

EpiQuik™ MBD2 Binding Activity Assay Kit

Base Catalog # P-3021

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The EpiQuik™ MBD2 Binding Activity Assay Kit is suitable for measuring MBD2 binding activity in human cells/tissues.

 **EPIGENETEK**
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KIT CONTENTS

| Components | 48 assays P-3021-48 | 96 assays P-3021-96 |
|--------------------------------------|------------------------|------------------------|
| MB1 (10X Wash Buffer) | 12 ml | 25 ml |
| MB2 (Assay Buffer) | 1.8 ml | 4 ml |
| MB3 (Methylated DNA, 25 µg/ml)* | 50 µl | 100 µl |
| MB4 (Affinity Antibody, 100 µg/ml)* | 25 µl | 50 µl |
| MB5 (Detection Antibody, 200 µg/ml)* | 10 µl | 20 µl |
| MB6 (Developing Solution) | 6 ml | 12 ml |
| MB7 (Stop Solution) | 3 ml | 6 ml |
| 8-Well Assay Strips (with Frame) | 6 | 12 |
| User Guide | 1 | 1 |

* For maximum recovery of the products, centrifuge the original vial after thawing 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: (1) Store **MB5** at -20°C away from light; (2) Store **MB1, MB3, MB4, MB6** and **8-Well Assay Strips** at 4°C away from light; and (3) Store **all other components (MB2, MB7)** at room temperature. The kit is stable for up to 6 months from the shipment date, when stored properly.

Note: Check if wash buffer, **MB1**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.

MATERIALS REQUIRED BUT NOT SUPPLIED

- Orbital shaker
- Pipettes and pipette tips
- Microplate reader
- 1.5 ml microcentrifuge tubes

GENERAL PRODUCT INFORMATION

Quality Control: Epigentek guarantees the performance of all products in the manner described in our product instructions.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design.

Usage Limitation: The *EpiQuik*[™] MBD2 Binding Activity Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The *EpiQuik*[™] MBD2 Binding Activity Assay Kit and method of use contain proprietary technologies by Epigenetek. *EpiQuik*[™] is a trademark of Epigenetek Group Inc.

A BRIEF OVERVIEW

MBD2 (methyl-CpG-binding domain protein 2) is a member of the MBD protein family. MBD2 selectively binds to methylated DNA and suppresses transcription from a methylated target gene through recruiting transcriptional repressor complexes, which contain Mi-2/NuRD or HDACs. MBD2 has also been shown to catalyze demethylation by directly removing methyl groups from 5-methylcytosine residues in DNA. MBD2 is demonstrated to be associated with tumorigenesis. For example, deficiency of MBD2 suppresses intestinal tumor formation, indicating that MBD2 is not necessary only for tumor development, but also for tumor growth. The binding activity of MBD2 to methylated DNA may be affected by MBD2 mutation and by biochemical or pharmacological intervention.

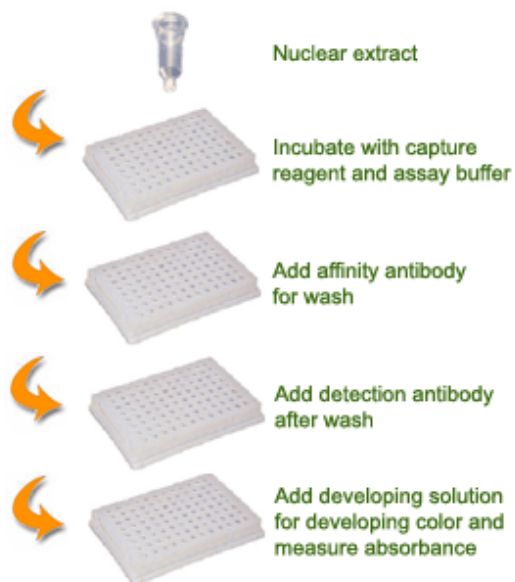
So far, there are few assays available for measuring MBD2 binding activity *in vitro*. The *EpiQuik*[™] MBD2 Binding Activity Assay Kit provides a unique procedure to measure binding activity of MBD2 to methylated DNA. The kit has following features:

- The fastest procedure, which can be finished within 3 hours.
- Innovative colorimetric assay to quantitatively measure MBD2 binding activity.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*[™] MBD2 Binding Activity Assay Kit is designed for measuring *in vitro* or intracellular MBD2 binding activity. In an assay with this kit, activated MBD2 protein contained in nuclear extract binds to methylated DNA and forms the MBD2/methylated DNA complex. The complex is then captured to a specifically-treated strip well plate. MBD2 is recognized with a specific antibody and measured colorimetrically.





Schematic Procedure for Using the *EpiQuik™* MBD2 Binding Activity Assay Kit

PROTOCOL

1. Prepare nuclear extracts from treated and untreated cells or tissues by using your own successful method. For your convenience and the best results, Epigentek offers the *EpiQuik™* Nuclear Extraction Kit (Cat. No. OP-0002-1) optimized for use in the *EpiQuik™* series. Nuclear extracts can be used immediately or stored at -80°C for future use.
2. 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). Dilute with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (e.g., 1 ml of **MB1** + 9 ml of distilled water). Wash the strip wells once with $150\ \mu\text{l}$ of the **diluted MB1**.
3. Add $36\ \mu\text{l}$ of **MB2**, $1\ \mu\text{l}$ of **MB3**, and then add $3\ \mu\text{l}$ of *nuclear extracts* (4-20 μg) or purified MBD2 protein to each strip well. Mix and cover the strip wells with Parafilm M and incubate at 37°C for 60-90 minutes. For the blank, add $3\ \mu\text{l}$ of **MB2** instead of nuclear extracts.
4. Aspirate and wash each well with $150\ \mu\text{l}$ of **diluted MB1** three times.
5. Dilute **MB4** (at a 1:100 ratio) to $1\ \mu\text{g}/\text{ml}$ with **diluted MB1**. Add $50\ \mu\text{l}$ of the **diluted MB4** to each strip well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).
6. Aspirate and wash each well with $150\ \mu\text{l}$ of **diluted MB1** four times.
7. Dilute **MB5** (at a 1:1000 ratio) to $1\ \mu\text{g}/\text{ml}$ with **diluted MB1**. Add $50\ \mu\text{l}$ of the **diluted MB5** to each strip well and incubate at room temperature for 30 minutes.
8. Aspirate and wash each well with $150\ \mu\text{l}$ of **diluted MB1** four times. Allow 3 minutes for last wash.
9. Add $100\ \mu\text{l}$ of **MB6** to each well and incubate at room temperature for 2-10 minutes away from light. Monitor the color development in the sample and control wells (blue).
10. Add $50\ \mu\text{l}$ of **MB7** 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 2-15 minutes.
11. Calculate % binding of MBD2:

$$\text{Binding \%} = \frac{\text{O.D (treated sample - blank)}}{\text{O.D (untreated control - blank)}} \times 100\%$$

TROUBLESHOOTING

No Signal for the Sample

The protein sample is not properly extracted.

Ensure the protein extraction protocol is suitable for nuclear protein extraction.

The protein amount is added into well insufficiently.

Ensure extract contains a sufficient amount of protein.

The sample is not prepared from fresh cells or tissues.

The nuclear extracts from frozen cells or tissues significantly lose enzyme activity. Fresh samples should be used.

Nuclear extracts are stored incorrectly.

Ensure the nuclear extracts are stored at -80°C .

Reagents are added incorrectly.

Check if reagents are added in the correct 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.

High Background Present for the Blank

The well is not washed sufficiently.

Check if wash at each step is performed according to the protocol.

Overdevelopment.

Decrease development time in step 9.

RELATED PRODUCTS

| | |
|--------|---|
| P-3001 | <i>EpiQuik</i> [™] DNA Methyltransferase Activity/Inhibition Assay Kit |
| P-3011 | <i>EpiQuik</i> [™] Dnmt1 Assay Kit |
| P-3012 | <i>EpiQuik</i> [™] Dnmt3A Assay Kit |
| P-3013 | <i>EpiQuik</i> [™] Dnmt3B Assay Kit |
| P-3019 | <i>EpiQuik</i> [™] DNA Demethylase Activity/Inhibition Assay Kit |