**Introduction and Background**

## A. 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 necessary not 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 few assays are available for measuring MBD2 binding activity in vitro. The MBD2 Binding Activity Assay Kit provides a unique procedure to measure binding activity of MBD2 to methylated DNA. In this assay, 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.

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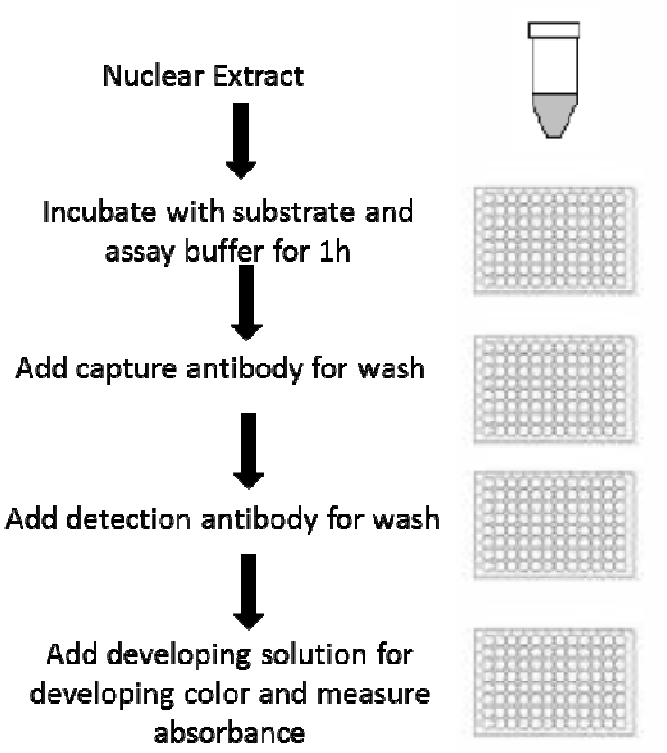
## B. Test Principle

The 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 specifically treated strip well plate. MBD2 is recognized with the specific antibody and measured colorimetrically.




## C. Notice for Application of Kit

✓ This kit has been configured for research use only and is not for diagnostic and clinical use.

## D. Application

For measuring MBD2 binding activity in human cells/tissues.

**Material and Method**

## A. List of component

| | 96 assays |
|---|---|
| MB1 (10X wash buffer) | **25 ml** |
| MB2 (assay buffer) | **3 ml** |
| MB3 (methylated DNA 25 µg/ml)* | **100 µl** |
| MB4 (affinity antibody 100 µg/ml)* | **50 µl** |
| MB5 (detection antibody 200 µg/ml)* | **20 µl** |
| MB6 (developing solution) | **12 ml** |
| MB7 (stop solution) | **6 ml** |
| 8 well sample strips (with frame) | **12** |

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

## B. Additional Required Materials But Not Provided

1. Orbital shaker
2. Pipettes and pipette tips
3. Microplate reader
4. 1.5 ml microcentrifuge tubes

## C. Stability and storage

Store MB3, MB4, MB5, MB6, and 8 well assay strips at 4℃ away from light. Store other components at room temperature. All components are stable for 6 months from date of shipment in proper storage.

## D. Protocol

1. Prepare nuclear extracts from treated and untreated cells or tissues by using you own successful method.
2. Determine number of the strip wells required. Leave these strips in the plate frame (remaining unused strips can be put back in the bag. Seal the bag tightly and store at 4℃). Dilute 10 X MB1 with distilled water (pH 7.2-7.5) to the 1 X MB1 and wash strip wells once with 150 µl of 1 X MB1.
3. Add 26 µl of MB2, 1 µl of MB3 and then add 3 µl 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℃ for 60 min. For blank, add 3 µl of MB2 instead of nuclear extracts.
4. Aspirate and wash each well with 150 µl of 1 X MB1 for 3 times.
5. Dilute the MB4 (at 1:100 ratio) to 1 µg/ml with 1 X MB1. Add 50 µl of the diluted MB4 to each strip well and incubate at room temperature for 60 min on an orbital shaker (50-100 rpm).
6. Aspirate and wash each well with 150 µl of 1 X MB1 for 4 times.

7. Dilute MB5 (at 1:1000 ratio) to 1 µg/ml with 1 X MB1. Add 50 µl of the diluted MB5 to each strip well and incubate at room temperature for 30 min.
8. Aspirate and wash each well with 150 µl of 1 X MB1 for 4 times. Allow 3 min for last wash.
9. Add 100 µl of MB6 to each well and incubate at room temperature for 2- 10 min away from light. Monitor color development in the sample and control well (blue).
10. Add 50 µl of MB7 to each well to stop enzyme reaction when 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 min
11. Calculate % binding of MBD2:

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

**Troubleshooting**

- **No Signal for the Sample**

| | |
|---|---|
| The protein sample is not properly extracted. | Ensure the nuclear protein extraction protocol is suitable for DNA demethylase protein extraction. |
| The protein amount is added into well insufficiently. | Ensure extract contains enough amount of proteins. |
| The sample is not prepared from fresh cells or tissues. | The nuclear extracts from frozen cells or tissues significantly lost enzyme activity. The fresh sample should be used. |
| Nuclear extracts are incorrectly stored. | Ensure the nuclear extracts are stored at –80℃. |
| Reagents are added incorrectly. | Check if reagents are added in order and if some steps of the procedure are omitted by mistake. |
| Incubation time and temperature is incorrect. | Ensure the incubation time and temperature described in the protocol are correctly followed. |

- **High Background Present for the Blank**

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|---|---|
| The well is not washed enough. | Check if wash at each step is performed according to the protocol. |
| Overdevelopment. | Decrease development time in protocol step 9. |