



**ZYMO RESEARCH**

*The Beauty of Science is to Make Things Simple*

# INSTRUCTION MANUAL

## **EZ DNA Methylation™ Kit**

Catalog Nos. **D5001 & D5002**

### **Highlights**

- Streamlined, proven procedure for bisulfite conversion of DNA.
- Desulphonation and recovery of bisulfite-treated DNA with a spin column.
- Recovered DNA is ideal for downstream analyses including PCR, endonuclease digestion, sequencing, microarrays, etc.

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**Product Contents:**

	<b>D5001</b>	<b>D5002</b>	<b>Storage Temperature</b>
<b>EZ DNA Methylation™ Kit</b>	50 rxns.	200 rxns.	
<b>CT Conversion Reagent*</b>	5 tubes	20 tubes	Room Temp.
<b>M-Dilution Buffer</b>	1.3 ml	5.2 ml	Room Temp.
<b>M-Binding Buffer</b>	20 ml	80 ml	Room Temp.
<b>M-Wash Buffer**</b>	6 ml	24 ml	Room Temp.
<b>M-Desulphonation Buffer</b>	10 ml	40 ml	Room Temp.
<b>M-Elution Buffer</b>	1 ml	4 ml	Room Temp.
<b>Zymo-Spin™ IC Columns</b>	50 columns	200 columns	Room Temp.
<b>Collection Tubes</b>	50 tubes	200 tubes	Room Temp.
<b>Instruction Manual</b>	1	1	–

Note - Integrity of kit components is guaranteed for one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

\* 750 µl water and 210 µl **M-Dilution Buffer** are added per tube of **CT Conversion Reagent** and mixed prior to use.

\*\* Add 24 ml of 100% ethanol to the 6 ml **M-Wash Buffer** concentrate (D5001) or 96 ml of 100% ethanol to the 24 ml **M-Wash Buffer** concentrate (D5002) before use.

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.

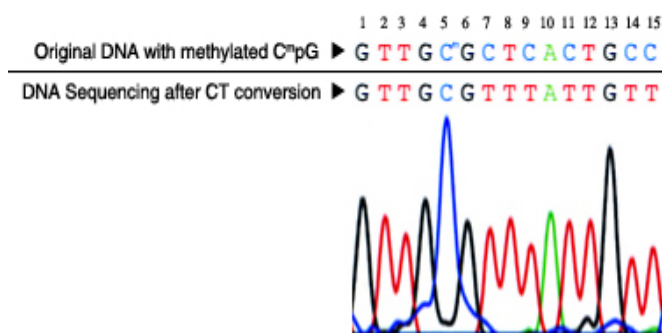
Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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## Introduction to DNA Methylation:

DNA methylation is a naturally occurring event in both prokaryotic and eukaryotic organisms. In prokaryotes DNA methylation provides a way to protect host DNA from digestion by restriction endonucleases that are designed to eliminate foreign DNA, and in higher eukaryotes DNA methylation functions in the regulation/control of gene expression (1). It has been demonstrated that aberrant DNA methylation is a widespread phenomenon in cancer and may be among the earliest changes to occur during oncogenesis (2). DNA methylation has also been shown to play a central role in gene imprinting, embryonic development, X-chromosome gene silencing, and cell cycle regulation. In many plants and animals, DNA methylation consists of the addition of a methyl group to the fifth carbon position of the cytosine pyrimidine ring via a methyltransferase enzyme (3). The majority of DNA methylation in mammals occurs in 5'-CpG-3' dinucleotides, but other methylation patterns do exist. In fact, about 80 percent of all 5'-CpG-3' dinucleotides in mammalian genomes are found to be methylated, whereas the majority of the twenty percent that remain unmethylated are within promoters or in the first exons of genes.

The ability to detect and quantify DNA methylation efficiently and accurately has become essential for the study of cancer, gene expression, genetic diseases, as well as many other important aspects of biology. To date, a number of methods have been developed to detect/quantify DNA methylation including: high-performance capillary electrophoresis (4) and methylation-sensitive arbitrarily primed PCR (5). However, the most common technique used today remains the bisulfite conversion method (6). This technique involves treating methylated DNA with bisulfite, which converts unmethylated cytosines into uracil. Methylated cytosines remain unchanged during the treatment. Once converted, the methylation profile of the DNA can be determined by PCR amplification followed by DNA sequencing (see below).



**DNA sequencing results following bisulfite treatment.** DNA with methylated C<sup>m</sup>pG at nucleotide position #5 was processed using the **EZ DNA Methylation™ Kit**. The recovered DNA was amplified by PCR and then sequenced directly. The methylated cytosine at position #5 remained intact while the unmethylated cytosines at positions #7, 9, 11, 14 and 15 were completely converted into uracil following bisulfite treatment and detected as thymine following PCR.

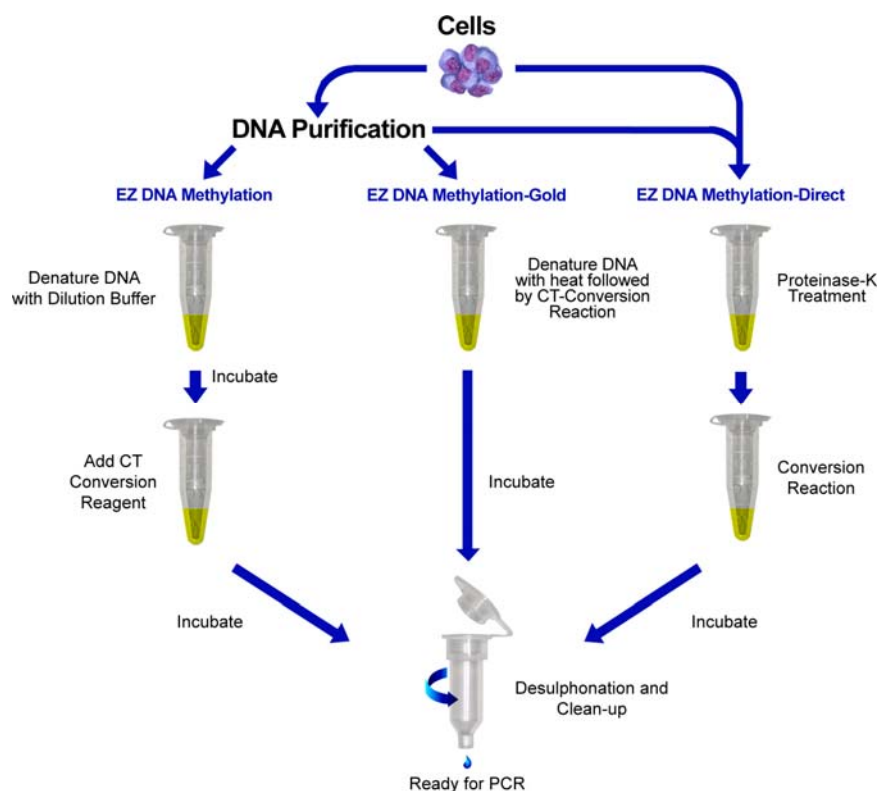
### References:

1. Costello JF, Plass CJ. *Med. Genet.* 2001; 38(5): 285-303.
2. Stirzaker C. *Cancer Res.* 1997; 57(11): 2229-2237.
3. Adams RL. *Bioessays.* 1995; 17(2): 139-145.
4. Fraga MF, *et al.* *Electrophoresis.* 2000; 21(14): 2990-2994.
5. Gonzalgo ML. *Cancer Res.* 1997; 57(4): 594-599.
6. Frommer M. *Proc. Natl. Acad. Sci. USA.* 1992; 89(5): 1827-1831.

## Product Description:

The **EZ DNA Methylation™ Kit** features a simplified procedure that streamlines bisulfite conversion of DNA. The kit is based on the three-step reaction that takes place between cytosine and sodium bisulfite where cytosine is converted into uracil. The product's innovative in-column desulphonation technology eliminates otherwise cumbersome precipitations. The kit is designed to reduce template degradation, minimize DNA loss during treatment and clean-up, while ensuring complete conversion of the DNA. Purified, converted DNA is ideal for PCR amplification for downstream analyses including endonuclease digestion, sequencing, microarrays, etc.

An outline comparing the **EZ DNA Methylation™ Kit** procedure to Zymo Research's other methylation kits is shown below.



Outline of the **EZ DNA Methylation™**, **EZ DNA Methylation-Gold™** and **EZ DNA Methylation-Direct™** Kit procedures.

**Note:** 96-Well spin-plate formats are available for processing larger numbers of samples. Also, MagPrep kits are available (p. 8) for adaptation to liquid handling robots (e.g., Tecan – Freedom EVO®) and automated sample prep.

### Selected EZ DNA Methylation™ Kit Citations:

1. Ehrich M, *et al.* *Nuc. Acids Res.* 2007; 35 (5): e29
2. Kaneda M, *et al.* *Nature.* 2004; 429: 900-903
3. Zhang F, *et al.* *Proc. Natl. Acad. Sci. USA.* 2007; 104 (11): 4395-4400.
4. Oda M, *et al.* *Genes & Dev.* 2006; 20: 3382-3394.
5. England RPM, *et al.* *Nature Meth.* 2005; 2: 1-2.

**Specifications:**

- **DNA Input:** Samples containing 500 pg - 2 µg of DNA. For optimal results, the amount of input DNA should be from 200 to 500 ng.
- **Conversion Efficiency:** > 99% of non-methylated C residues are converted to U; > 99% protection of methylated cytosines.
- **DNA Recovery:** > 80%

**Reagent Preparation:**

- **Preparation of CT Conversion Reagent**

The **CT Conversion Reagent** supplied within this kit is a solid mixture and must be prepared prior to first use. Prepare as follows:

1. Add 750 µl water and 210 µl of **M-Dilution Buffer** to a tube of **CT Conversion Reagent**.
2. Mix at room temperature with frequent vortexing or shaking for 10 minutes.

**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** should be used immediately following preparation. If not used immediately, the **CT Conversion Reagent** solution can be stored overnight at room temperature, one week at 4°C, or up to one month at -20°C. Stored **CT Conversion Reagent** solution must be warmed to 37°C, then vortexed prior to use.

- **Preparation of M-Wash Buffer**

Add 24 ml of 100% ethanol to the 6 ml **M-Wash Buffer** concentrate (D5001) or 96 ml of 100% ethanol to the 24 ml **M-Wash Buffer** concentrate (D5002) before use.

### Protocol:

1. Add 5 µl of **M-Dilution Buffer** to the DNA sample and adjust the total volume to 50 µl with water. Mix the sample by flicking or pipetting up and down.

**Example:** For 14 µl of a DNA sample add 5 µl M-Dilution Buffer and 31 µl water.

2. Incubate the sample at 37° C for 15 minutes.
3. After the above incubation, add 100 µl of the prepared **CT Conversion Reagent** to each sample and mix.

4. Incubate the sample in the dark at 50° C for 12-16 hours.

Please see **Appendix** (page 6) for alternative incubation conditions (e.g., when using the Illumina Infinium® Methylation Assay)

5. Incubate the sample at 0-4° C (e.g., on ice) for 10 minutes. Samples may be kept at 4° C for up to 20 hours.

6. Add 400 µl of **M-Binding Buffer** to a **Zymo-Spin™ IC Column** and place the column into a provided **Collection Tube**.

7. Load the sample (from Step 5) into the **Zymo-Spin™ IC Column** containing the **M-Binding Buffer**. Close the cap and mix by inverting the column several times.

8. Centrifuge at full speed ( $\geq 10,000 \times g$ ) for 30 seconds. Discard the flow-through.

9. Add 100 µl of **M-Wash Buffer** to the column. Centrifuge at full speed for 30 seconds.

10. Add 200 µl of **M-Desulphonation Buffer** to the column and let stand at room temperature (20-30° C) for 15-20 minutes. After the incubation, centrifuge at full speed for 30 seconds.

11. Add 200 µl of **M-Wash Buffer** to the column. Centrifuge at full speed for 30 seconds. Add another 200 µl of **M-Wash Buffer** and centrifuge for an additional 30 seconds.

12. Place the column into a 1.5 ml microcentrifuge tube. Add 10 µl of **M-Elution Buffer** directly to the column matrix. Centrifuge for 30 seconds at full speed to elute the DNA.

The DNA is ready for immediate analysis or can be stored at or below -20° C for later use. For long term storage, store at or below -70° C. We recommend using 1-4 µl of eluted DNA for each PCR, however, up to 10 µl can be used if necessary. The elution volume can be > 10 µl depending on the requirements of your experiments, but small elution volumes will yield more concentrated DNA.

The **CT Conversion reagent** is light sensitive, so try to minimize the reaction's exposure to light whenever possible.

The capacity of the collection tube with the column inserted is 800 µl. Empty the collection tube whenever necessary to prevent contamination of the column contents by the flow-through.

Alternatively, water or TE (pH  $\geq 6.0$ ) can be used for elution if required for your experiments.





**Frequently Asked Questions:**

**Q: Should the input DNA be dissolved in TE, water, or some other buffer prior to its conversion?**

**A:** *Water, TE or modified TE buffers can be used to dissolve the DNA and do not interfere with the conversion process.*

**Q: Which Taq polymerase(s) do you recommend for PCR amplification of converted DNA?**

**A:** *We recommend a “hot start” DNA polymerase (e.g., ZymoTaq™, page 9).*

**Q: Why are there two different catalog numbers for the EZ-96 DNA Methylation™ Kit?**

**A:** *The two different catalog numbers are used to differentiate between the binding plates that are included in the kit. Deep and shallow-well binding plates are available to accommodate most rotors and microplate carriers. Below is a comparison of the two binding plates.*



Binding Plate	Silicon-A™ Plate	Zymo-Spin™ I-96 Plate
Style	Shallow-Well	Deep-Well
Height of Binding Plate	19 mm (0.75 inches)	35 mm (1.38 inches)
Binding Plate/Collection Plate Assembly	43 mm (1.69 inches)	60 mm (2.36 inches)
Binding Cap./Minimum Elution Volume	5 µg/30 µl	5 µg/15 µl
Catalog Numbers	<b>D5003</b>	<b>D5004</b>



**Ordering Information:**

Product Description	Catalog No.	Kit Size
<b>EZ DNA Methylation™ Kit</b>	D5001	50 rxns.
	D5002	200 rxns.
<b>EZ-96 DNA Methylation™ Kit</b> (Shallow-Well)	D5003	2 x 96 rxns.
<b>EZ-96 DNA Methylation™ Kit</b> (Deep-Well)	D5004	2 x 96 rxns.
<b>EZ-96 DNA Methylation™ MagPrep*</b>	D5040	4 x 96 rxns.
	D5041	8 x 96 rxns.

\* **MagPrep** kits are adaptable to liquid handling robots (e.g., Tecan – Freedom EVO®) making them ideal for automated sample prep.

For Individual Sale	Catalog No.	Amount(s)
<b>CT Conversion Reagent</b>	D5001-1	1 tube
	D5003-1	1 bottle
<b>M-Dilution Buffer</b>	D5001-2	1.3 ml
	D5002-2	5.2 ml
<b>M-Binding Buffer</b>	D5005-3	30 ml
	D5006-3	125 ml
	D5040-3	250 ml
<b>M-Wash Buffer</b>	D5001-4	6 ml
	D5002-4	24 ml
	D5007-4	36 ml
	D5040-4	72 ml
<b>M-Desulphonation Buffer</b>	D5001-5	10 ml
	D5002-5	40 ml
	D5040-5	80 ml
<b>M-Elution Buffer</b>	D5001-6	1 ml
	D5002-6	4 ml
	D5007-6	8 ml
	D5041-6	40 ml
<b>Zymo-Spin™ IC Columns</b> (capped)	C1004-50	50 columns
	C1004-250	250 columns
<b>Collection Tubes</b>	C1001-50	50 tubes
	C1001-500	500 tubes
	C1001-1000	1,000 tubes
<b>MagBinding Beads</b>	D4100-2-6	6ml
	D4100-2-8	8 ml
	D4100-2-12	12 ml
	D4100-2-16	16 ml
	D4100-2-24	24 ml
<b>Zymo-Spin™ I-96 Binding Plates</b>	C2004	2 plates
<b>Silicon-A™ Binding Plates</b>	C2001	2 plates
<b>Conversion Plates w/ Pierceable Cover Film</b>	C2005	2 plates/films
<b>Collection Plates</b>	C2002	2 plates
<b>Elution Plates</b>	C2003	2 plates

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## Epigenetics Products From Zymo Research

Product	Description	Kit Size	Cat No. (Format)
<b>Bisulfite Kits for DNA Methylation Detection</b>			
<b>EZ DNA Methylation™ Kit</b>	For the conversion of unmethylated cytosines in DNA to uracil via the <u>chemical-denaturation</u> of DNA and a specially designed CT Conversion Reagent. <i>Fast-Spin</i> technology ensures ultra-pure, converted DNA for subsequent DNA methylation analysis. Magnetic bead format for adaptation to automated liquid handling platforms.	50 Rxns. 200 Rxns. 2x96 Rxns. 2x96 Rxns. 4x96 Rxns. 8x96 Rxns.	<b>D5001</b> (spin column) <b>D5002</b> (spin column) <b>D5003</b> (shallow-well plate) <b>D5004</b> (deep-well plate) <b>D5040</b> (magnetic bead) <b>D5041</b> (magnetic bead)
<b>EZ DNA Methylation-Gold™ Kit</b>	For the fast (3 hr.) conversion of unmethylated cytosines in DNA to uracil via <u>heat/chemical-denaturation</u> of DNA and a specially designed CT Conversion Reagent. <i>Fast-Spin</i> technology ensures ultra-pure, converted DNA for subsequent DNA methylation analysis. Magnetic bead format for adaptation to automated liquid handling platforms.	50 Rxns. 200 Rxns. 2x96 Rxns. 2x96 Rxns. 4x96 Rxns. 8x96 Rxns.	<b>D5005</b> (spin column) <b>D5006</b> (spin column) <b>D5007</b> (shallow-well plate) <b>D5008</b> (deep-well plate) <b>D5042</b> (magnetic bead) <b>D5043</b> (magnetic bead)
<b>EZ DNA Methylation-Direct™ Kit</b>	Features simple and reliable DNA bisulfite conversion directly from blood, tissue (FFPE/LCM), and cells without the prerequisite for DNA purification in as little as 4-6 hrs. The increased sensitivity of this kit makes it possible to amplify bisulfite converted DNA from as few as 10 cells or 50 pg DNA. Magnetic bead format for adaptation to automated liquid handling platforms.	50 Rxns. 200 Rxns. 2x96 Rxns. 2x96 Rxns. 4x96 Rxns. 8x96 Rxns.	<b>D5020</b> (spin column) <b>D5021</b> (spin column) <b>D5022</b> (shallow-well plate) <b>D5023</b> (deep-well plate) <b>D5044</b> (magnetic bead) <b>D5045</b> (magnetic bead)
<b>EZ DNA Methylation-Lightning™ Kit</b>	Complete bisulfite conversion in about an hour using a unique liquid format conversion reagent that requires no preparation. <i>Fast-Spin</i> technology ensures ultra-pure, converted DNA for subsequent DNA methylation analysis. Magnetic bead format for adaptation to automated liquid handling platforms.	50 Rxns. 200 Rxns. 2x96 Rxns. 2x96 Rxns. 4x96 Rxns. 8x96 Rxns.	<b>D5030</b> (spin column) <b>D5031</b> (spin column) <b>D5032</b> (shallow-well plate) <b>D5033</b> (deep-well plate) <b>D5046</b> (magnetic bead) <b>D5047</b> (magnetic bead)
<b>EZ DNA Methylation-Startup™ Kit</b>	Designed for the first time user requiring a consolidated product to perform DNA methylation analysis. Includes technologies for sample processing, bisulfite treatment of DNA, and PCR amplification of "converted" DNA for methylation analysis.	1 Kit	<b>D5024</b>
<b>Methylated DNA Standards</b>			
<b>Universal Methylated Human DNA Standard</b>	Human (male) genomic DNA having all CpG sites methylated. To be used for the evaluation of bisulfite-mediated conversion of DNA. Supplied with a control primer set.	1 set	<b>D5011</b>
<b>Universal Methylated Mouse DNA Standard</b>	Mouse (male) DNA having all CpG sites methylated. To be used for the evaluation of bisulfite-mediated conversion of DNA. Supplied with a control primer set.	1 set	<b>D5012</b>
<b>Other...</b>			
<b>ChIP DNA Clean &amp; Concentrator™</b>	Clean and concentrate DNA from any reaction or "crude" preparation in 2 min. A 6 µl minimum elution volume allows for highly concentrated DNA. Designed for samples containing up to 5 µg of DNA.	50 Preps. 50 Preps.	<b>D5201</b> (uncapped column) <b>D5205</b> (capped column)
<b>Genomic DNA Clean &amp; Concentrator™</b>	Genomic DNA clean-up in minutes. Unique spin column technology for recovery of ultra-pure large-sized DNA (100 bp to ≥200 kb) DNA from any impure preparation (e.g., Proteinase K digestion).	25 Preps. 100 Preps.	<b>D4010</b> <b>D4011</b>
<b>ZymoTaq™ DNA Polymerase</b>	ZymoTaq™ "hot start" DNA Polymerase is specifically designed for the amplification of "difficult" DNA templates including: bisulfite-treated DNA for methylation detection. The product generates specific amplicons with little or no by-product formation. Available either as a single buffer premix or as a polymerase system with components provided separately.	50 Rxns. 200 Rxns. 50 Rxns. 200 Rxns.	<b>E2001</b> (system) <b>E2002</b> (system) <b>E2003</b> (premix) <b>E2004</b> (premix)
<b>Methylated-DNA IP Kit</b>	IP with a highly specific anti-5-methylcytosine monoclonal antibody. Designed for the enrichment of 5-methylcytosine-containing DNA from any pool of fragmented genomic DNA for use in genome-wide methylation analysis.	10 Rxns.	<b>D5101</b>
<b>Services</b>			
Available for <b>DNA Methylation</b> and <b>Hydroxymethylation</b> at <a href="http://www.zymoresearch.com/services">http://www.zymoresearch.com/services</a> or inquire at <a href="mailto:services@zymoresearch.com">services@zymoresearch.com</a> ...powered by the latest Next-Gen Sequencing technologies!			

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