Technical Manual

MethylEdge™ Bisulfite Conversion System

INSTRUCTIONS FOR USE OF PRODUCT N1301.

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MethylEdge™ Bisulfite Conversion System

All technical literature is available on the Internet at: www.promega.com/protocols/ Please visit the web site to verify that you are using the most current version of this Technical Manual. Please contact Promega Technical Services if you have questions on use of this system. E-mail: techserv@promega.com

1. Description

Methylation of cytosines in CpG dinucleotides is an epigenetic mechanism which contributes to regulation of gene expression. The bisulfite conversion reaction is a common technique used to study the methylation patterns of genomic DNA. Unmethylated cytosines react with bisulfite and are converted to uracils, while methylated cytosines are unaffected. This allows for sequence specific determination of cytosine methylation.

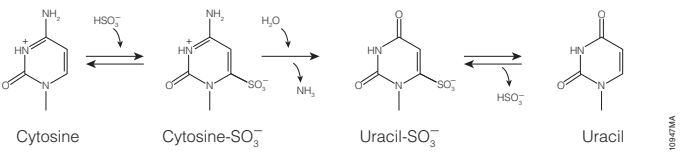

Figure 1. Bisulfite conversion of unmethylated cytosines to uracil.

1. Description (continued)

Methylated cytosines can be detected after bisulfite conversion using a variety of techniques such as bisulfite-specific PCR (end-point and real-time), cloning and sequencing, and arrays.

The MethylEdge™ Bisulfite Conversion System[a] offers a novel and efficient bisulfite conversion reagent with a protocol that can be completed in less than two hours, including desulphonation and clean-up. Typical bisulfite conversion protocols involve long incubation times under harsh conditions resulting in highly fragmented DNA. The ME Conversion Reagent is supplied ready-to-use, and all components can be stored at room temperature. The kit offers efficient conversion and recovery of converted DNA as well as reduced template fragmentation.

2. Product Components and Storage Conditions

Product	Size	Cat. #
MethylEdge™ Bisulfite Conversion System	50 reactions	N1301

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

- 5 vials ME Conversion Reagent
- 30ml ME Binding Buffer
- 6ml ME Wash Buffer (concentrated)
- 10ml ME Desulphonation Buffer
- 1ml ME Elution Buffer
- 1 pack ME Spin Columns (50/pack)
- 1 pack Collection Tubes (50/pack)

Storage Conditions: All components may be stored at 15–30°C.

3. General Considerations

To avoid introducing contamination:

1. Wear gloves at all times during the conversion and clean-up procedure.
2. Use sterile, disposable, aerosol-resistant pipette tips and tubes.

4. Before You Begin

Materials to Be Supplied by the User

- 95–100% ethanol
- heat blocks, water baths or thermocycler set to 98°C and 54°C
- 200µl PCR tubes and/or 96-well plates
- 1.5ml microcentrifuge tubes

4.A. Prepare 1X ME Wash Buffer

1. Add 24ml of 95–100% ethanol to the bottle containing 6ml of concentrated ME Wash Solution.
2. After adding ethanol, mark on the bottle that you have performed this step. This reagent is stable at room temperature when capped tightly.

4.B. Prepare Samples

1. Prepare 20µl aliquots of purified DNA. One reaction can convert 100pg–2µg; the optimal range is 200–500ng.

   Note: If your sample volume is less than 20µl, adjust the volume to 20µl with nuclease-free water. If your sample volume is greater than 20µl, divide the sample into 20µl replicate reactions before performing the conversion.

2. Prepare control reactions using pre-qualified methylated and unmethylated DNA from a source that is similar to the source of the experimental samples.

5. Bisulfite Conversion

1. Place 20µl of each DNA sample into a 200µl PCR tube. Reactions may also be performed in a 96 well plate.
2. Add 130µl of Bisulfite ME Conversion Reagent to each DNA sample, and pipet gently to mix. Centrifuge briefly to collect the sample at the bottom of the tube.

   Note: If you do not use all the ME Conversion Reagent contained in the tube, ensure that the cap is closed tightly prior to storing the remaining reagent.

3. Program a thermocycler or preheat water baths or heat blocks and incubate the reaction as follows:

   8 minutes at 98°C
   60 minutes at 54°C
   Hold at 4°C

4. Following incubation, the samples can be stored at 4°C or on ice, protected from light for up to 20 hours until you are ready to proceed to Section 6, *DNA Desulphonation and Cleanup*.

6. DNA Desulphonation and Cleanup

1. For each sample to be processed, place a ME Spin Column into one of the provided Collection Tubes.

   Note: Wear gloves when handling the columns and tubes.

2. Add 600µl of ME Binding Buffer to the ME Spin Column. Transfer the entire bisulfite-treated sample to the column. Close the cap, and mix by inverting the tube several times.

3. Spin at maximum speed (≥10,000 × *g*) for 30 seconds. Discard the flowthrough and re-insert the ME Spin Column into the same Collection Tube.

4. Add 100µl of 1X ME Wash Buffer (with ethanol added). Spin at maximum speed (≥10,000 × *g*) for 30 seconds.

5. Add 200µl of ME Desulphonation Buffer to each ME Spin Column. Close the caps and incubate at room temperature for 15 minutes.

6. Spin at maximum speed (≥10,000 × *g*) for 30 seconds.

7. Add 200µl of ME Wash Buffer (ethanol added.) Spin at maximum speed for 30 seconds. Repeat this wash step once more.

8. Place the ME Spin Column into a clean 1.5 ml microcentrifuge tube.

9. Add 10µl of ME Elution Buffer and spin at maximum speed (≥10,000 × *g*) for 30 seconds.

   Note: DNA may be eluted with up to 20µl of ME Elution Buffer if desired. Nuclease-free water or TE buffer (≥ pH 6.0) may be used.

10. Remove and discard the ME Spin Column. Cap the tube and store the eluted DNA at 4°C for use within one week, or store the eluted DNA at ≤–20°C for long-term storage. All samples should be stored protected from light.

11. Process the bisulfite-converted DNA according to your usual procedures.

7. Considerations for Downstream Analysis

Assessing DNA Quality Following Bisulfite Conversion

We recommend analyzing the converted DNA using either an absorbance scan or Agilent Bioanalyzer, if possible, to assess purity and recovery. While the nature of bisulfite-converted DNA makes it difficult to accurately quantify, performing this check provides an estimate of DNA concentration and confirmation that the sample is pure. If you are using a NanoDrop® spectrophotometer, set the Sample Type to "RNA-40" because bisulfite-converted DNA contains uracil and is largely single stranded.

To assess the level of fragmentation following conversion, 100ng of each sample can be run on a 1% agarose gel and stained with Diamond™ Nucleic Acid Dye (Cat.# H1181) according to the manufacturer's instructions.

PCR

We recommend using 20–50ng of bisulfite-converted template DNA for each real-time or end-point PCR. Because bisulfite conversion results in highly fragmented DNA, smaller amplicons will yield better results. Amplicons for real-time PCR should be 75–200bp. When the experimental DNA samples are of poor quality (e.g., DNA isolated from FFPE tissue), amplicons should be designed to be smaller than 200bp. Because the MethylEdge™ Bisulfite Conversion System yields bisulfite-converted DNA with significantly less fragmentation than other bisulfite conversion kits, end-point PCR can be designed for amplicons up to 500bp when high-quality, purified genomic DNA is used. Amplicons larger than 700bp have been successfully amplified with highly optimized primer models. Amplification of longer sequences may require more template DNA and/or higher primer concentration.

Primer design is key to analyzing bisulfite-converted DNA using PCR-based methods. Primers must be carefully designed based on the converted sequence to avoid PCR bias. Keep in mind that following conversion, DNA strands are no longer complementary and, because the DNA sequence is now reduced to essentially three bases (A, U, G), there is higher probability for non-specific interaction. Unconverted DNA should be run in parallel with bisulfite-converted DNA to ensure the primers are specific to the bisulfite-converted sequence. Several tools are available online to assist in developing primers specific to bisulfite-converted DNA, such as MethPrimer (**www.urogene.org/methprimer/index1.html**).

7. Considerations for Downstream Analysis (continued)

Because the presence of a cytosine following bisulfite-conversion indicates methylation, it is essential that control reactions are run at every step in the process. Prequalified methylated and unmethylated control DNA should be bisulfite-converted in parallel with experimental samples to ensure that >99% of cytosines are converted and >99% of methylated CpGs are protected. Impurities carried over during purification of source DNA or the presence of secondary structure can affect the efficiency of conversion. Pre-qualified bisulfite-converted methylated and bisulfite-converted unmethylated control DNA also should be run in parallel with experimental samples in downstream analysis to prevent false-positive identification of methylated cytosines. Promega offers pre-qualified fully methylated (Methylated Human Control, Cat# N1231) and fully methylated, bisulfite-converted (Converted Methylated Human Control, Cat# N1221) control DNA, each sold separately.

8. Reference

1. Harrison A and Parle-McDermott A (2011) DNA methylation: a timeline of methods and applications. *Front. Gene.* **2**, 74.

9. Related Products

Product	Size	Cat. #
Methylated Human Control	5µg	N1231
Converted Methylated Human Control	1µg	N1221
Diamond™ Nucleic Acid Gel Stain	500µl	H1181
ReliaPrep™ FFPE gDNA Miniprep System	10 reactions	A2351
	100 reactions	A2352
ReliaPrep™ Blood gDNA Miniprep System	100 reactions	A5081
	250 reactions	A5082
ReliaPrep™ gDNA Tissue Miniprep System	100 reactions	A2051
	250 reactions	A2052

[a]Use of Methylation Specific PCR (MSP) is protected by U.S. Pat. Nos. 5,786,146; 6,017,704; 6,200,756 & 6,265,171. No license under these patents to use the MSP process is conveyed expressly or by implication to the purchaser by the purchase of this product..

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Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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