# DNA Methylation Detection Kit

# (Cat# K5082100)

***An Instructional Guide for***
***Bisulfite Conversion of Methylated DNA***

## Features

- *Human Positive Control*
- *Conversion Rate: > 99 %*
- *CpG protection: > 99 %*
- *Range: 2 µg to 500 pg*
- *Total time: 3 hrs*

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**Contents**



## Kit Contents

| Methylated DNA Conversion Kit | Part No. | Amount | Storage |
|---|---|---|---|
| Conversion Reagent (CVR)* | K5082100-1 | 5 x 0.4 g | Room Temperature |
| Activator Solution (ACS)* | K5082100-2 | 5.0 ml | Room Temperature |
| Stabilizer Solution (STS)* | K5082100-3 | 500 µl | Room Temperature |
| Equilibration Buffer (EQB) | K5082100-4 | 20 ml | Room Temperature |
| Desulfonation Buffer (DSB) | K5082100-5 | 4 ml | Room Temperature |
| Elution Buffer (TE) | K5082100-6 | 2 x 1 ml | Room Temperature |
| Control MetPrimers, 5 µM (CMP) | K5082100-7 | 100 µl | -20ºC |
| Control Human DNA (2 µg) | K5082100-8 | 100 µl | -20ºC |
| Column | K5082100-9 | 50 | Room Temperature |

*After mixing ACS, STS, and CVR to prepare Conversion Buffer (CVB), store at -20ºC.

BioChain®

Catalog: K5082100

## Introduction

Methylated DNA occurs naturally in prokaryotes and eukaryotes. In humans, it has been implicated in epigenetics, genomic imprinting, embryonic development, X-chromosome inactivation and carcinogenesis. In humans, a majority of all cytosines in the dinucleotide 5'-CpG-3' are methylated, though non-CpG methylation patterns have also been found. A breakthrough in the detection of DNA methylation was made with the discovery that bisulfite has higher specificity for cytosine than 5-methyl cytosine (Fig1). The DNA after conversion is single stranded and non-complementary (Fig2).

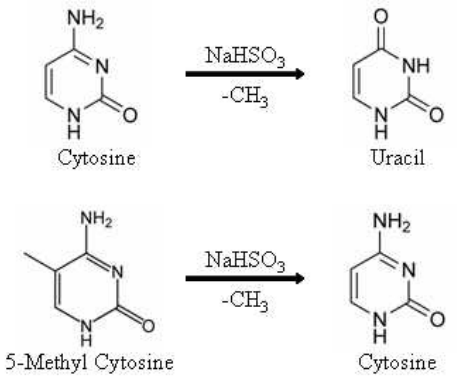


**Fig 1:** Bisulfite ($NaHSO_3$) Conversion of Cytosine and 5-Methyl Cytosine. Cytosine is converted to uracil and 5-methyl cytosine is converted is cytosine.

| | DNA Before Conversion | DNA After Conversion |
|---|---|---|
| **Forward Strand** | 5'TG**C**GCAAG**C**GCATGC**C**GCT | 5'TG**C**GUAAG**C**GUATGU**C**GUT |
| **Reverse Strand** | 3'ACG**C**GTTCG**C**GTACGG**C**GA | 3'AUG**C**GTTUG**C**GTAUGG**C**GA |

**Fig 2:** DNA before and after bisulfite conversion. Methylated cytosine is indicated as **C.** Non-methylated cytosine is indicated as **C**. After conversion, methylated cytosine remains unchanged, indicated as **C;** while non-methylated cytosine changes to uracil, indicated as **U**. Conversion generates non-complementary strands [(U)racil does not pair with (G)uanine].

## Product Description

BioChain's DNA methylation detection kit offers a ***fast, efficient and convenient*** alternative to traditional bisulfite conversions. Our kit provides users with a ***true endogenous human positive control*** which eliminates any ambiguity in validating results. The positive control has been ***tested in over 15 different human tissues***. We have optimized our protocol to minimize DNA degradation, reagent preparation, and conversion time. Our protocol guarantees **> 99 % conversion** of cytosine to uracil and **> 99 % CpG protection**, as calculated by sequencing, and is **sensitive over a range of 2 µg – 500 pg** of DNA. In less than **3 hours**, users can obtain high quality bisulfite modified DNA ready for analysis by PCR, sequencing, or microarrays. The kit is enough for 50 reactions.

## Quality Control

Each lot of BCI- DNA Methylation Detection kit is tested under stringent conditions to ensure consistent high quality products.

## Required Materials

- Heating block or Thermocycler with heating lid
- 96-100 % ethanol
- 1.5 ml microfuge tubes
- Microcentrifuge
- Pipettes and pipette tips
- Sterile dd-water
- *Optional:* PCR tubes

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**Reagent preparation**

- Conversion Buffer (CVB): Add 900 µl activator solution (ACS) and 100 µl stabilizer solution (STS) to pre-measured conversion reagent (CVR). Vortex vigorously till CVR is completely dissolved. Incubate for 10 min at room temperature. CVB is stable at -20ºC for 1 month. Solubilize any precipitation by vortexing before use.
- Equilibration Buffer (EQB): Add 30 ml of 96 – 100 % ethanol to 20 ml buffer solution. Store at room temperature. Do not use if precipitated.
- Desulfonation Buffer (DSB): Add 6 ml of 96 – 100 % ethanol to 4 ml buffer solution. Store at room temperature. Do not use if precipitated.

**Protocol**

***All centrifugations are performed at room temperature for 1 min at ≥ 10,000 g.***

1. Add **90 µl** of **Conversion Buffer (CVB)** to **10 µl** of **DNA** sample. Mix well.
2. Incubate at 92ºC for 10 min, followed by 64ºC for 2.5 hr.
   ***Note:*** The sample can be stored at 4ºC overnight if needed.
3. Add **600 µl** of **Equilibration Buffer (EQB)** to the sample and mix well.
4. Apply to the column. Centrifuge. Discard the flow through.
5. Add **200 µl** of **Equilibration Buffer (EQB)** to the column. Centrifuge.
6. Add **200 µl** of **Desulfonation Buffer (DSB)** to the column.
7. Incubate for 15 – 20 min at room temperature. Centrifuge.
8. Add **200 µl** of **Equilibration Buffer (EQB)** to the column. Centrifuge. Discard the flow-through.
9. Centrifuge. Transfer the column to a 1.5 ml microfuge tube.
10. Apply **20 µl** of **Elution Buffer (TE)** to the column. Incubate for 1 min. Centrifuge. Store eluate at -20ºC. Eluted DNA is stable at -20ºC for 3 months.
    ***Note:*** If starting with less than 5 ng of DNA, elution with sterile dd-water is recommended. Eluted DNA is stable for 1 week at -20ºC. Elution volume can be decreased to 10 µl to increase concentration. DNA recovery can be improved by increasing incubation up to 5 min.

**Technical Notes**

**Amount of DNA:** This kit gives optimal results within the range of 500 – 50 ng. When using over 500 ng of DNA, shredding the genomic DNA using a small gauge needle or restriction enzymes is recommended. When using under 5 ng of DNA elution in sterile dd-water instead of elution buffer is recommended.

**Determination of DNA Concentration:** Since the converted DNA is likely to have a high percentage of uracil nucleotides, measurement of DNA concentration should be calculated as

$$\text{DNA Concentration (ng/µl)} = 40 \times O.D_{260}$$

If measuring DNA concentration using a Nanodrop, select RNA40 instead of DNA50.

**Designing MSP primers:** Since bisulfite treatment generates random nicks in the DNA strand during conversion, it is recommended to design primers to amplify short target sequences (100 -200 bp). The converted DNA is non-complementary, so the primers should be designed for a single strand. Uracil (U) residues are complementary to adenine (A).

**How to use the Control Primers:** BioChain Institute's DNA methylation detection kit provides a pair of positive control primers. The positive primers are designed to target a human genomic DNA fragment that is methylated in normal tissues. If customers work on human DNA, it can be a internal positive control. The final converted DNA sample can be evaluated by PCR with the control primers. The PCR product is 127 bp that can be detected by 2.5% agarose gel. The optimal PCR conditions for the control primers has been determined as: 4 min. @ 92ºC; 45 x [30 s @ 92ºC, 30 s @ 60ºC, 30 s @ 72ºC]; 4 min. @ 72ºC; hold @ 4ºC. For 5 ng to2 µg DNA, use 100 – 200 nM control primer; for less than 5 ng, use 50 nM control primers. Agarose gel electrophoresis can visualize results from 5 ng DNA starting material. For less than 5 ng, the results can be visualized via QPCR.

**Control human DNA:** This human genomic DNA provided in the kit is used as PCR template after it goes through the bisulfite conversion. The control primer should generate the 127 bp PCR fragment.