample 15	Sample 19
E	DEC 1.0 μ l	Sample 4	Sample 8	Sample 12	Sample 16	Sample 20
F	DEC 1.0 μ l	Sample 4	Sample 8	Sample 12	Sample 16	Sample 20
G	Sample 1	Sample 5	Sample 9	Sample 13	Sample 17	Sample 21
H	Sample 1	Sample 5	Sample 9	Sample 13	Sample 17	Sample 21

- Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (include blank and positive control) 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).
- Blank Wells:** Add 50 μ l of **Working DAB** Buffer per well.
- Positive Control Wells:** Add 50 μ l of **Working DAB** Buffer and then 0.5 μ l and 1 μ l of DEC per well.
- Sample Wells Without Inhibitor:** Add 45 μ l to 49 μ l of **Working DAB** Buffer, and 1 μ l to 5 μ l of nuclear extracts or 1 to 5 μ l of purified DNMT enzymes per well. The total volume should be 50 μ l per well.
- Sample Wells With Inhibitor:** Add 40 μ l to 44 μ l of **Working DAB** Buffer, 1 to 5 μ l of nuclear extracts or 1 to 5 μ l of purified DNMT enzymes, and 5 μ l of inhibitor solution per well. The total volume should be 50 μ l per well.



Take Note! (1) To reduce cross variation between replicates, it is important to load the wells in vertical formation according to the plate layout depicted above. (2) It is recommended to use 5 μ g to 10 μ g of nuclear extract per well or 10 ng to 100 ng of purified enzyme per well; (3) The concentration of inhibitors to be added into the sample wells can be varied (e.g., 1 μ M to 1000 μ M). However, the final concentration of the inhibitors before adding to the wells should be prepared

with **DAB** Buffer at a 1:10 ratio (e.g., add 0.5 µl of inhibitor to 4.5 µl of **DAB** Buffer), so that the original solvent of the inhibitor can be reduced to 1% of the reaction solution or less.

- f. Tightly cover the strip-well microplate with **Adhesive Covering Film** to avoid evaporation, and incubate at 37°C for 60-90 min.



Take Note! (1) The incubation time may depend on intrinsic DNMT activity. In general, 60 min incubation is suitable for active purified DNMT enzymes and 90 min incubation may be required for nuclear extracts; (2) The **Adhesive Covering Film** can be cut to the required size to cover the strips based on the number of strips utilized.

- g. Remove the reaction solution from each well. Wash each well three times with 150 µl of **Diluted WB** 1X Wash Buffer each time. This can be done by simply pipetting **Diluted WB** in and out of the wells.



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

3. Antibody Binding & Signal Enhancing

- a. During the last 10 minutes of sample incubation, prepare the **Detection Complex Solution**: In each 1 ml of **Diluted WB** add 1 µl of **CA**, mix and then add 1 µl of **SI** and 0.5 µl of **ES**. Mix well.
- b. After 60-minute incubation, remove the assay buffer from each well. Wash each well with 150 µl of the **Diluted WB** each time for three times. This can be done by simply pipetting **Diluted WB** in and out of the wells.
- c. Add 50 µl of the **Detection Complex Solution** to each, then cover and incubate at room temperature for 50 minutes.
- d. Remove the **Detection Complex Solution** from each well.
- e. Wash each well with 150 µl of the **Diluted WB** each time for five times.

4. Signal Detection

- a. Add 100 µl of **DS** (Developer Solution) to each well in a column, not row, simultaneously in a vertical fashion with a multi-channel pipette so that replicates are developed at the same time. Gently shake the plate against a flat surface for 5-10 seconds and incubate at room temperature for 3-4 minutes. Monitor color development in the sample wells and control wells. After a few minutes, the **DS** will turn blue in the presence of sufficient methylated DNA. The color in the **Blank** wells will remain generally unchanged.
- b. When the color in the positive control wells turns medium blue, stop the enzyme reaction by adding 100 µl of **SS** (Stop Solution) to each well in a column, not row, simultaneously in a vertical fashion with a multi-channel pipette so that replicates are stopped at the same time. 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 **SS** and absorbance should be read on a microplate reader within 2 to 10 minutes– at 450 nm with an optional reference wavelength of 655 nm.



Take Note! (1) Most microplate readers have the capability to carry out dual wavelength analysis and will automatically subtract the 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 stripwell microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.

5. DNMT Activity Calculation

- Calculate the average duplicate readings for sample wells and blank wells.
- Calculate DNMT activity using the following formula:

$$\text{DNMT Activity (OD/h/mg)} = \frac{(\text{Sample OD} - \text{Blank OD})}{(\text{Protein amount } (\mu\text{g}) \times \text{hour}^{**})} \times 1000$$

* Protein amount added into the reaction at step 2d in μg .

** Incubation time at step 2f.

Example calculation:

Average OD₄₅₀ of sample is 0.25

Average OD₄₅₀ of blank is 0.05

Protein amount is 5 μg

Incubation time is 1 hour (60 min)

$$\text{DNMT activity} = \frac{(0.25 - 0.05)}{(5 \times 1)} \times 1000 = 40 \text{ OD/h/mg}$$

- Calculate DNMT inhibition using the following formula:

$$\text{DNMT Inhibition \%} = 1 - \left[\frac{\text{Inhibitor Sample OD} - \text{Blank OD}}{\text{No Inhibitor Sample OD} - \text{Blank OD}} \right] \times 100\%$$

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 in the protocol may have been omitted by mistake.
	The well is incorrectly washed before enzyme reaction.	Ensure the well is not washed prior to adding the standard control and sample.
	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 filter) 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 positive control wells	The DNMT enzyme control is insufficiently added to the well in Step 2c.	Ensure a sufficient amount of DNMT enzyme control is added.
	The quality of the DNMT enzyme control has been degraded due to improper storage conditions.	Follow the Shipping & Storage guidance for storage instructions of DEC (DNMT Enzyme Control).
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 positive control.	Ensure the well is not contaminated from adding sample or positive control accidentally or from using contaminated tips.
	Incubation time with detection antibody is too long.	The incubation time at Step 3c should not exceed 75 min.
	Over development of color.	Decrease the development time in Step 4a before adding SS (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 DNMT protein extraction. For the best results, it is advised to use EpigenTek's Nuclear Extraction Kit (Cat. No. OP-0002). Also, use fresh cells or tissues for protein extraction, as frozen cells or tissues could lose enzyme activity.
	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of purified enzymes or 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 weeks for nuclear extracts and 6 months for purified enzymes. Avoid repeated freezing/thawing.
	Little or no activity of DNMT contained 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 or purified enzymes.
Uneven color development	Insufficient washing of the wells.	Ensure the wells are washed according to the protocol. Ensure any residues from the wash buffer are removed as much as possible.
	Delayed color development or delayed stopping of color development in the wells.	Ensure color development solution is added sequentially and consistent with the order you added the other reagents (e.g., from well A to well H or from well 1 to well 12).

Large variation between replicate wells	Horizontal positioning of well replicates causes inconsistent delays in pipetting and loading of reagents.	Follow the vertical layout example provided in Step 2 . Ensure loading of reagents is also in vertical order with a multi-channel pipette, especially when adding DS (Developer Solution) and SS (Stop Solution) in Step 4 .
	Color reaction is not evenly stopped due to an inconsistency in pipetting time.	Ensure that DS (Developer Solution) and SS (Stop Solution) are added at the same time between replicates or otherwise maintain a consistent timing in between each addition of solutions.
	Color reaction is not evenly stopped due to an inconsistent order of adding solutions.	Ensure all solutions, particularly DS (Developer Solution) and SS (Stop Solution), are added in the same order each time as all other solutions.
	The solutions are not evenly added due to 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 (e.g., 1 ul) are completely added into the wells. Pay special attention at the steps of adding samples and preparing Detection Complex Solution .
	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 to prevent solutions from sticking to the surface.
	Did not sufficiently shake the solutions in the wells evenly after adding SS Stop Solution in Step 4b.	Gently and evenly shake the plate frame 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.
Capture Antibody vial appears to be empty or insufficient in volume	Buffer evaporated due to the very small volumes, resulting in a higher concentrated antibody.	Add 1X PBS buffer into the CA (Capture Antibody) vial until you restore the correct, intended volume according to the Kit Contents described in this User Guide. Mix and centrifuge prior to use.

RELATED PRODUCTS

Nuclear Extract Preparation

OP-0002-1 EpiQuik™ Nuclear Extraction Kit

DNMT Activity/Inhibition Assay and Content Quantification

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
 P-3013 EpiQuik™ DNMT3B Assay Kit