ZYMO RESEARCH

Epigenetics Made Simple™

# EZ-96 DNA Methylation-Direct™ MagPrep

Bisulfite conversion of DNA directly from the source in a scalable, high-throughput format

## Highlights

- High throughput, 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.
- High throughput (96-well), automated desulphonation and recovery of bisulfite-treated DNA.
- Well-suited for FFPE and LCM-derived samples.

Catalog Numbers:
D5044, D5045

Scan with your smart-phone camera to view the online protocol/video.

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# Table of Contents

# Product Contents

| EZ-96 DNA Methylation-Direct™ MagPrep | D5044 (4x96 rxns) | D5045 (8x96 rxns) | Storage Temperature |
|---|---|---|---|
| Proteinase K and Storage Buffer[1] | 2 x 20 mg set | 4 x 20 mg set | −20°C (after mixing) |
| M-Digestion Buffer (2X) | 2 x 15 ml | 4 x 15 ml | Room Temp. |
| CT Conversion Reagent[2] | 4 bottles | 8 bottles | Room Temp. |
| M-Dilution Buffer | 2 x 7 ml | 4 x 7 ml | Room Temp. |
| M-Solubilization Buffer | 2 x 18 ml | 4 x 18 ml | Room Temp. |
| M-Reaction Buffer | 2 x 4 ml | 4 x 4 ml | Room Temp. |
| M-Binding Buffer | 250 ml | 2 x 250 ml | Room Temp. |
| M-Wash Buffer[3] | 2 x 72 ml | 4 x 72 ml | Room Temp. |
| M-Desulphonation Buffer | 80 ml | 2 x 80 ml | Room Temp. |
| M-Elution Buffer | 2 x 8 ml | 40 ml | Room Temp. |
| EZ-Methylation Magprep Beads | 8 ml | 16 ml | Room Temp. |
| Conversion Plates w/ Pierceable Cover Film | 4 plates/films | 8 plates/films | Room Temp. |
| Collection Plates[4] | 6 plates | 10 plates | Room Temp. |
| Elution Plates | 4 plates | 8 plates | Room Temp. |
| Instruction Manual | 1 | 1 | – |

[1] 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.
[2] 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.
[3] Add 288 mL of 95-100% ethanol to the 72 mL **M-Wash Buffer** concentrate prior to use.
[4] Two additional **Collection Plates** are provided as stands for the **Conversion Plates** during processing.

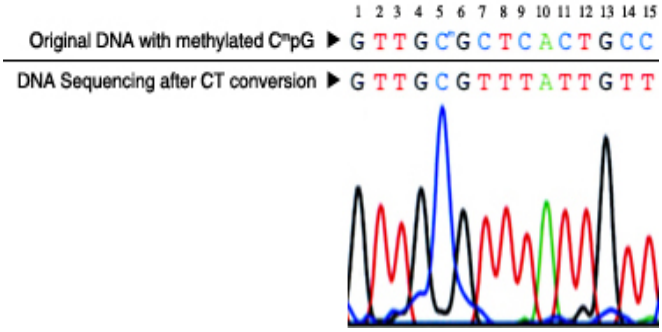
# Introduction to DNA Methylation

Cytosine methylation is a naturally occurring base modification, in both prokaryotic and eukaryotic organisms, consisting of the addition of a methyl group to the fifth carbon position of the cytosine pyrimidine ring via a methyltransferase enzyme (1). In prokaryotes DNA methylation provides a way to protect host DNA from digestion by restriction endonucleases that are designed to eliminate foreign DNA. DNA methylation in higher eukaryotes functions in the regulation/control of gene expression (2).

The majority of DNA methylation in mammals occurs in 5′-CpG-3′ dinucleotides, although other patterns do exist. About 80 percent of all 5′-CpG-3′ dinucleotides in mammalian genomes are found to be methylated, and the majority of the twenty percent that remain unmethylated are within promoters or in the first exons of genes. 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 (3). DNA methylation has also been shown to play a central role in gene imprinting, embryonic development, X-chromosome gene silencing, and cell cycle regulation.

The ability to detect and quantify DNA methylation efficiently and accurately has become essential for the study of cancer, gene expression, genetic diseases, and 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 techniques used today still rely on bisulfite conversion (6).

Treating DNA with bisulfite chemically modifies non-methylated cytosines into uracil, methylated cytosines remain unchanged. Once converted, the methylation profile of the DNA can be determined using the desired downstream application. For single locus analysis, the region of interest is generally amplified following bisulfite conversion (i.e., bisulfite PCR) and then sequenced or processed for Pyrosequencing®. Recent advances in methylation detection also allow the investigation of genome-wide methylation using technologies including array-based methods, reduced representation bisulfite sequencing (RRBS), and whole genome bisulfite sequencing (7).



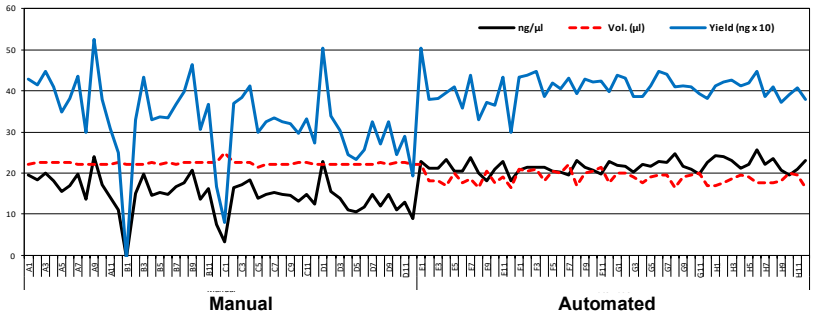

**DNA sequencing results following bisulfite treatment.** DNA with methylated C at nucleotide position #5 was processed using the **EZ DNA Methylation™ Kit**. The recovered DNA was amplified by PCR and then sequenced directly. The methylated cytosine at position #5 remains intact while the unmethylated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

## References:

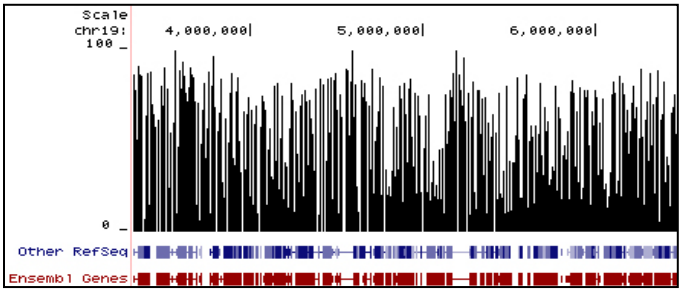
1. Adams RL. Bioessays. 1995; 17(2): 139-145.
2. Costello JF, Plass CJ. Med. Genet. 2001; 38(5): 285-303.
3. Stirzaker C. Cancer Res. 1997; 57(11): 2229-2237.
4. Fraga MF, et al. Electrophoresis. 2000; 21(14): 2990-2994.
5. Gonzalgo ML. Cancer Res. 1997; 57(4): 594-599.
6. Frommer M. Proc. Natl. Acad. Sci. USA. 1992; 89(5): 1827-1831.
7. Rakyan VK, et al. Nat. Rev. 2011, 12(8): 529-541.

# Product Description

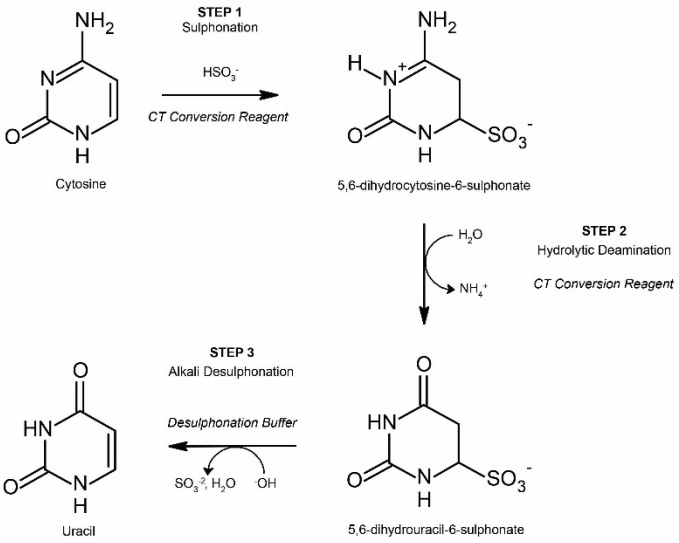
The **EZ-96 DNA Methylation-Direct™ MagPrep**[1] features simple and reliable DNA bisulfite conversion directly from blood, tissue, and cells without the prerequisite for DNA purification. The increased sensitivity of this kit makes it possible to amplify bisulfite converted DNA from as few as 10 cells or 50 pg DNA. These innovations have been coupled to a magnetic bead based clean-up for high-throughput methylation analysis. Desulphonation and clean-up of the converted DNA is performed while bound to the **EZ-Methylation Magprep Beads**. 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, array, bisulfite and next generation sequencing, etc.




**Comparison of Manual vs. Automated Processing.** Data show concentration, volume and total yield for DNA samples across a 96-well plate. Half of the samples (rows A-D) were processed manually. The other half of the samples "Automated" (rows E-H) were processed using the Tecan – Freedom EVO® platform and a dedicated script.




**Methylation Plot From Reduced Representation Bisulfite Sequencing (RRBS).** Data shows the relative percentage of methylation at individual CpG sites in mouse DNA. Methylation percentage is shown across a ~3 Mb region of mouse chromosome 19. Bisulfite sequencing libraries were prepared using mouse genomic DNA prepped with the **Genomic Clean & Concentrator™** (D4010, D4011 – Zymo Research) and bisulfite converted using **EZ DNA Methylation™** technology prior to Next-Gen sequencing.




**Overview of Bisulfite Conversion.** Steps 1 and 2 occur during bisulfite conversion, while Step 3 is performed as the DNA is bound to the column matrix. For the reaction to proceed to completion, it is essential the DNA be fully denatured.

## Select 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.
6. Berman BP, et al. Nature Gen. 2012; 44: 40-46.
7. Leung DC, et al. Proc. Natl. Acad. Sci. USA. 2011; 108 (14): 5718-5723.
8. Hesselink AT, et al. Clin. Cancer Res. 2011; 17: 2459-2465.
9. Campan M, et al. PLoS ONE. 2011, 6 (12): e28141.

[1] Single spin-column and 96-Well spin-plate formats are available.

# 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 x $10^3$-8 x $10^4$ 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.

- **Required Additional Equipment:** Magnetic Stand[1], Heating element for 96-well plate.

[1] A strong-field magnetic stand is recommended (e.g., ZR-96 MagStand, Cat. No. P1005)

# Protocol

## Reagent Preparation

### A. Proteinase K Preparation

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

### B. CT Conversion Reagent Preparation

✓ 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 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 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.

### C. M-Wash Buffer Preparation

✓ Add 288 ml of 100% ethanol to the 72 ml **M-Wash Buffer** concentrate before use.

## Sample Processing

✓ 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 9).

If blood, tissue, or cells are used, see **Appendix I** (page 12) for sample-specific recommendations (e.g., FFPE and LCM samples). For optimal results, the cell number should be between 1 x $10^3$-8 x $10^4$ 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.

1. **A**. Setup the following digestion for samples containing up to 2 x $10^3$ cells.

| | |
|---|---|
| 10 µl | M-Digestion Buffer (2X) |
| Up to 9 µl | Sample (≤ 2 x $10^3$ cells) |
| 1 µl | Proteinase K |
| X µl | $H_2O$ |
| 20 µl | 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 x $10^5$ cells. This should also include all "difficult to digest" samples that form debris or precipitate following **Proteinase K** digestion– see **Appendix I**.

| | |
|---|---|
| 13 µl | M-Digestion Buffer (2X) |
| Up to 12 µl | Sample (≤ $10^5$ cells) |
| 1 µl | Proteinase K |
| X µl | $H_2O$ |
| 26 µl | Total Volume |

2. 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**).

3. 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 µl of the supernatant to a new **Conversion Plate** and proceed to **Section II**.

## Section II. Bisulfite Conversion and Cleanup of DNA

1. Add 130 µl of **CT Conversion Reagent** to 20 µl of a DNA sample in a **Conversion Plate**. Mix the samples by pipetting up and down.

   **Note**: If the volume of DNA is less than 20 µl, compensate with water.

   **Note**: If procedure **A** is used (p. 8), 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.

2. Seal the plate with the provided film. Transfer the **Conversion Plate** to 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

   **Note:** The 4 °C storage step is *optional*.

3. Pre-heat a plate heating element to 55 °C.

   **Note:** Alternatively, depending on the time necessary for the element to reach temperature, this can be performed any time prior to step 10.

4. Add 600 µl of **M-Binding Buffer** and 10 µl of **EZ-Methylation Magprep Beads** to each well of a **Collection Plate**.

   **Note: EZ-Methylation Magprep Beads** settle very quickly, ensure that beads are kept suspended in the reservoir while adding to the plate.

5. Transfer the samples from the **Conversion Plate** into the **Collection Plate** containing the **M-Binding Buffer** and **EZ-Methylation Magprep Beads**. Mix by pipetting up and down 3-6 times and, if available, shaking the plate at 1,300-1,500 rpm for 30 seconds (e.g. Tecan - Te-Shake™).

   **Note:** Transfer may be accomplished by either piercing or removing the cover foil on the **Conversion Plate**. If using a **Collection Plate** as a stand for the **Conversion Plate** it may be necessary to secure the plates together using the tabs on the cover foil to prevent lifting of the **Conversion Plate**.

6. Let plate stand at room temperature for 5 minutes, then transfer plate to a magnetic stand for an additional 5 minutes or until beads pellet and supernatant is cleared. With the plate on the magnetic stand remove the supernatant and discard.

   **Note:** Some beads will adhere to the sides of the well. Remove supernatant slowly to allow these beads to be pulled to the magnet as the liquid level is lowered.

7. Remove the **Collection Plate** from the magnetic stand for this and each subsequent buffer addition. Add 400 µl of **M-Wash Buffer** to the beads. Re-suspend the beads by pipetting up and down or shaking the plate at 1,300-1,500 rpm for 30 seconds. Replace the plate on the magnetic stand for 3 minutes or until beads pellet. Remove and discard supernatant.

8. Add 200 µl of **M-Desulphonation Buffer** to the beads. Re-suspend the beads by pipetting up and down or shaking the plate for 30 seconds. Let plate stand at room temperature (20°C-30°C) for 15-20 minutes. After the incubation, replace the plate on the magnetic stand for 3 minutes or until beads pellet. Remove and discard supernatant.

   **Note**: Take time for handling/re-suspension into account for the total incubation time. Adjust time as necessary to ensure that no sample remains in the **M-Desulphonation Buffer** for more than 20-25 minutes.

9. Add 400 µl of **M-Wash Buffer** to the beads. Re-suspend the beads by pipetting up and down or shaking the plate for 30 seconds. Replace the plate on the magnetic stand for 3 minutes or until beads pellet. Remove and discard supernatant. Repeat this wash step.

   **Note:** Remove as much buffer as possible after final wash to aid in the drying of the beads.

10. Transfer the plate to a heating element at 55°C for 20-30 minutes to dry the beads and remove residual **M-Wash Buffer**.

    **Note:** Beads will change in appearance from glossy black when still wet to a dull brown when fully dry.

11. Add 25 µl of **M-Elution Buffer** directly to the dried beads and pipette or shake the plate for 30 seconds to re-suspend. Heat the elution at 55°C for 4 minutes then transfer the plate to the magnetic stand for 1 minute or until beads pellet. Remove the supernatant and transfer to a clean **Elution Plate**.

    **Note:** If beads are removed with the elution, slowly pipetting up and down one or two times will allow them to be pulled to the magnet.

    **Note**: Alternatively, water or TE (pH ≥ 6.0) can be used for elution if required for your experiments.

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 25 µl can be used if necessary. The elution volume can be > 25 µl depending on the requirements of your experiments, but small elution volumes will yield higher DNA concentrations.

## Automation Scripts:

Various automation scripts are available and can be obtained free of charge by contacting Zymo Research at tech@zymoresearch.com. Include "Automation Scripts" in the subject line and provide kit catalog number and the automation platform desired in the email.

# Appendix

## 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 x $10^3$-8 x $10^4$ 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 8. 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 µl whole blood per **Proteinase K** digestion (procedure **A** or **B**, page 8) However, the volume of the **Proteinase K** digestion can be adjusted when processing replicate samples or for convenient sample manipulation. For example, to increase the sample volume 5-fold for digestion procedure **A**: add 2.5 µl of blood to 50 µl **M-Digestion Buffer**, 42.5 µl $H_20$, and 5 µl of **Proteinase K**.

- ✓ **Solid Tissue (Fresh or Frozen):** Use up to 0.1 mg or 0.1 µl tissue per **Proteinase K** digestion (procedure **A** or **B**). However, the volume of the **Proteinase K** digestion can be adjusted when processing replicate 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 µl of tissue to 65 µl **M-Digestion Buffer**, 59.5 µl $H_20$, and 5 µl 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. Bisulfite Conversion of Double Stranded DNA Templates.

The following illustrates what occurs to a DNA template during bisulfite conversion.

Template:
**A:** 5′-GACCGTTCCAGGTCCAGCAGTGCGCT-3′
**B:** 3′-CTGGCAAGGTCCAGGTCGTCACGCGA-5′

Bisulfite Converted:
**A:** 5′-GATCGTTTTAGGTTTAGTAGTGCGTT-3′
**B:** 3′-TTGGCAAGGTTTAGGTTGTTATGCGA-5′

**Note**: Methylated "C" is underlined in the examples.

**Note**: Following bisulfite conversion, the strands are no longer complementary.

### 2. PCR Primer Design.

Generally, primers 26 to 32 bases are required for amplification of bisulfite converted DNA. In general, all Cs should be treated as Ts for primer design purposes, unless they are in a CpG context. See example below.

Bisulfite Converted: **A:** 5′-GATCGTTTTAGGTTTAGTAGTGCGTT-3′
Primers: Reverse: 3′-ATCATCACRCAA-5′ **R**= G/A
Forward: 5′-GATYGTTTTAGGT-3′ **Y**= C/T

Zymo Research provides primer design assistance with its Bisulfite Primer Seeker Program, available at: www.zymoresearch.com/tools/bisulfite-primer-seeker

**Note**: Only one strand (**A**) is amplified by a given primer set. Only the reverse primer binds to the converted DNA, the forward primer will bind the strand generated by the reverse primer. If the primer contains CpG dinucleotides with uncertain methylation status, then mixed bases with C and T (or G and A) can be used. Usually, there should be no more than one mixed position per primer, and it 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.

## 3. 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 100 pg. The optimal amount of DNA per bisulfite treatment is 200 to 500 ng. Although up to 2 µg of DNA can be processed, it should be noted that high input levels of DNA may result in incomplete bisulfite conversion for some GC-rich regions.

## 4. 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 by optimizing the PCR conditions. 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. 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.

**Note**: **Zymo*Taq*™** is a "hot start" DNA polymerase specifically designed for the amplification of bisulfite treated DNA.

## 5. Quantifying Bisulfite Treated DNA.

Following bisulfite treatment of genomic DNA, the original base-pairing no longer exists since non-methylated cytosine residues are converted into uracil. Recovered DNA is typically A, U, and T-rich and 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 $Ab_{260}$ = 1.0 when determining the concentration of the recovered bisulfite-treated DNA.

# 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™ DNA Polymerase).*

**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** | **D5022** | **D5023** |

# Ordering Information

| Product Description | Catalog No. | Size |
|---|---|---|
| **EZ DNA Methylation-Direct™ Kit** | D5020<br>D5021 | 50 rxns.<br>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. |
| **EZ-96 DNA Methylation-Direct™ MagPrep** | D5044<br>D5045 | 4 x 96 rxns.<br>8 x 96 rxns. |

| Individual Kit Components | Catalog No. | Amount |
|---|---|---|
| **CT Conversion Reagent** | D5001-1<br>D5003-1 | 1 tube<br>1 bottle |
| **M-Dilution Buffer** | D5005-2<br>D5006-2 | 1.5 ml<br>7 ml |
| **M-Binding Buffer** | D5005-3<br>D5006-3<br>D5040-3 | 30 ml<br>125 ml<br>250 ml |
| **M-Wash Buffer** | D5001-4<br>D5002-4<br>D5007-4<br>D5040-4 | 6 ml<br>24 ml<br>36 ml<br>72 ml |
| **M-Desulphonation Buffer** | D5001-5<br>D5002-5<br>D5040-5 | 10 ml<br>40 ml<br>80 ml |
| **M-Elution Buffer** | D5001-6<br>D5002-6<br>D5007-6<br>D5041-6 | 1 ml<br>4 ml<br>8 ml<br>40 ml |
| **M-Solubilization Buffer** | D5020-7<br>D5021-7 | 4.5 ml<br>18 ml |

| | | |
|---|---|---|
| **M-Reaction Buffer** | D5020-8<br>D5021-8 | 1 ml<br>4 ml |
| **M-Digestion Buffer** | D5020-9<br>D5021-9 | 4 ml<br>15 ml |
| **Proteinase K and Storage Buffer** | D3001-2-5<br>D3001-2-20 | 5 mg set<br>20 mg set |
| **Zymo-Spin™ IC Columns** (capped) | C1004-50<br>C1004-250 | 50 columns<br>250 columns |
| **Collection Tubes** | C1001-50<br>C1001-500<br>C1001-1000 | 50 tubes<br>500 tubes<br>1,000 tubes |
| **EZ-Methylation Magprep Beads** | D4100-5-8<br>D4100-5-16 | 8 ml<br>16 ml |
| **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 |

# Notes

# Notes

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

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. 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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