ab113467

EpiSeeker DNMT Activity Quantification Kit (Colorimetric)

Instructions for Use

For the measurement of activity/inhibition of DNMT using nuclear extracts or purified enzymes from a broad range of species

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

ab113467 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 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.

Demonstration of high sensitivity and specificity of the DNMT activity assay achieved by using nuclear extracts with ab113467. Nuclear extracts were prepared from MCF-7 cells by using the EpiSeeker Nuclear Extraction Kit (ab113474).
Demonstration of high sensitivity and specificity of the DNMT activity/inhibition assay achieved by using recombinant DNMT1 with ab113467.

ab113467 is suitable for measuring total DNMT activity or inhibition using nuclear extracts or purified enzymes from a broad range of species such as mammalians, plants, fungi, bacteria, and viruses in a variety of forms including, but not limited to, cultured cells and fresh and frozen tissues. Nuclear extracts can be prepared by using your own successful method. For your convenience and the best results, Abcam also offers a nuclear extraction kit (ab113474) 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 materials can be nuclear extracts or purified DNMT enzymes. The amount of nuclear extracts for each assay can be between 0.5 µg - 20 µg with an optimal range of 5-10 µg. The amount of purified enzymes can be 0.5 ng - 200 ng, depending on the purity and catalytic activity of the enzymes.

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.

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.

2. Background

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.

![Figure 1. Methylation of cytosine in DNA via DNA methyltransferase and Sadenosylmethionine](image)

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.
ab113467 has the following features:

- Colorimetric assay with easy-to-follow steps for convenience and speed. The entire procedure can be completed within 3 hours and 45 minutes.
- Safe and innovative fluorometric assay without radioactivity, extraction, and chromatography.
- The ultra-sensitive detection limit can be as low as 0.5 µg of nuclear extract or 0.5 ng of purified enzymes.
- Optimized antibody & enhancer solutions allow high specificity to 5-mC without cross-reactivity to unmethylated cytosine.
- 96 stripwell microplate format allows for either low or high throughput analysis.
3. Components and Storage

A. Kit Components

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity (48 tests)</th>
<th>Quantity (96 tests)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDM1 (10X Wash Buffer)</td>
<td>14 mL</td>
<td>28 mL</td>
</tr>
<tr>
<td>EDM2 (DNMT Assay Buffer)</td>
<td>4 mL</td>
<td>8 mL</td>
</tr>
<tr>
<td>EDM3 (Adomet, 50X)*</td>
<td>60 µL</td>
<td>120 µL</td>
</tr>
<tr>
<td>EDM4 (DNMT Enzyme Control, 50 µg/ml)*</td>
<td>6 µL</td>
<td>12 µL</td>
</tr>
<tr>
<td>EDM5 (Capture Antibody, 1000 µg/ml*)</td>
<td>5 µL</td>
<td>10 µL</td>
</tr>
<tr>
<td>EDM6 (Detection Antibody, 400 µg/ml)*</td>
<td>6 µL</td>
<td>12 µL</td>
</tr>
<tr>
<td>EDM7 (Enhancer Solution)*</td>
<td>6 µL</td>
<td>12 µL</td>
</tr>
<tr>
<td>EDM8 (Developer Solution)</td>
<td>5 mL</td>
<td>10 mL</td>
</tr>
<tr>
<td>EDM9 (Stop Solution)</td>
<td>5 mL</td>
<td>10 mL</td>
</tr>
<tr>
<td>8-Well Assay Strip (with Frame)</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Adhesive Covering Film</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Spin the solution down to the bottom prior to use.
B. Additional Materials Required

- 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 aluminium foil
C. Storage

- Store EDM3, EDM4, EDM6, and EDM7 at −20°C away from light.

- Store EDM1, EDM5, EDM8, and the 8-Well Assay Strips at 4°C away from light.

- Store all remaining components (EDM2, EDM9, 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.

Notes:

(1) Check if EDM1 (10X 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.

(2) Transfer the amount of EDM8 required into a secondary container (tube or vial) before adding EDM8 into the assay wells in order to avoid contamination. Check if a blue color is present in EDM8 (Developer Solution) before each use, as this would indicate contamination of the solution and should not be used.
4. Protocol

Protocol Summary

Prepare nuclear extracts or purified enzymes

Incubate with substrate and assay buffer for 90 min

Wash wells, then add capture antibody

Wash wells, then add detection antibody and enhancer solution

Add color developing solution for color development, then measure absorbance

A. 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 - 10 µg. The amount of purified enzymes can be 0.5 ng - 200 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. Abcam also offers a nuclear extraction kit (ab113474) 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.

**B. Buffer and Solution Preparation**

1. Prepare Diluted EDM1 1X Wash Buffer: Add 13 ml of EDM1 10X Wash Buffer to 117 ml of distilled water and adjust pH to 7.2-7.5. This Diluted EDM1 1X Wash Buffer can now be stored at 4°C for up to six months.

2. Prepare Diluted EDM3 Working Buffer: Freshly prepare the Diluted EDM3 Working Buffer required for the assay by adding 2 µl of EDM3 into 98 µl of EDM2 (DNMT Assay Buffer). About 50 µl of this Diluted EDM3 will be required for each assay well.

3. Prepare Diluted EDM5 Capture Antibody Solution: Dilute EDM5 (Capture Antibody) with Diluted EDM1 at a ratio of 1:1000 (i.e., add 1 µl of EDM5 to 1000 µl of Diluted EDM1). About 50 µl of Diluted EDM5 will be required for each assay well.

4. Prepare Diluted EDM6 Detection Antibody Solution: Dilute EDM6 (Detection Antibody) with Diluted EDM1 at a ratio of 1:2000 (i.e., add 1 µl of EDM6 to 2000 µl of
Diluted EDM1). About 50 µl of this Diluted EDM6 will be required for each assay well.

5. Prepare Diluted EDM7 Enhancer Solution: Dilute EDM7 (Enhancer Solution) with Diluted EDM1 at a ratio of 1:5000 (i.e., add 1 µl of EDM7 to 5000 µl of Diluted EDM1). About 50 µl of this Diluted EDM7 will be required for each assay well.

6. About the EDM4 DNMT Enzyme Control: The EDM4 (DNMT Enzyme Control) is an enzyme with activity of both maintenance and de novo DNMTs and is used as the positive control of the 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.

*Note: Keep each of the diluted solutions (except Diluted EDM1 1X Wash Buffer) on ice until use. Any remaining diluted solutions, other than Diluted EDM1, should be discarded if not used within the same day.*

C. Enzymatic Reaction

1. 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 unneeded 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 50 µl of Diluted EDM3 per well.

3. Positive Control Wells: Add 50 µl of Diluted EDM3 and 1 µl of EDM4 per well.

4. Sample Wells Without Inhibitor: Add 45 µl - 49 µl of Diluted EDM3, and 1 µl - 5 µl of nuclear extracts or 1 µl - 5 µl of purified DNMT enzymes per well. Total volume should be 50 µl/well.

5. Sample Wells With Inhibitor: Add 40 µl - 44 µl of Diluted EDM3, 1 µl - 5 µl of nuclear extracts or 1 µl - 5 µl of purified DNMT enzymes, and 5 µl of inhibitor solution per well. Total volume should be 50 µl/well.

Notes:
(1) Follow the suggested well setup diagrams on page 19;
(2) It is recommended to use 5 µg - 10 µg of nuclear extract per well or 10 ng - 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 µM - 1000 µM). However, the final concentration of the inhibitors before adding to the wells should be prepared with EDM2 at a 1:10 ratio (e.g., add 0.5 µl of inhibitor to 4.5 µl of EDM2), so that the original solvent of the inhibitor can be reduced to 1% of the reaction solution or less.

6. Tightly cover the strip-well microplate with the Adhesive Covering Film to avoid evaporation, and incubate at 37°C for 90-120 min.

Note: (1) The incubation time may depend on intrinsic DNMT activity. In general, 90 min incubation is suitable for active purified DNMT enzymes and 120 min incubation is 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 to be used.

7. Remove the reaction solution from each well. Wash each well with 150 µl of the Diluted EDM1 1X Wash Buffer each time for three times.
D. Antibody Binding & Signal Enhancing

1. Add 50 µl of the Diluted EDM5 to each well, then carefully cover with Parafilm M or aluminium foil and incubate at room temperature for 60 min.

2. Remove the Diluted EDM5 solution from each well.

3. Wash each well with 150 µl of the Diluted EDM1 each time for three times.

4. Add 50 µl of the Diluted EDM6 to each well, then carefully cover with Parafilm M or aluminium foil and incubate at room temperature for 30 min.

5. Remove the Diluted EDM6 solution from each well.

6. Wash each well with 150 µl of the Diluted EDM1 each time for four times.

7. Add 50 µl of the Diluted EDM7 to each well, then carefully cover with Parafilm M or aluminium foil and incubate at room temperature for 30 min.

8. Remove the Diluted EDM7 solution from each well.

9. Wash each well with 150 µl of the Diluted EDM1 each time for five times.
Note: Ensure any residual wash buffer in the wells is thoroughly removed as much as possible at each wash step.

E. Signal Detection

1. Add 100 µl of EDM8 to each well and incubate at room temperature for 1 - 10 min away from direct light. Monitor color change in the sample wells and control wells. The EDM8 solution will turn blue in the presence of sufficient methylated DNA.

2. Add 100 µl of EDM9 to each well to stop enzyme reaction when the color in the positive control wells turns medium blue. The color will change to yellow after adding EDM9 and 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 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.

F. DNMT Activity Calculation

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

2. Calculate DNMT activity or inhibition using the following formula:

$$\text{DNMT activity (OD/h/µg)} = \frac{(\text{Sample OD} - \text{Blank OD})}{(\text{Protein Amount (µg)} \times \text{hour}^*)} \times 1000$$

* Protein amount added into the reaction at section C, step 4 in µg. ** Incubation time at section C, step 6.

Example calculation:

Average OD450 of sample is 0.55
Average OD450 of blank is 0.05
Protein amount is 5 µg
Incubation time is 2 hours (120 min)

$$\text{DNMT activity} = \frac{(0.55 - 0.05)}{(5 \times 2)} \times 1000$$
$$= 50 \text{ OD/h/mg}$$
3. Calculate DNMT inhibition using the following formula:

\[
\text{Inhibition} \% = \left( \frac{\text{Inhibitor Sample OD} - \text{Blank OD}}{\text{No inhibitor sample OD} - \text{Blank OD}} \right) \times 100\%
\]

G. Suggested Working Buffer and solution Setup

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

<table>
<thead>
<tr>
<th>Reagents</th>
<th>1 well</th>
<th>8 wells (1 strip)</th>
<th>16 wells (2 strips)</th>
<th>48 wells (6 strips)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluted EDM1</td>
<td>2.5 mL</td>
<td>20 mL</td>
<td>40 mL</td>
<td>120 mL</td>
</tr>
<tr>
<td>Diluted EDM3</td>
<td>50 µL</td>
<td>400 µL</td>
<td>800 µL</td>
<td>2400 µL</td>
</tr>
<tr>
<td>Diluted EDM5</td>
<td>50 µL</td>
<td>400 µL</td>
<td>800 µL</td>
<td>2400 µL</td>
</tr>
<tr>
<td>Diluted EDM6</td>
<td>50 µL</td>
<td>400 µL</td>
<td>800 µL</td>
<td>2400 µL</td>
</tr>
<tr>
<td>Diluted EDM7</td>
<td>50 µL</td>
<td>400 µL</td>
<td>800 µL</td>
<td>2400 µL</td>
</tr>
<tr>
<td>Developer Solution</td>
<td>0.1 mL</td>
<td>0.8 mL</td>
<td>1.6 mL</td>
<td>4.8 mL</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>0.1 mL</td>
<td>0.8 mL</td>
<td>1.6 mL</td>
<td>4.8 mL</td>
</tr>
<tr>
<td>DNMT Enzyme Control</td>
<td>N/A</td>
<td>0.25 µL – 1 µL</td>
<td>0.5 µL – 2 µL</td>
<td>1 µL – 4 µL</td>
</tr>
</tbody>
</table>
### H. Suggested Strip Well Setup

Table 2. 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.

<table>
<thead>
<tr>
<th>Well #</th>
<th>Strip 1</th>
<th>Strip 2</th>
<th>Strip 3</th>
<th>Strip 4</th>
<th>Strip 5</th>
<th>Strip 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Blank</td>
<td>Blank</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
</tr>
<tr>
<td>B</td>
<td>EDM4 0.5µl</td>
<td>EDM4 0.5µl</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
</tr>
<tr>
<td>C</td>
<td>EDM4 1µl</td>
<td>EDM4 1µl</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
</tr>
<tr>
<td>D</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
</tr>
<tr>
<td>E</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
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<td>F</td>
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<td>Sample</td>
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<td>Sample</td>
<td>Sample</td>
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<tr>
<td>G</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
</tr>
<tr>
<td>H</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
</tr>
</tbody>
</table>
## 5. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No signal or weak signal in both the positive control and sample wells.</td>
<td>Reagents are added incorrectly.</td>
<td>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.</td>
</tr>
<tr>
<td>The well is incorrectly washed before enzyme reaction.</td>
<td></td>
<td>Ensure the well is not washed prior to adding the positive control and sample.</td>
</tr>
<tr>
<td>Incubation time and temperature are incorrect.</td>
<td></td>
<td>Ensure the incubation time and temperature described in the protocol is followed correctly.</td>
</tr>
<tr>
<td>Incorrect absorbance reading.</td>
<td></td>
<td>Check if appropriate wavelength (450 nm filter) is used.</td>
</tr>
<tr>
<td>No signal or weak signal in both the positive control and sample wells.</td>
<td>Kit was not stored or handled properly.</td>
<td>Ensure all components of the kit were stored at the appropriate temperature and caps are tightly capped after each opening or use.</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
<td>----------</td>
</tr>
<tr>
<td>No signal or weak signal in both the positive control and sample wells.</td>
<td>The DNMT enzyme control is insufficiently added to the well in Step C3.</td>
<td>Ensure a sufficient amount of DNMT enzyme control is added.</td>
</tr>
<tr>
<td></td>
<td>The quality of the DNMT enzyme control has been degraded due to improper storage conditions.</td>
<td>Follow the Storage guidance on Page 9 of this User Guide for storage instructions of EDM4 (DNMT Enzyme Control).</td>
</tr>
<tr>
<td>High Background Present for the Blank</td>
<td>Insufficient washing of wells.</td>
<td>Check if washing recommendations at each step is performed according to the protocol.</td>
</tr>
<tr>
<td></td>
<td>Contaminated by sample or positive control.</td>
<td>Ensure the well is not contaminated from adding sample or positive control accidentally or from using contaminated tips.</td>
</tr>
<tr>
<td></td>
<td>Incubation time with detection antibody is too long.</td>
<td>The incubation time at section D, step 4 should not exceed 45 min.</td>
</tr>
<tr>
<td></td>
<td>Over development of color.</td>
<td>Decrease the development time in section E, step 1 and before adding EDM9 (stop solution).</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>--------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>No signal or weak signal only in sample wells</td>
<td>Protein sample is not properly extracted or purified.</td>
<td>Ensure your protocol is suitable for DNMT protein extraction. For the best results, it is advised to use Abcam’s Nuclear Extraction Kit (ab113474). Also, use fresh cells or tissues for protein extraction, as frozen cells or tissues could lose enzyme activity.</td>
</tr>
<tr>
<td>Sample amount added into the wells is insufficient.</td>
<td></td>
<td>Ensure a sufficient amount of purified enzymes or nuclear extracts is used as indicated in section C. The sample can be titrated to determine the optimal amount to use in the assay.</td>
</tr>
<tr>
<td>Sample was not stored properly or has been stored for too long.</td>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Little or no activity of</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>DNMT contained in the</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sample.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uneven color development</td>
<td>Insufficient washing of the wells.</td>
<td>Ensure the wells are washed according to the protocol. Ensure any residues from the wash buffer are removed as much as possible.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed color development</td>
<td>Ensure color development solution is added sequentially and consistent with the order you added the other reagents (e.g., from well A to well G or from well 1 to well 12).</td>
<td></td>
</tr>
<tr>
<td>or delayed stopping of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>color development in the</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wells.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).
6. Related Products

- EpiSeeker Nuclear Extraction Kit (ab113474)
- EpiSeeker DNMT Activity Quantification Kit (Fluorometric) (ab113468)
- EpiSeeker DNMT1 Assay Kit (ab113469)
- EpiSeeker DNMT3A Assay Kit (ab113470)
- EpiSeeker DNMT3B Assay Kit (ab113471)
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