



**ZYMO RESEARCH**

*The Beauty of Science is to Make Things Simple*

# INSTRUCTION MANUAL

## **EZ-96 DNA Methylation-Direct™ Kit**

Catalog No. **D5022 (Shallow-Well Format)**

### **Highlights**

- High throughput (96-well), complete *bisulfite conversion* of DNA directly\* from blood, tissue, or cells.
- Compatible with small sample inputs – as few as 10 cells or 50 pg DNA.
- Well-suited for FFPE and LCM-derived samples.

### **Contents**

Product Contents .....	1
Introduction to DNA Methylation.....	2
Product Description.....	3
Product Specifications.....	4
Reagent Preparation .....	4
Protocol.....	5, 6
Appendices .....	7, 8
Frequently Asked Questions .....	9
Ordering Information .....	10
List of Related Products .....	11

\* Patent pending; for research use only

Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

### Product Contents:

<b>EZ-96 DNA Methylation-Direct™ Kit</b>	<b>D5022</b>	<b>Storage Temperature</b>
	2 x 96 rxns.	
<b>Proteinase K and Storage Buffer*</b>	20 mg set	-20°C(after mixing)
<b>M-Digestion Buffer (2X)</b>	15 ml	Room Temp.
<b>CT Conversion Reagent**</b>	2 bottles	Room Temp.
<b>M-Dilution Buffer</b>	7 ml	Room Temp.
<b>M-Solubilization Buffer</b>	18 ml	Room Temp.
<b>M-Reaction Buffer</b>	4 ml	Room Temp.
<b>M-Binding Buffer</b>	125 ml	Room Temp.
<b>M-Wash Buffer***</b>	2 x 36 ml	Room Temp.
<b>M-Desulphonation Buffer</b>	40 ml	Room Temp.
<b>M-Elution Buffer</b>	8 ml	Room Temp.
<b>Silicon-A™ Binding Plates</b>	2 plates	Room Temp.
<b>Conversion Plates w/Pierceable Cover Film</b>	4 plates/films	Room Temp.
<b>Collection Plates</b>	2 plates	Room Temp.
<b>Elution Plates</b>	2 plates	Room Temp.
<b>Instruction Manual</b>	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.

\* Add 1040 µl **Proteinase K Storage Buffer** to the tube of **Proteinase K** prior to use. The final concentration of **Proteinase K** after the addition of **Proteinase K Storage Buffer** is 20 mg/ml.

\*\* 7.9 ml **M-Solubilization Buffer** and 3 ml **M-Dilution Buffer** are added per bottle of **CT Conversion Reagent**, mixed, and then 1.6 ml **M-Reaction Buffer** is added prior to use.

\*\*\* Add 144 ml of 100% ethanol to the 36 ml **M-Wash Buffer** concentrate before use.

The EZ DNA Methylation-Direct™ Kit is 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.

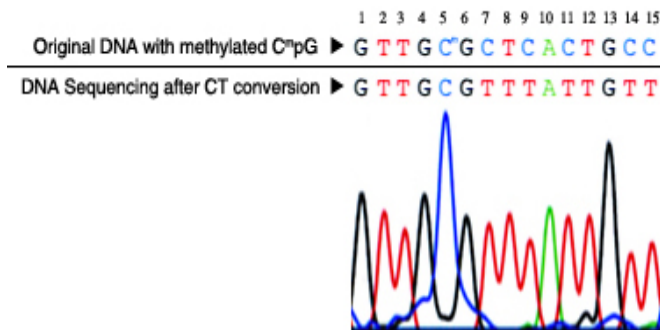
Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution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

#### ZYMO RESEARCH CORP.

## 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.

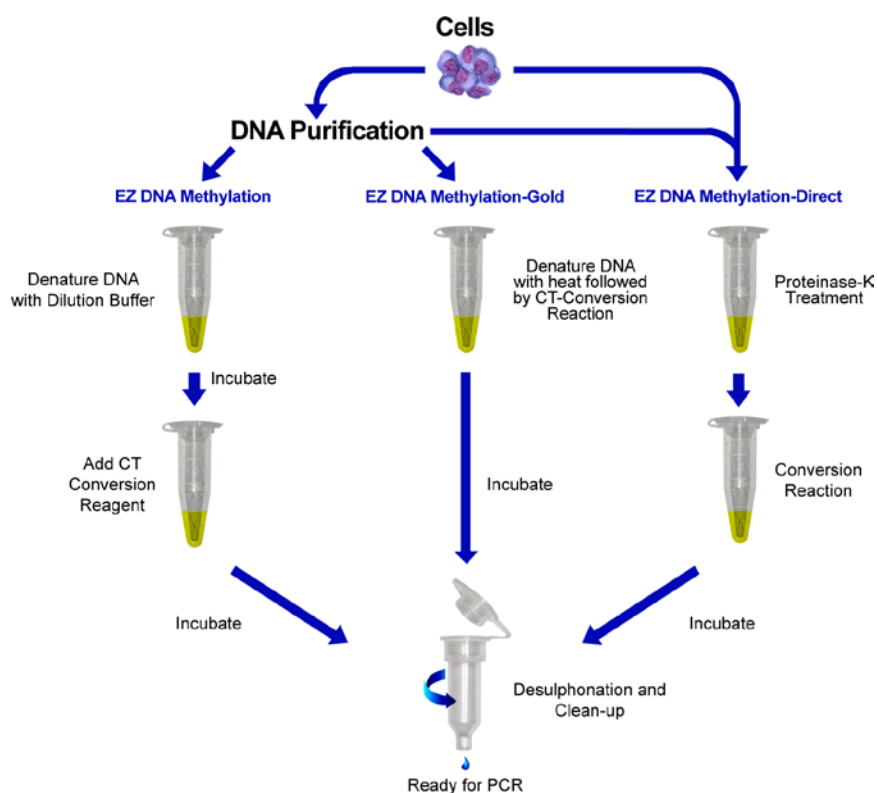
## Product Description:

### Selected EZ DNA Methylation Kit Citations:

1. Ehrlich 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.

The **EZ DNA Methylation-Direct™ Kit** is a further refinement of our popular **EZ DNA Methylation™** and **EZ DNA Methylation-Gold™ Kits**. The **EZ DNA Methylation-Direct™ Kit** features simple and reliable DNA bisulfite conversion directly from blood, tissue, and cells without the prerequisite for DNA purification. The increased sensitivity of this kit makes it possible to amplify bisulfite converted DNA from as few as 10 cells or 50 pg DNA. Like the **EZ DNA Methylation-Gold™ Kit**, DNA denaturation and bisulfite conversion processes are combined into a single step (see below). All kits streamline the three step process of bisulfite conversion of non-methylated cytosine in DNA into uracil. In addition the methylation kits share innovative in-column desulphonation technology that eliminates otherwise cumbersome DNA precipitation steps while ensuring researchers consistent results every time. The kits have 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 amplification for downstream analyses including restriction endonuclease digestion, sequencing, microarrays, etc.

An outline comparing the **EZ DNA Methylation-Direct™ 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.

## Specifications:

- **Starting Materials:**

**Cells:** Compatible with cells from solid tissue, tissue culture, whole blood, buffy coat, biopsies, LCM (Laser-Capture Micro-Dissection) and FFPE samples, etc. The number of cells per standard treatment can range from  $10 - 10^5$  cells. For optimal results, the cell number should be from  $1 \times 10^3 - 8 \times 10^4$  cells.

**Purified DNA:** Samples containing 50 pg - 2 µg of DNA. For optimal results, the amount of input DNA should be from 200 to 500 ng.

- **Conversion Efficiency:** > 99.5% of non-methylated C residues are converted to U; > 99.5% protection of methylated cytosines.
- **DNA Recovery:** > 80%
- **Sensitivity of Detection (Lower Limit):** 10 cells for successful PCR amplification.

## Reagent Preparation:

- **Preparation of Proteinase K**

Add 1040 µl of **Proteinase K Storage Buffer** to the tube containing **Proteinase K**. Dissolve completely then store at -20°C.

- **Preparation of CT Conversion Reagent**

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

1. Add 7.9 ml of **M-Solubilization Buffer** and 3 ml of **M-Dilution Buffer** to a bottle of **CT Conversion Reagent**.
2. Mix at room temperature with frequent vortexing or shaking for 15 minutes.
3. Add 1.6 ml of **M-Reaction Buffer** and mix an additional 4 minutes.

**Note:** It is normal to see trace amounts of undissolved reagent in the **CT Conversion Reagent**. Each bottle of **CT Conversion Reagent** is designed for 96 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 its 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 144 ml of 100% ethanol to the 36 ml **M-Wash Buffer** concentrate before use.

**Protocol:**

Either blood, tissue, cells, or purified DNA can be used as the starting material for the **EZ DNA Methylation-Direct™ Kit**. If purified DNA is used, then proceed directly to **Section II** (page 6).

If blood, tissue, or cells are used, see **Appendix I** (page 7) for sample-specific recommendations (e.g., FFPE and LCM samples). For optimal results, the cell number should be between  $1 \times 10^3$  -  $8 \times 10^4$  per treatment, although the cell number can range from  $10$  -  $10^5$  cells. Using more cells than the recommended limit may result in incomplete bisulfite conversion of the DNA.

**Section I: Sample Digestion with Proteinase K.**

Digestions should be performed in a **Conversion Plate** (provided) using procedure **A** or **B** (below) based on the number of cells and/or tissue type. Digestions are scalable to facilitate multiple samples or to increase the ease of manipulation. Sufficient volumes of reagents are included with this kit to increase the overall **Proteinase K** digestion volume 5-fold.

- A.** Setup the following digestion for samples containing up to  $2 \times 10^3$  cells.

10 $\mu$ l	M-Digestion Buffer (2X)
Up to 9 $\mu$ l	Sample ( $\leq 2 \times 10^3$ cells)
1 $\mu$ l	Proteinase K
<u>X <math>\mu</math>l</u>	<u>H<sub>2</sub>O</u>
20 $\mu$ l	Total Volume

**Important!** "Difficult to digest" samples result in the formation of visible debris following digestion. These should be processed according to procedure **B**.

- B.** Setup the following digestion for samples containing up to  $1 \times 10^5$  cells. This should also include all "difficult to digest" samples that form debris or precipitate following **Proteinase K** digestion— see **Appendix I**.

13 $\mu$ l	M-Digestion Buffer (2X)
Up to 12 $\mu$ l	Sample ( $\leq 10^5$ cells)
1 $\mu$ l	Proteinase K
<u>X <math>\mu</math>l</u>	<u>H<sub>2</sub>O</u>
26 $\mu$ l	Total Volume

- Seal the **Conversion Plate** with **Cover Film** and incubate the samples for 20 minutes at 50°C.

**Note:** For FFPE, LCM and other "fixed" tissue samples, adjust the incubation time to 4 hours (see **Appendix I**).

- If following procedure **A**, proceed directly to **Section II**.

If following procedure **B**, mix the contents the **Conversion Plate** thoroughly, then mount on a **Collection Plate** and centrifuge for 10 minutes at 1,000 x *g*. Pierce or remove film, then transfer 20  $\mu$ l of the supernatant to a new **Conversion Plate** and proceed to **Section II**.

Proteinase K digested material can be stored for several months at -20°C.

**Protocol (continued):****Section II. Bisulfite Conversion of DNA**

1. Add 130  $\mu\text{l}$  of **CT Conversion Reagent** solution to each 20  $\mu\text{l}$  sample in the **Conversion Plate** from **Section I**. Mix the samples by pipetting up and down.

**Note:** If purified DNA is used, add up to 20  $\mu\text{l}$  of DNA to 130  $\mu\text{l}$  of **CT Conversion Reagent** solution. If the volume of DNA is less than 20  $\mu\text{l}$ , compensate with water.

2. Seal the **Conversion Plate** with **Cover Film**. Place the plate in a thermal cycler and perform the following steps:

1. 98°C for 8 minutes
2. 64°C for 3.5 hours
3. 4°C storage for up to 20 hours

3. Add 400  $\mu\text{l}$  of **M-Binding Buffer** to the wells of a **Silicon-A™ Binding Plate** on a provided **Collection Plate**.

4. Load the samples (from Step 2) into the wells of the **Silicon-A™ Binding Plate** containing the **M-Binding Buffer**. Mix by pipetting up and down.

5. Centrifuge at  $\geq 3,000 \times g$  for 5 minutes. Discard the flow-through and reuse the **Collection Plate** for the following wash steps.

6. Add 400  $\mu\text{l}$  of **M-Wash Buffer** to each well of the plate. Centrifuge at  $\geq 3,000 \times g$  for 5 minutes.

7. Add 200  $\mu\text{l}$  of **M-Desulphonation Buffer** to each well and let the plate stand at room temperature (20°C - 30°C) for 15 - 20 minutes, then centrifuge at  $\geq 3,000 \times g$  for 5 minutes. Discard the flow-through.

8. Add 400  $\mu\text{l}$  of **M-Wash Buffer** to each well of the plate. Centrifuge at  $\geq 3,000 \times g$  for 5 minutes. Add another 400  $\mu\text{l}$  of **M-Wash Buffer** and centrifuge for an additional 5 minutes.

9. Place the **Silicon-A™ Binding Plate** onto a provided **Elution Plate**. Add 30  $\mu\text{l}$  of **M-Elution Buffer** directly to each well. Wait 5 minutes, then centrifuge for 3 minutes at  $\geq 3,000 \times g$  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  $\leq -70^\circ\text{C}$ . We recommend using 2 - 8  $\mu\text{l}$  of eluted DNA for each PCR, however, up to 30  $\mu\text{l}$  can be used if necessary. The elution volume can be  $> 30 \mu\text{l}$  depending on the requirements of your experiments, but small elution volumes will yield higher concentrations of DNA.

If procedure A is used (p. 5), the CT-Conversion Reagent can be added directly to the samples in the Conversion Plate by either piercing the Cover Film or carefully removing it. Use a new Cover Film to re-seal the plate.

The capacity of each well of the collection plate is approximately 1 ml. Empty the collection plate whenever necessary to prevent contamination with the flow-through.

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

## **Appendix I: Recommendations for Specific Cells and Tissues**

The following guidelines are provided as recommendations when sampling specific cell and tissue sources. *Most importantly*, the optimal amount of DNA used for bisulfite treatment (**Section II**) should be from  $1 \times 10^3 - 8 \times 10^4$  cells, although DNA from as few as 10 to as many as  $10^5$  cells may be used. *Caution: using more cells than the recommended maximum may result in incomplete bisulfite conversion of the DNA.*

**Important!** “Difficult to digest” samples result in the formation of visible debris following digestion and should be processed according to digestion procedure **B** on page 5. This can occur with samples that are large or resistant to **Proteinase K** digestion, including: connective tissue (e.g., cartilage), adipose tissue, some fixed tissue, etc. *If debris is not removed by centrifugation, it may interfere with the bisulfite conversion process resulting in incomplete conversion of the DNA.*

**Whole Blood:** Use up to 0.5  $\mu$ l whole blood per **Proteinase K** digestion (procedure **A** or **B**, page 5) However, the volume of the **Proteinase K** digestion can be adjusted when processing multiple samples or for convenient sample manipulation. For example, to increase the sample volume 5-fold for digestion procedure **A**: add 2.5  $\mu$ l of blood to 50  $\mu$ l **M-Digestion Buffer**, 42.5  $\mu$ l H<sub>2</sub>O, and 5  $\mu$ l of **Proteinase K**.

**Solid Tissue (Fresh or Frozen):** Use up to 0.1 mg or 0.1  $\mu$ l tissue per **Proteinase K** digestion (procedure **A** or **B**). However, the volume of the **Proteinase K** digestion can be adjusted when processing multiple samples or for convenient sample manipulation. For example, to increase the sample volume 5-fold for digestion procedure **B**: add 0.5 mg or 0.5  $\mu$ l of tissue to 65  $\mu$ l **M-Digestion Buffer**, 59.5  $\mu$ l H<sub>2</sub>O, and 5  $\mu$ l of **Proteinase K**.

**Cultured Cells and Other Cell-Containing Liquids:** Both monolayer and cells in suspension may be processed either directly from the culture container or after harvesting. Small amounts of culture medium do not adversely affect the procedure but should be kept to a minimum. Ideally, cells should be suspended in PBS or Tris-buffered solutions prior to **Proteinase K** digestion.

Other cell-containing liquids (e.g., those derived from FACS or buffy coat) may also be used directly as sample sources. If the composition of the liquid is not “defined”, then pellet the cells by centrifugation and remove the supernatant. Cells should be resuspended in PBS or Tris-buffered solutions. Generally, cells in body fluids can be used directly for **Proteinase K** digestion.

**FFPE (Formalin-Fixed Paraffin-Embedded) and Other “Fixed” Tissues:** Paraffin-embedded tissues must be deparaffinized prior to use. This can be accomplished according to conventional xylene-ethanol protocols. The **Proteinase K** digestion must be extended from 20 minutes to 4 hours for FFPE and any other fixed tissue samples.

**LCM (Laser Capture Micro-Dissection):** Tissue samples from LCM should be in PBS or Tris-buffered solutions. The **Proteinase K** digestion must be extended from 20 minutes to 4 hours for LCM and any other fixed tissue samples.

## **Appendix II: Bisulfite Conversion and PCR Optimization**

- 1. PCR Primer Design.** Generally, primers of 24 to 32 bases are required for amplification of bisulfite converted DNA. For most eukaryotes, all non-methylated cytosine residues will be converted into uracil during the bisulfite treatment. These Cs should be treated as Ts for primer design purposes. For example, for the sequence 5'-AACCTTACAGGCAC-3', the corresponding primer should be 5'-AATTTTATAGGGTAT-3'.

If the primer contains CpG dinucleotides with uncertain methylation status, then mixed bases with C and T can be used. Usually, there should be no more than three mixed positions per primer and they should be located toward the 5' end of the primer. It is not recommended to have mixed bases located at the 3' end of the primer.

- 2. Amount of DNA Required for Bisulfite Conversion.** The minimal amount of human or mouse genomic DNA required for bisulfite treatment and subsequent PCR amplification is 50 pg. The optimal amount of DNA per bisulfite treatment is 200 to 500 ng. Although, up to 2 µg of DNA can also be processed, it should be noted that high input levels of DNA may result in incomplete bisulfite conversion for some GC-rich regions.
- 3. PCR Conditions.** Usually, 35 to 40 cycles are required for successful PCR amplification of bisulfite converted DNA. Optimal amplicon size should be between 150 - 300 bp; however larger amplicons (up to 1 kb) can be generated with optimization of the bisulfite reaction and PCR conditions. We have found that annealing temperatures between 55 - 60°C typically work well. As most non-methylated cytosine residues are converted into uracil, the bisulfite-treated DNA usually is AT-rich and has low GC composition. Thus, it may be necessary to reduce the annealing temperature accordingly.

Non-specific PCR amplification is relatively common with bisulfite treated DNA due to its AT-rich nature. PCR using "hot start" polymerases is strongly recommended for the amplification of bisulfite-treated DNA.

- 4. Quantifying Bisulfite Treated DNA.** Following bisulfite treatment of genomic DNA, non-methylated cytosine residues are converted into uracil. The recovered DNA is typically A, U, and T-rich. The original base-pairing no longer exists. Instead, it is single stranded with limited non-specific base-pairing at room temperature. The absorption coefficient at 260 nm resembles that of RNA. Use a value of 40 µg/ml for  $A_{260} = 1.0$  when determining the concentration of the recovered bisulfite-treated DNA.

**ZymoTaq™** is a "hot start" DNA polymerase specifically designed for the amplification of bisulfite treated DNA. (see page 11 for details)

### 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 DNA and do not interfere with the conversion process.*

**Q: At what temperature and for how long can the converted DNA be stored?**

**A:** *The sample should be stored at  $\leq -20^{\circ}\text{C}$  whenever possible. The quality of the DNA should remain relatively unchanged for up to 3 months.*

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

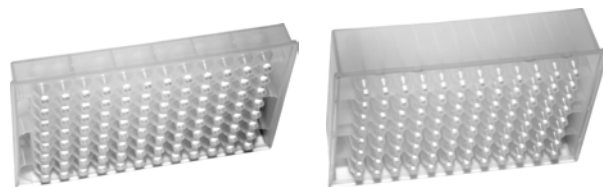
**A:** *We recommend a “hot start” DNA polymerase (e.g., ZymoTaq™, page 11).*

**Q: Why am I not getting “complete” conversion of the DNA?**

**A:** *1) If sampling solid tissue, then it is most likely that too much sample was processed, resulting in incomplete DNA conversion. 2) If sampling FFPE tissue, then it is probable that the DNA was extensively damaged and/or cross-linked resulting in incomplete DNA conversion. 3) If debris is not removed by centrifugation from the Proteinase K digestion, it may interfere with the bisulfite conversion process resulting in incomplete conversion of the DNA.*

**Q: Why are there two different catalog numbers for the EZ-96 DNA Methylation-Direct™ 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>D5022</b>	<b>D5023</b>

#### ZYMO RESEARCH CORP.

**Ordering Information:**

Product Description	Catalog No.	Kit Size
EZ DNA Methylation-Direct™ Kit	D5020	50 rxns.
EZ DNA Methylation-Direct™ Kit	D5021	200 rxns.
EZ-96 DNA Methylation-Direct™ Kit (Shallow-Well)	D5022	2 x 96 rxns.
EZ-96 DNA Methylation-Direct™ Kit (Deep-Well)	D5023	2 x 96 rxns.

For Individual Sale	Catalog No.	Amount(s)
CT Conversion Reagent	D5001-1	1 tube
	D5003-1	1 bottle
M-Dilution Buffer	D5005-2	1.5 ml
	D5006-2	7 ml
M-Binding Buffer	D5005-3	30 ml
	D5006-3	125 ml
M-Wash Buffer	D5001-4	6 ml
	D5002-4	24 ml
	D5007-4	36 ml
M-Desulphonation Buffer	D5001-5	10 ml
	D5002-5	40 ml
M-Elution Buffer	D5001-6	1 ml
	D5002-6	4 ml
	D5007-6	8 ml
M-Solubilization Buffer	D5020-7	4.5 ml
	D5021-7	18 ml
M-Reaction Buffer	D5020-8	1 ml
	D5021-8	4 ml
M-Digestion Buffer	D5020-9	4 ml
	D5021-9	15 ml
Proteinase K and Storage Buffer	D3001-2-5	5 mg set
	D3001-2-20	20 mg set
Zymo-Spin™ IC Columns (capped)	C1004-50	50 columns
	C1004-250	250 columns
Collection Tubes	C1001-50	50 tubes
	C1001-500	500 tubes
	C1001-1000	1,000 tubes
Zymo-Spin™ I-96 Binding Plates	C2004	2 plates
Silicon-A™ Binding Plates	C2001	2 plates
Conversion Plates w/ Pierceable Cover Film	C2005	2 plates/films
Collection Plates	C2002	2 plates
Elution Plates	C2003	2 plates

**ZYMO RESEARCH CORP.**

## 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.	50 Rxns. 200 Rxns. 2x96 Rxns. 2x96 Rxns.	<b>D5001</b> (spin column) <b>D5002</b> (spin column) <b>D5003</b> (shallow-well plate) <b>D5004</b> (deep-well plate)
<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.	50 Rxns. 200 Rxns. 2x96 Rxns. 2x96 Rxns.	<b>D5005</b> (spin column) <b>D5006</b> (spin column) <b>D5007</b> (shallow-well plate) <b>D5008</b> (deep-well plate)
<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.	50 Rxns. 200 Rxns. 2x96 Rxns. 2x96 Rxns.	<b>D5020</b> (spin column) <b>D5021</b> (spin column) <b>D5022</b> (shallow-well plate) <b>D5023</b> (deep-well plate)
<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>EZ Bisulfite DNA Clean-up Kit™</b>	Desulphonation and purification of DNA from any "homebrew" or commercially derived reaction mixture containing bisulfite.	50 Preps. 200 Preps. 2x96 Preps. 2x96 Preps.	<b>D5025</b> (spin column) <b>D5026</b> (spin column) <b>D5027</b> (shallow-well plate) <b>D5028</b> (deep-well plate)
<b>Methylated DNA Standards</b>			
<b>Universal Methylated DNA Standard</b>	pUC19 plasmid 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>D5010</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>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>Anti-5-Methylcytosine Monoclonal Antibody (clone 10G4)</b>	Mouse monoclonal antibody developed to facilitate the differentiation between methylated and non-methylated cytosines in DNA. Can be used in immunoprecipitation-based procedures including Methylated DNA Immunoprecipitation (MeIP).	50 µg/50 µl 200 µg/200 µl	<b>A3001-50</b> <b>A3001-200</b>
<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>

**ZYMO RESEARCH CORP.**