Coupled Heat-Denaturation/Bisulfite Treatment for Rapid Conversion of Non-Methylated C to U in DNA

In-Column Desulphonation Technology

Input
500 pg – 2 µg DNA per treatment with 200 – 500 ng being optimal

Converted DNA is Ideal for Downstream Analysis Including…
• PCR
• Real-Time PCR
• Microarray
• Sequencing

Formats
• Single Column
• 96-well Format

The EZ DNA Methylation-Gold™ Kit is a refinement of our popular EZ DNA Methylation™ Kit. The EZ DNA Methylation-Gold™ Kit integrates DNA denaturation and bisulfite conversion processes into a single step (see figure below). Also, the kit has been streamlined for high yield recovery of DNA following bisulfite treatment. Both kits are based on a three step reaction process between cytosine and sodium bisulfite resulting in cytosine being converted into uracil. The EZ DNA Methylation-Gold™ Kit features innovative in-column desulphonation technology that eliminates cumbersome DNA precipitation steps and provides consistent results every time. The kit has been designed to minimize template degradation, loss of DNA during treatment and clean-up, and to provide complete conversion of unmethylated cytosines. Recovered DNA is ideal for PCR, sequencing, microarrays, etc.

Comparative Overview

<table>
<thead>
<tr>
<th></th>
<th>EZ DNA Methylation-Gold™</th>
<th>Suppliers C &amp; H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing Time</td>
<td>3 hrs.</td>
<td>16-20 hrs.</td>
</tr>
<tr>
<td>Protocol</td>
<td>Simple</td>
<td>Complicated</td>
</tr>
<tr>
<td>Conversion</td>
<td>Rapid, Complete - integrates denaturation/conversion into one step.</td>
<td>Slow, Incomplete - denaturation and conversion steps are separate.</td>
</tr>
<tr>
<td>Desulphonation</td>
<td>Convenient In-Column</td>
<td>Cumbersome Precipitation</td>
</tr>
<tr>
<td>DNA Recovery</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>96-well Format</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

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Fax 1-714-288-9643
Email info@zymoresearch.com
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DNA sequencing results after bisulfite treatment. DNA with methylated C<sup>m</sup>CpG (at nucleotide position #5) was processed using the EZ DNA Methylation-Gold™ Kit. The recovered DNA was amplified by PCR and then sequenced directly. The methylated cytosine at position #5 remained intact while the unmethylated cytosines (i.e., positions #7, 9, 11, 14 and 15) were completely converted into uracil following bisulfite treatment and detected as thymine following PCR.

Original DNA with methylated C<sup>m</sup>CpG

DNA Sequencing after CT conversion

Selected Citations:

1. Takahiro Arima, et al. ZAC, LIT1 (KCNQ1OT1) and p57<sup>KIP2</sup> (CDKN1C) are in an imprinted gene network that may play a role in Beckwith–Wiedemann syndrome. *Nucleic Acids Res.*, May 2005; 33: 2650 - 2660.


**EZ DNA Methylation-Gold™ Kit**

**Short Protocol**

**Preparation of CT Conversion Reagent:**

Add 900 µl water, 50 µl of M-Dissolving Buffer and 300 µl of M-Dilution Buffer to one tube of CT Conversion Reagent. Mix for 10 minutes.

1. Add 130 µl of the prepared CT Conversion Reagent to 20 µl of DNA sample. Mix.

2. Perform the following temperature steps: 98°C for 10 minutes, 64°C for 2.5 hours, then hold at 4°C.

3. Add 600 µl of M-Binding Buffer to a Zymo-Spin IC Column, then add the sample. Close the cap and mix by inverting several times.

4. Centrifuge at full speed (>10,000 x g) for 30 seconds. Discard the flow-through!

5. Add 100 µl of M-Wash Buffer to the column, spin 30 seconds.

6. Add 200 µl of M-Desulphonation Buffer to the column and wait for 15-20 minutes.

7. Spin at full speed for 30 seconds.

8. Add 200 µl of M-Wash Buffer to the column, spin 30 seconds. Repeat this wash step one more time.

9. Add 10 µl of M-Elution Buffer directly to the column matrix. Place into a 1.5 ml tube. Spin briefly to elute the DNA.

**Ordering Information**

<table>
<thead>
<tr>
<th>Kit</th>
<th>Catalog No.</th>
<th>Kit Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>EZ DNA Methylation-Gold™ Kit</td>
<td>D5005</td>
<td>50 Rxns.</td>
</tr>
<tr>
<td>EZ DNA Methylation-Gold™ Kit</td>
<td>D5006</td>
<td>200 Rxns.</td>
</tr>
<tr>
<td>EZ-96 DNA Methylation-Gold™ Kit</td>
<td>D5007</td>
<td>2x96 Rxns., 2x96 Shallow-well Silicon-A Plate™</td>
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<tr>
<td>EZ-96 DNA Methylation-Gold™ Kit</td>
<td>D5008</td>
<td>2x96 Rxns., 2x96 Deep-well Zymo-Spin I-96 Plate™</td>
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</table>

<table>
<thead>
<tr>
<th>Methylated DNA Standards</th>
<th>Catalog No.</th>
<th>Kit Size</th>
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</thead>
<tbody>
<tr>
<td>Universal Methylated DNA Standard</td>
<td>D5010</td>
<td>1 set</td>
</tr>
<tr>
<td>Universal Methylated Human DNA Standard</td>
<td>D5011</td>
<td>1 set</td>
</tr>
<tr>
<td>Universal Methylated Mouse DNA Standard</td>
<td>D5012</td>
<td>1 set</td>
</tr>
</tbody>
</table>

The EZ DNA Methylation-Gold™ and EZ DNA Methylation-Direct™ Kits are patent pending. The Polymerase Chain Reaction (PCR) process is covered by U.S. Pat. Nos. 4,683,195 and 4,683,202 assigned to Hoffmann-La Roche. Patents pending in other countries. No license under these patents to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of Zymo Research’s EZ DNA Methylation kits. Further information on purchasing licenses to practice the PCR process can be obtained from the director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501. Use of Methylation Specific PCR (MSP) is protected by US Patents 5,786,146 & 6,017,704 & 6,200,756 & 6,265,171 and International Patent WO 97/46705. 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. Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.