

MethylEdge™ Bisulfite Conversion System

INSTRUCTIONS FOR USE OF PRODUCT N1301.

Quick
PROTOCOL

Bisulfite Conversion

Before You Begin

1. Add 24ml of 95–100% ethanol to the bottle containing 6ml of concentrated ME Wash Solution to make 1X ME Wash Buffer. Invert to mix.
2. Prepare 20 μ l aliquots of purified DNA. One reaction can convert 100pg–2 μ g; the optimal range is 200–500ng. Smaller volume samples should be adjusted to 20 μ l with nuclease-free water. Larger volume samples should be divided into 20 μ l replicate reactions.
3. Prepare control reactions using pre-qualified methylated and unmethylated DNA from a source that is similar to the source of the experimental samples.

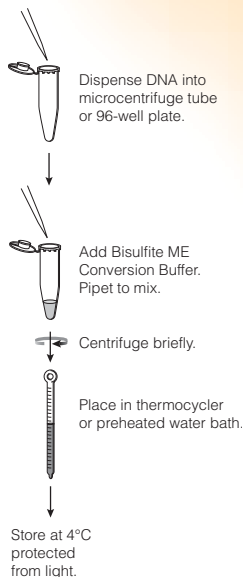
Bisulfite Conversion

1. Place 20 μ l of each DNA sample into a 200 μ l microcentrifuge tube.

Note: Reactions can be performed in a 96-well plate.

2. Add 130 μ l 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.
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, store the samples at 4°C or on ice, protected from light, for up to 20 hours.

Continued on back



For a detailed protocol and additional information please see Technical Manual #TM381, available at:
www.promega.com/protocols

ORDERING/TECHNICAL INFORMATION:

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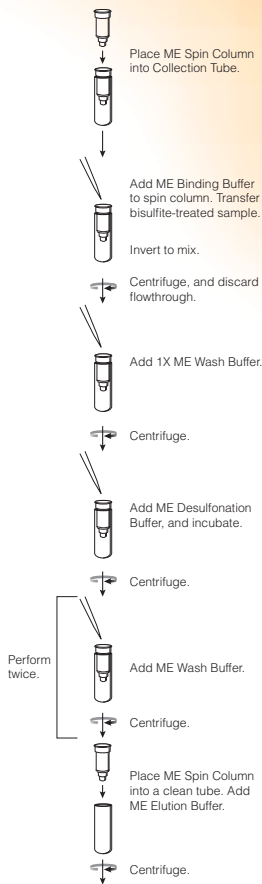
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DNA Desulfonation and Cleanup

DNA Desulfonation 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 invert to mix.
3. Spin at maximum speed ($\geq 10,000 \times g$) 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 for 30 seconds.
5. Add 200µl of ME Desulfonation Buffer to each ME Spin Column. Close the caps and incubate at room temperature for 15 minutes.
6. Spin at maximum speed for 30 seconds.
7. Add 200µl of ME Wash Buffer. 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 for 30 seconds.
Note: DNA may be eluted with up to 20µl of ME Elution Buffer if desired. Nuclease-free water or TE buffer (\geq pH 6.0) may be used as an alternative elution buffer.
10. Remove and discard the ME Spin Column. Cap the tube, and store the eluted DNA at 4°C. Use the DNA within one week, or place the DNA at $\leq -20^\circ\text{C}$ for long-term storage. All samples should be stored protected from light.
11. Process the bisulfite-converted DNA according to your usual procedures.



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