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DNA Bisulfite Conversion Kit I

08/20

(Catalog # K1478-50, -200; 50 or 200 Rxns; Spin Column based bisulfite DNA purification; Store at RT)

I. Introduction:

BioVision's DNA Bisulfite Conversion Kit I 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 I** integrates the DNA denaturation and bisulfite conversion processes into a single step. The kit has been developed for the high recovery of DNA following DNA bisulfite conversion. This kit provides innovative in-column desulphonation technology that eliminates cumbersome DNA precipitation steps while providing researchers with consistent results every time. 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
- Results ready in less than 2 h
- **High recovery** of DNA
- **High quality spin columns** to recover bisulfite-treated 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	K1478-50 (50 Rxns)	K1478-200 (200 Rxns)	Part Number
*CT Conversion Reagent	5 tubes	20 tubes	K1478-XX-1
Dilution Buffer	1.5 ml	7 ml	K1478-XX-2
Dissolving Buffer	500 µl	1.2 ml	K1478-XX-3
Binding Buffer	30 ml	120 ml	K1478-XX-4
**Wash Buffer	6 ml	25 ml	K1478-XX-5
Desulphonation Buffer	10 ml	40 ml	K1478-XX-6
Elution Buffer	1.2 ml	5 ml	K1478-XX-7
Mini Column	50	200	K1478-XX-8
Collection Tube 2 ml	50	200	K1478-XX-9

***To each tube of CT Conversion Reagent, add 900 µl water, 300 µl Dilution buffer and 50 µl Dissolving Buffer to prepare CT conversion Reagent solution.**

****Wash Buffer must be diluted with 100% Ethanol before starting. Add 24 ml (K1478-50) or 100 ml (K1478-200) of 100% Ethanol to the Wash Buffer bottle 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
- PCR tubes
- Nuclease-free Water
- Sterile, nuclease-free 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Thermal Cycler

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 **900 µl water, 300 µl of Dilution Buffer and 50 µl Dissolving Buffer** to a tube of CT Conversion Reagent. Mix at RT with frequent vortexing or shaking for 10 min.

   Note: It is normal to see trace amounts of undissolved reagent in the CT Conversion Reagent. Each tube of CT Conversion Reagent is designed for 10 separate DNA treatments.

   Storage: The CT Conversion Reagent 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 24 ml (K1478-50) or 100 ml (K1478-200) of 100% Ethanol to Wash Buffer bottle before use.

IX. Protocol:

1. Add 130 µl of the **CT Conversion Reagent** solution to 20 µl of your **DNA sample** in a PCR tube. If the volume of the DNA sample is less than 20 µl, make up the difference with water. Mix the sample by flicking the tube or pipetting the sample up and down, then centrifuge to bring the liquid to the bottom of the tube.
2. Place the sample tube 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 hr
   Note: The 4°C storage step is optional.
3. Add 600 µl of **Binding Buffer** to a **Mini Column** and place the Column in the Collection Tube (provided).
4. Load the sample (from Step 2) into the **Mini Column** containing the Binding Buffer. Close the cap and mix by inverting the column several times.
5. Centrifuge at 10,000 x g for 30 sec. Discard the flow-through.
6. Add 100 µl of **Wash Buffer** to the Column. Centrifuge at 10,000 x g for 30 sec.
7. Add 200 µl of **Desulphonation Buffer** to the column and let stand at RT (20-30°C) for 15-20 min. After the incubation, centrifuge at 10,000 x g for 30 sec.
8. Add 200 µl of **Wash Buffer** to the column. Centrifuge at 10,000 x g for 30 sec. Add another 200 µl of **Wash Buffer** and centrifuge for an additional 30 sec.
9. Empty the filtrate, and place the column back in the Collection Tube. Centrifuge at 12,000 x g for 2 min to **remove the residual Ethanol**.
10. Place the column into a new sterile nuclease-free 1.5 ml microcentrifuge tube (not provided). Add 20 µl of **Elution Buffer** directly to the column matrix. Centrifuge for 30 sec at 10,000 x g to elute the DNA.
11. Discard the column and **store the DNA** at -20°C**.**

X. Related Products:

Product Name	Cat. No.	Size
DNA Bisulfite Conversion Kit II	K1479	1, 4 plates
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.