Global DNA Methylation ELISA Kit

Enzyme Immunoassay for the quantification of Global DNA Methylation in urine, cell lysate and tissue lysate.

Catalog number: ARG82223

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

DNA methylation is a biological process by which methyl groups are added to the DNA molecule. Methylation can change the activity of a DNA segment without changing the sequence. When located in a gene promoter, DNA methylation typically acts to repress gene transcription. In mammals, DNA methylation is essential for normal development and is associated with a number of key processes including genomic imprinting, X-chromosome inactivation, repress of transposable elements, aging, and carcinogenesis.

Two of DNA's four bases, cytosine and adenine, can be methylated. Cytosine methylation is widespread in both eukaryotes and prokaryotes, even though the rate of cytosine DNA methylation can differ greatly between species. Adenine methylation has been observed in bacterial, plant, and recently in mammalian DNA, but has received considerably less attention. [Provide by Wikipedia: DNA methylation]

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. First, a 5MedCyd DNA Conjugate is coated on the DNA Binding microplate. The 5MedCyd samples or Standards (5MedCyd Standards) are then added to the 5MedCyd DNA Conjugate pre-coated plate. After a brief incubation, the Antibody Conjugate is added. The 5MedCyd DNA Conjugate competes with the DNA samples / Standards for the limited number of antibody sites. After incubation, the wells are washed with Wash Buffer to remove unbound material. After adding an HRP Conjugate for incubation, the wells are washed with Wash Buffer to remove unbound material. Then the TMB Substrate is added to the wells and color develops in inversely proportional to the amount of 5MedCyd DNA content in the samples competition in the initial step. The color development is stopped by the addition of STOP Solution and the intensity of the color is measured at a wavelength of 450nm. The concentration of 5MedCyd DNA content in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, aliquot and store the Standard, 1000X 5MedCyd DNA Conjugate and Antibody Conjugate at-20°C to avoid multiple freeze/thaw cycles. Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
DNA Binding microplate	8 X 12 strips	4°C
Standard (4 mM 5MedCyd in 1X PBS)	100 µL	–20°C
1000X 5MedCyd DNA Conjugate	10 µL	–20°C
Diluent Buffer	50 ml (ready to use)	4°C
Antibody Conjugate	5 µL	–20°C
HRP Conjugate	20 µL	4°C
10X Wash Buffer	100 ml	4°C
TMB substrate	12 ml (ready to use)	4°C (protect from light)
STOP solution	12 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- DNA Extraction Kit
- Sodium Acetate, pH 5.2
- 1X PBS, 1X PBS containing 0.1% BSA
- Nuclease P1, Alkaline Phosphatase
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The Stock Standard and 5MedCyd DNA Conjugate should be aliquoted into smaller portions before use to ensure product integrity and store the aliquoted material above at-20°C. Avoid repeated freeze-thaw cycles.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.

- Take care not to contaminate the TMB Substrate. Do not expose the TMB Substrate to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

Urine: Clear urine samples can be diluted 5-50 fold in 1X PBS containing 0.1% BSA before use in the assay. Samples containing precipitates should be centrifuged at 3000 g for 10 minutes, or filtered through 0.45 µm filter, prior to use in the assay.

Cell or Tissue DNA: following the user manual of commercial DNA Extraction kit to extract DNA from cells or tissues. Dissolved the DNA in distilled water at 0.1-1 mg/ml. Convert DNA sample to single-stranded DNA by incubating the sample at 95°C for 5 minutes and then rapidly chilling on ice. Then digest DNA sample to nucleosides by incubating the denatured DNA with 5-10 units of nuclease P1 for 2 hours at 37°C in 20 mM Sodium Acetate, pH 5.2, and following with treatment of 5-10 units of alkaline phosphatase for 1 hour at 37°C in 100 mM Tris, pH 7.5. Centrifuge the reaction mixture for 5 minutes at 6000 g and use the supernatant for the Global DNA Methylation ELISA assay.

Note:

1. Based on LC/MS analysis, there are about 10 ng 5MedCyd per µg of normally methylated human genomic DNA or 1 ng 5MedCyd per µg of hypomethylated human genomic DNA. Therefore, we recommend using at least 1 µg of digested normally methylated human DNA or 10 µg of digested hypomethylated human DNA per assay.
2. Aliquot samples for testing and store at -80°C. Avoid repeated freeze-thaw cycles.
3. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 5MedCyd DNA Conjugate Coated Plate:

  1. Dilute 1000X 5MedCyd DNA Conjugate 1:1000 in 1X PBS.
  2. Add 100 µL of diluted 5MedCyd DNA Conjugate to the wells.
  3. Incubate overnight at 4°C.
  4. Remove the 5MedCyd Conjugate coating solution and wash once with distilled water. Blot plate on paper towels to remove excess fluid.
  5. Add 200 µL of Diluent Buffer to each well and block for 1 hour at room temperature. Transfer the plate to 4°C and remove the Diluent Buffer immediately before use.

  Note: The 5MedCyd DNA Conjugate coated wells are not stable and should be used within 24 hours after coating. Only coat the number of wells to be used immediately.

- 1X Wash Buffer: Dilute 10X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 50 ml of 10X Wash Buffer into 450 ml of distilled water to a final volume of 500 ml) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C.
- Antibody Conjugate and HRP Conjugate: Immediately before use dilute the Antibody Conjugate 1:1000 and the HRP Conjugate 1:1000 with Diluent Buffer. Do not store diluted solutions.

- **Standards (5MedCyd standards):** Prepare a series dilution of 5MedCyd standards. The Diluent Buffer serves as zero standard (0 nM), and the rest of the standard serial dilution can be diluted with Diluent Buffer as according to the suggested concentration table below:

Standard tubes	Final 5MedCyd Conc. (µM)	Diluent Buffer (µL)	Standards (µL)
S1	20	995	5 of 4 mM 5MedCyd standards
S2	10	250	250 of S1
S3	5	250	250 of S2
S4	2.5	250	250 of S3
S5	1.25	250	250 of S4
S6	0.625	250	250 of S5
S7	0.313	250	250 of S6
S8	0.156	250	250 of S7
S9	0.078	250	250 of S8
S0	0	250	0

Note: Dilutions for the standards must be made and applied to the plate immediately. S0 serves as background.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Each 5MedCyd sample including Standards should be assayed in duplicate. High content 5MedCyd samples should be diluted in 1X PBS containing 0.1% BSA.

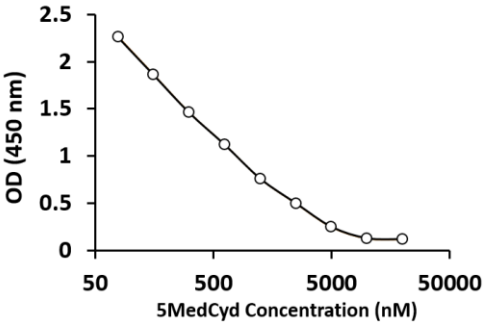
1. Remove **Diluent Buffer** from the **5MedCyd DNA Conjugated Coated plate.**
2. Add **50 µL** of **DNA sample or standards** to the **5MedCyd DNA Conjugate Coated microplate**. Incubate at **RT** for **10 minutes** on a microplate shaker. Each DNA sample and standards should be assayed in duplicate.
3. Add **50 µL** of **diluted Antibody Conjugate** to each tested well.
4. Incubate at **RT** for **2 hour** on a microplate shaker.
5. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **100 µL** of the **diluted HRP Conjugate** to each well. Incubate at **RT** for **1 hour** on a microplate shaker.
7. Aspirate each well and **wash as step 5**.
8. Warm **TMB Substrate** to **RT**. Add **100 µl** of **TMB Substrate** to each well, including the blank wells. Incubate for **2-30 minutes** on a microplate shaker at **RT** in the dark.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9. Add 100 µl of Stop Solution to each well, including the blank wells. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at 450nm immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance within 30 minutes after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Global DNA Methylation ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV value of intra-assay and inter-assay precision was ≤ 10%.

Sensitivity

39 nM