FOR RESEARCH USE ONLY!

DNA Bisulfite Conversion Kit II

Rev 03/21

(Catalog # K1479-96-1, -4; 1 or 4 Plates; Magnetic Beads based bisulfite DNA purification; Store at RT)

I. Introduction:

BioVision's DNA Bisulfite Conversion Kit II is used for the bisulfite conversion of DNA that can be used to study the expression 5-methylcytosine. 5-methylcytosine is an epigenetic marker that plays an important role in differentiation, neurodegeneration, cancer etc. In this kit, DNA is treated with sodium bisulfite which leads to the deamination of cytosine residues and conversion to uracil, while 5-methyl cytosine residues remain unaffected. **DNA Bisulfite Conversion Kit II** integrates DNA denaturation and bisulfite conversion processes into a single step coupled to a magnetic bead based clean-up for high-throughput methylation analysis. The kit has been developed for high recovery of DNA following DNA bisulfite conversion. Desulphonation and clean-up of the converted DNA is performed while bound to the MagPure Beads. The kit is optimized to minimize template degradation, loss of DNA during treatment and clean-up and to provide complete conversion of unmethylated cytosine residues. The DNA recovered from this kit is used for downstream applications such as endonuclease digestion, Next Generation Sequencing, microarrays, PCR amplification, etc.

II. Application:

- An ideal tool for bisulfite modification of DNA and purification

III. Key Features:

- **Reliable** and Ready-to-use
- **High-throughput (96-well), bisulfite conversion** of DNA in less than 2 h
- **High recovery** of DNA
- Many downstream applications such as Next Generation Sequencing, PCR amplification, etc.

IV. Sample Types:

Purified genomic DNA, endonuclease-digested DNA, linearized plasmid DNA, etc. **High-quality and RNA-free DNA is required**.

V. Kit Contents:

Components	K1479-96-1 (1 pack)	K1479-96-4 (4 packs)	Part Number
*CT Conversion Reagent	1 bottle	4 bottles	K1479-96-X-1
Dilution Buffer	3.5 ml	14 ml	K1479-96-X-2
Dissolving Buffer	600 µl	2 x 1.2 ml	K1479-96-X-3
Binding Buffer	60 ml	2 x 120 ml	K1479-96-X-4
**Wash Buffer	15 ml	2 x 25 ml	K1479-96-X-5
Desulphonation Buffer	20 ml	80 ml	K1479-96-X-6
Elution Buffer	2 x 1.5 ml	15 ml	K1479-96-X-7
MagPure Beads	2 ml	8 ml	K1479-96-X-8

To each bottle of CT Conversion Reagent, add 9 ml water, 3 ml Dilution buffer and 500 µl Dissolving Buffer to prepare **CT Conversion Reagent solution.*

***Wash Buffer must be diluted with 100% Ethanol before starting. Add 60 ml of 100% Ethanol (K1479-96-1) to 15 ml of Wash Buffer concentrate or 100 ml of 100% Ethanol (K1479-96-4) to 25 ml of Wash Buffer concentrate before use. Be sure to close the bottle tightly after each use to avoid Ethanol evaporation.*

VI. User Supplied Reagents and Equipment:

- Pipettes, Pipette tips
- 100% Ethanol
- Thermal Cycler
- 96-well PCR plate
- Nuclease-free Water
- Deep-well plates
- Magnetic Separation Stand for plates

VII. Shipping and Storage Conditions:

The kit should be stored at room temperature (RT). The kit reagents are stable for 12 months, if stored as recommended.

VIII. Reagent Preparation and Storage Conditions:

1. Preparation of **CT Conversion Reagent solution:**

   The CT Conversion Reagent supplied in this kit is a solid mixture and must be prepared prior to first use. Add **9 ml water, 3 ml of Dilution Buffer and 500 µl Dissolving Buffer** to a tube of CT Conversion Reagent. Mix at RT with frequent vortexing or shaking for 15 min.

   Note: It is normal to see trace amounts of undissolved reagent in the CT Conversion Reagent solution. Each tube of CT Conversion Reagent is designed for 96 separate DNA treatments.

   Storage: The CT Conversion Reagent solution is light sensitive, so minimize its exposure to light. For best results, the CT Conversion Reagent solution should be used immediately following preparation. If not used immediately, the CT Conversion Reagent solution can be stored overnight at RT or one week at 4°C or up to one month at -20°C. Stored CT Conversion Reagent solution must be warmed to 37°C and then vortexed prior to use.

2. Preparation of **Wash Buffer:**

   Wash Buffer must be diluted with 100% Ethanol before starting. Add 60 ml of 100% Ethanol (K1479-96-1) to 15 ml of Wash Buffer concentrate or 100 ml of 100% Ethanol (K1479-96-4) to 25 ml of Wash Buffer concentrate before use.

IX. Protocol:

1. Add 130 µl of the **CT Conversion Reagent** solution to 20 µl of your **DNA sample** in a 96-well PCR plate. If the volume of the DNA sample is less than 20 µl, make up the difference with water. Mix the sample by pipetting the sample up and down.
2. Seal the plate and place in a **Thermal Cycler** and perform the following steps:
   Step 1. 95°C for 5 min
   Step 2. 54°C for 30 min
   Step 3. 95°C for 1 min
   Step 4. 54°C for 30 min
   Step 5. 95°C for 1 min
   Step 6. 54°C for 30 min
   Step 7. 4°C storage for up to 20 h
   Note: The 4°C storage step is optional.
3. Add 600 µl of **Binding Buffer** and 20 µl of **MagPure Beads** to each well of a deep-well plate (not provided).
4. **Transfer** the samples from the 96-well PCR plate into the deep-well plate containing the Binding Buffer and MagPure Beads. Mix by pipetting up and down 3-6 times.
5. Let the plate stand at RT for 5 min, then transfer the plate to a **Magnetic stan**d for an additional 5 min or until the beads pellet and the supernatant is clear. With the plate on the Magnetic stand remove the supernatant and discard.
6. Remove the plate from the Magnetic stand. Add 400 µl of **Wash Buffer** to the beads. Re-suspend the beads by pipetting up and down or vortexing the plate at 1,500 rpm for 30 sec. Replace the plate on the Magnetic stand for 3 min or until the beads pellet. Remove and discard the supernatant.
7. Add 200 µl of **Desulphonation Buffer** to the beads. Re-suspend the beads by pipetting up and down or vortexing for 30 sec. Let the plate stand at RT for 15-20 min. After the incubation, place the plate on the Magnetic stand for 3 min or until the beads pellet. Remove and discard the supernatant.
8. Add 200 µl of **Wash Buffer** to the beads. Re-suspend the beads by pipetting up and down or vortexing for 30 sec. Replace the plate on the Magnetic stand for 3 min or until the beads pellet. Remove and discard the supernatant. Repeat this wash step.
9. **Dry the MagPure Beads** by placing the plate at 50°C for 10-30 min until the beads become brittle and brown.
10. Add 25 µl of **Elution Buffer** directly to the dried beads and pipette or vortex for 30 sec to resuspend.
11. Heat the plate with elution buffer at 50°C for 4 min. Then transfer the plate to the Magnetic stand for 1 min or until the beads pellet. Pipette the supernatants and transfer to a clean plate or tubes.

X. Related Products:

Product Name	Cat. No.	Size
DNA Bisulfite Conversion Kit I	K1478	50, 200 Rxns
DNA Library Prep Kit for Illumina Sequencing	K1475	12 Rxns
Magnetic Beads for DNA Purification	K1476	5 ml
Cell & tissue genomic DNA extraction Kit	K1442	100 Preps
Mammalian Cell Genomic DNA Isolation Kit	K967	100 preps
PCR DNA extraction Kit	K1444	100 Preps
Gel and PCR DNA Purification Kit	K1455	50 Preps
Genomic DNA Isolation Kit	K281	50 Preps
Whole Blood DNA Isolation Kit	K528	100 preps
Mitochondrial DNA Isolation Kit	K280	50 Preps

FOR RESEARCH USE ONLY! Not to be used on humans.