EpiQuik™ MBD2 Binding Activity/Inhibition Assay Ultra Kit

Base Catalog # P-3099

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ MBD2 Binding Activity/Inhibition Assay Ultra Kit is suitable for measuring the activity/inhibition of MBD2 binding using nuclear extracts or purified MBD2 protein from a broad range of species such as mammalian, plant, fungal, and bacterial, in a variety of forms including, but not limited to, cultured cells and fresh/frozen tissues.

Starting Materials: Input materials can be nuclear extracts or purified MBD2 proteins. The amount of nuclear extracts for each assay can be 2 µg to 20 µg with an optimal range of 5-10 µg. The amount of purified proteins can be 10 ng to 500 ng, depending on the purity and catalytic activity of the proteins.

Internal Control: The MBD2 assay standard (MBD2 recombinant protein) is provided in this kit for the quantification of MBD2 binding activity. Because MBD2 binding activity can vary from tissue to tissue and from normal and diseased states, it is advised to run replicate samples to ensure that the generated signal 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.

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KIT CONTENTS

Component	48 Assays Cat. #P-3099-48	96 Assays Cat. #P-3099-96	Storage Upon Receipt
WB (10X Wash Buffer)	14 ml	28 ml	4°C
MAB (MBD2 Assay Buffer)	3 ml	6 ml	RT
meDNA (Methylated DNA)*	100 µl	200 µl	–20°C
BS (Binding Solution)	5 ml	10 ml	RT
MPS (MBD2 Protein Standard, 200 µg/ml)*	10 µl	20 µl	–20°C
MCA (MBD2 Capture Antibody, 1000 µg/ml)*	4 µl	8 µl	–20°C
SI (Signal Indicator**)***	8 µl	16 µl	–20°C
ES (Enhancer Solution)*	8 µl	16 µl	–20°C
DS (Developer Solution)	5 ml	10 ml	4°C
SS (Stop Solution)	3 ml	6 ml	RT
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: the first part at ambient room temperature, and the second part on frozen ice packs at 4°C. **Upon receipt:** (1) Store **meDNA**, **MPS**, **MCA, SI**, and **ES** at –20°C away from light; (2) Store **WB**, **DS**, and **8-Well Assay Strips** at 4°C away from light; (3) Store remaining components (**MAB, BS,** and **SS**) at room temperature away from light.

***Note**: Check if **WB** (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.*

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

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 or purified proteins
- ☐ Parafilm M or aluminum foil

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of EpiQuik™ MBD2 Binding Activity/Inhibition Assay Ultra 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.

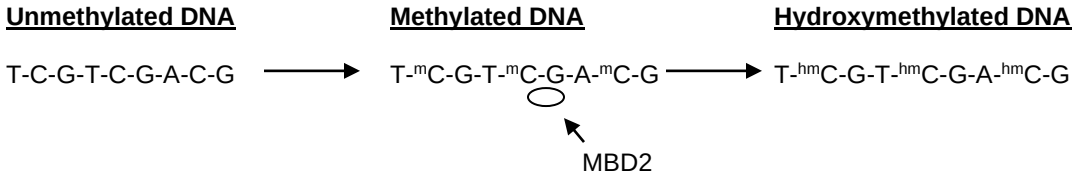
Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The EpiQuik™ MBD2 Binding Activity/Inhibition Assay Ultra Kit is for research use only and is not intended for diagnostic or therapeutic application

Intellectual Property: The EpiQuik™ MBD2 Binding Activity/Inhibition Assay Ultra Kit and methods of use contain proprietary technologies by EpigenTek.

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. As an epigenetic reader protein to methylated CpG DNA, MBD2 is a novel target that has been accredited for cancer therapy. Some evidence also linked this protein to neurodevelopment. The binding activity of MBD2 to methylated DNA may be affected by MBD2 mutation and biochemical or pharmacological intervention. Selective and potent MBD2 inhibition can induce gene re-expression, as seen with genetic disruption of MBD2 in cancer cells. Thus, detecting binding activity and inhibition of MBD2 is important in elucidating mechanisms of epigenetic regulation of gene activation and silencing, as well as benefiting cancer and neuro disease therapeutics.



There are few assays available for measuring MBD2 binding activity/inhibition *in vitro.* To address this issue, EpigenTek developed and offers the EpiQuik™ MBD2 Binding Activity/Inhibition Assay Ultra Kit. The kit has the following advantages and features:

- **Quick:** Colorimetric assay completed within 3 hours
- **Direct:** Measures MBD2 binding activity in the absence or presence of inhibitors
- **Versatile:** Works with cell/tissue extracts or purified MBD2 proteins, detecting TET hydroxylase inhibitor effects *in vivo* and *in vitro*
- **Sensitive:** Detects activity from as low as 10 ng of purified MBD2 proteins
- **Convenient:** Includes MBD2 protein standard for quantification
- **Flexible:** Allows manual or high-throughput analysis with strip microplate format

PRINCIPLE & PROCEDURE

In this assay, methylated DNA is stably coated onto microplate wells. Active MBD2 proteins bind to the methylated DNA. The bound MBD2 proteins can be recognized with specific antibodies. The signal intensity of bound MBD2-antibody complex, which is proportional to binding activity, can then be amplified with enhancer and colorimetrically measured by reading the absorbance in a microplate spectrophotometer at a wavelength of 450 nm. The binding activity of the MBD2 is, in turn, proportional to the optical density intensity measured.

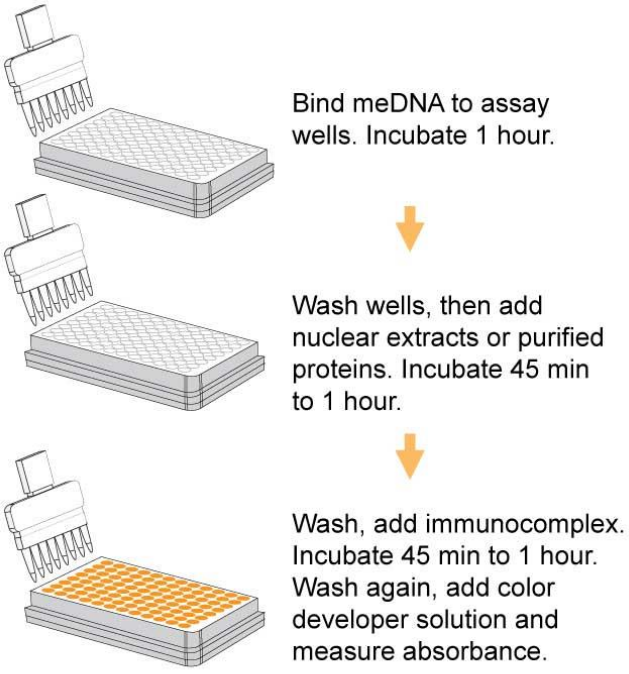


Fig 1. Schematic procedure for EpiQuik™ MBD2 Binding Activity/Inhibition Assay Ultra Kit.

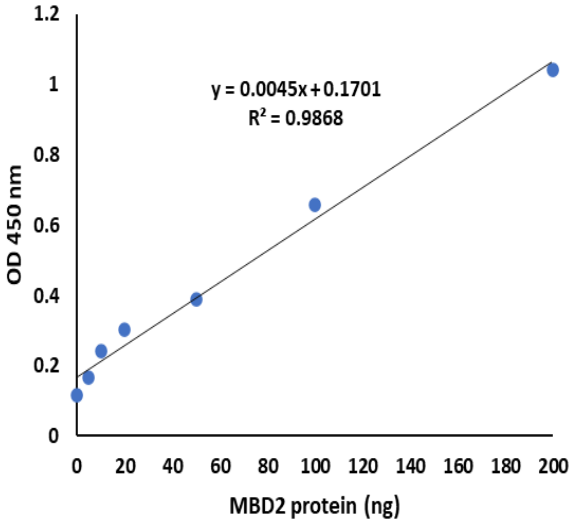


Fig. 2. Illustrated standard curve generated with the MBD2 protein standard.

ASSAY 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 2 µg to 20 µg with an optimal range of 5-10 µg. The amount of purified enzymes can be 10 ng to 500 ng, depending on the purity and catalytic activity of the enzymes.

Nuclear Extraction: You can use your method of choice for preparing nuclear extracts. EpigenTek offers a nuclear extraction kit (Cat # OP-0002) optimized for use with this kit.

Nuclear Extract or Purified MBD2 Protein Storage: Nuclear extract or purified MBD2 proteins should be stored in aliquots at –80°C until use.

1. Working Buffer and Solution Preparation

For a 48-reaction size kit, prepare **Diluted WB** (1X Wash Buffer) by adding 13 ml of **WB** (10X Wash Buffer) to 117 ml of distilled water and adjust pH to 7.2-7.5.

For the 96-reaction size kit, add 26 ml of **WB** (10X Wash Buffer) to 234 ml of distilled water and adjust pH to 7.2-7.5.

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

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
BS	100 µl	800 µl	1600 µl	4800 µl	9600 µl
MBD2 Detection Complex	50 µl	400 µl	800 µl	2400 µl	4800 µl
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
PC	N/A	N/A	Optional	6 µl	6-12 µl

2. Standard or Positive Control (PC) Curve Preparation

Suggested Standard Curve Preparation: Prepare five concentrations by combining the 200 ng/µl **MPS** (MBD2 Protein Standard) with **MAB** (MBD2 Assay Buffer) into final concentrations of 10, 20, 50, 100, and 200 ng/µl according to the following dilution chart:

Tube	MPS (200 ng/µl)	MAB	Resulting MPS Concentration
1	1.0 µl	19.0 µl	10 ng/µl
2	1.0 µl	9.0 µl	20 ng/µl
3	1.0 µl	3.0 µl	50 ng/µl
4	2.0 µl	2.0 µl	100 ng/µl
5	4.0 µl	0.0 µl	200 ng/µl

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

3. MBD2 Binding Strip Well Setup

Review the configuration of the strip-well plate setup for standard curve preparation in 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	PC 100 ng/well	Sample 3	Sample 7	Sample 11	Sample 15
B	Blank	PC 100 ng/well	Sample 3	Sample 7	Sample 11	Sample 15
C	PC 10 ng/well	PC 200 ng/well	Sample 4	Sample 8	Sample 12	Sample 16
D	PC 10 ng/well	PC 200 ng/well	Sample 4	Sample 8	Sample 12	Sample 16
E	PC 20 ng/well	Sample 1	Sample 5	Sample 9	Sample 13	Sample 17
F	PC 20 ng/well	Sample 1	Sample 5	Sample 9	Sample 13	Sample 17
G	PC 50 ng/well	Sample 2	Sample 6	Sample 10	Sample 14	Sample 18
H	PC 50 ng/well	Sample 2	Sample 6	Sample 10	Sample 14	Sample 18

4. Enzymatic Reaction

a. Predetermine the number of strip wells required for your experiment. 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. Add 80 µl of **BS** (Binding Solution) to each well.

c. Add 2 µl of **meDNA** (Methylated DNA) into each blank well, each sample well and each standard well.

d. Cover strip plate with plate seal or Parafilm M and incubate at 37°C for 60 min.

e. Remove the **BS** (Binding Solution) from each well.

f. Wash each well one time with 150 µl of the **Diluted WB** (1X Wash Buffer) each time.

g. Blank Wells: Add 50 µl of **MAB** (MBD2 Assay Buffer) to each blank well.

h. Standard Wells: Add 49 µl of **MAB** (MBD2 Assay Buffer) followed by adding 1 µl of **Diluted MPS** (MBD2 Protein Standard) to each standard well (see the designated wells depicted in the Table under "MBD2 Binding Strip Well Setup" above).

i. Sample Wells Without Inhibitor: Add 46 to 49 µl of **MAB** (MBD2 Assay Buffer) and 1 to 4 µl of nuclear extracts or purified MBD2 proteins to each sample well without inhibitor. Total volume should be 50 µl per well.

j. Sample Wells With Inhibitor: Add 41 to 44 µl of **MAB** (MBD2 Assay Buffer), 1 to 4 µl of nuclear extracts or purified MBD2 proteins, and 5 µl of inhibitor solution. Total volume should be 50 µl per well.

k. Mix solution by gently tilting from side to side or shaking the plate several times. Ensure the solution coats the bottom of the well evenly.

***Note**: (1) Follow the suggested well setup diagram under "MBD2 Binding Strip Well Setup"; (2) It is recommended to use 5 µg to 10 µg of nuclear extract per well or 50 ng to 200 ng of purified protein per well; (3) The concentration of inhibitor to be added into the sample wells can be varied (1 µM to 1000 µM). However, the final concentration of the inhibitors before adding to the wells should be prepared with **MAB** (MBD2 Assay Buffer) at a 1:10 ratio (i.e., add 0.5 µl of inhibitor to 4.5 µl of **MAB**) so that the original solvent of the inhibitor can be reduced to 1% of the reaction solution or less.*

l. Tightly cover strip plate with **Parafilm M** to avoid evaporation and incubate at 37°C for 45-60 min.

***Note**: (1) The incubation time may depend on intrinsic MBD2 activity. However, in general, 45 min incubation is suitable for active purified MBD2 proteins and 60 min incubation is required for nuclear extract.*

m. During the last 10 minutes of sample incubation, prepare the **MBD2 Detection Complex Solution**: In each 1 ml of **Diluted WB** add 1 µl of **MCA** (MBD2 Capture Antibody), mix and then add 1 µl of **SI** (Signal Indicator**)** and 1 µl of **ES** (Enhancer Solution). Mix well.

n. Remove the reaction solution from each well. Wash each well one time with 150 µl of the **Diluted WB** (1X Wash Buffer) each time.

5. MBD2 Binding Activity Detection and Signal Measurement

a. Add 50 µl of the **MBD2 Detection Complex Solution** to each well, then cover and incubate at room temperature for 50 minutes.

b. Remove the **MBD2 Detection Complex Solution** from each well.

c. Wash each well with 150 µl of the **Diluted WB** (1X Wash Buffer) each time for four times.

d. 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** (Developer Solution) will turn blue in the presence of sufficient bound MBD2. The color in the **blank** wells will remain generally unchanged or slightly changed.

e. When the color in the 200 ng/µl **PC** wells turns deep 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 plate against a flat surface and wait 1-2 minutes to allow the color reaction to be completely stopped. The color will change to yellow after adding **SS** (Stop Solution) and the absorbance should be read on a microplate reader at 450 nm within 2-15 minutes.

Take Note! *(1) The color development time may vary from 1-10 minutes based on the speed of color change, but is typically 4-5 minutes. (2) If the strip-well plate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.*

6. Binding Activity Calculation

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

b. Calculate Binding activity or inhibition using the following formulas:

For simple calculation:

$$\textit{Binding Activity (OD/min/mg)} = \frac{\textit{(Sample OD – Blank OD)}}{\textit{(Protein Amount (µg)* x min**)}} \textbf{ x } \textit{1000}$$

* Protein amount added into the reaction at step 4i.
** Incubation time at step 4l (in minutes).

Example calculation:

Average OD450 of sample is 0.65
Average OD450 of blank is 0.05
Protein amount is 5 µg
Incubation time is 1 hour (60 min)

$$\textit{Binding activity} = \frac{\textit{(0.65 – 0.05)}}{\textit{(5 x 60)}} \textbf{ x 1000 = 2 OD/min/mg}$$

For accurate or specific activity calculation:

First, generate a standard curve and plot the OD values versus the amount of **MPS** (MBD2 Protein Standard) at each concentration point. Then determine the slope as OD/ng using linear regression (*Microsoft Excel's* linear regression or slope functions are suitable for such calculation) and the most linear part (include at least 4 concentration points) of the standard curve for optimal slope calculation. Now calculate the amount of **bound MBD2** using the following formulas:

$$\textit{Bound MBD2 (ng)} = \frac{\textit{(Sample OD – Blank OD)}}{\textit{Slope}}$$

$$\textit{Binding Activity (ng/min/mg)} = \frac{\textit{Bound MBD2 (ng)}}{\textit{(Protein Amount (µg)* x min**)}} \textbf{ x } \textit{1000}$$

* Protein amount added into the reaction at step 4i.
** Incubation time at step 4l (in minutes).

For inhibition calculation:

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

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
No signal or weak signal in both the standard 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 meDNA (Methylated DNA) and standard are not properly bound to the wells.	Ensure that (1) the **meDNA** (Methylated DNA) and **MPS** (MBD2 Protein Standard) are added into the wells; (2) the wells are completely covered with sufficient **BS** (Binding Solution); and (3) binding time is sufficient (60 min).
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
	Incorrect absorbance reading.	Check if the 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 temperatures and the cap is tightly capped after each opening or use.
No signal or weak signal in only the standard curve wells	The standard amount is insufficiently added to the well in Step 4h.	Ensure a sufficient amount of standard is added.
	The standard is degraded due to improper storage conditions.	Follow the Shipping & Storage guidance of this User Guide for storage of **MPS** (MBD2 Protein Standard).
High background present in the blank wells	Insufficient washing of wells.	Check if washing at step 5c is performed according to the protocol.
	Contaminated by sample or standard.	Ensure the well is not contaminated from adding sample or standard accidentally or from using contaminated tips.

	Incubation time with detection complex is too long.	The incubation time at Step 5a should not exceed 50 minutes.
	Over development of color.	Decrease the development time in Step 5d before adding **SS** (Stop Solution) in Step 5e.
No signal or weak signal only in sample wells	Protein sample is not properly extracted or purified.	Ensure your protocol is suitable for MBD2 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 proteins or nuclear extracts is used as indicated in Steps 4i and 4j. 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 proteins. Avoid repeated freezing/thawing.
	Little or no activity of MBD2 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 wash of the wells.	Ensure the wells are washed according to the guidance of washing and residue wash buffer is removed as much as possible.
	Delayed color development or delayed stopping of color development in the wells.	Ensure color development and stop solutions are added sequentially and consistent with the order you added the other reagents (e.g., from well A to G or from well 1 to 12).

RELATED PRODUCTS

Nuclear Extract Preparation

OP-0002-1 EpiQuik™ Nuclear Extraction Kit

DNA Modification Enzyme Activity Assay

P-3009 EpiQuik™ DNMT Activity/Inhibition Assay Ultra Kit (Colorimetric)
P-3039 EpiQuik™ DNMT Activity/Inhibition ELISA Easy Kit (Colorimetric)
P-3086 Epigenase™ 5mC Hydroxylase TET Activity/Inhibition Assay Kit