ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

EZ DNA Methylation-Direct™ Kit

Catalog Nos. **D5020 & D5021**

Highlights

- 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

* Patent pending; For research use only

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Product Contents:

EZ DNA Methylation-Direct™ Kit	D5020 50 rxns.	D5021 200 rxns.	Storage Temperature
Proteinase K and Storage Buffer*	5 mg set	20 mg set	−20°C(after mixing)
M-Digestion Buffer (2X)	4 ml	15 ml	Room Temp.
CT Conversion Reagent**	5 tubes	20 tubes	Room Temp.
M-Dilution Buffer	1.5 ml	7 ml	Room Temp.
M-Solubilization Buffer	4.5 ml	18 ml	Room Temp.
M-Reaction Buffer	1 ml	4 ml	Room Temp.
M-Binding Buffer	30 ml	125 ml	Room Temp.
M-Wash Buffer***	6 ml	24 ml	Room Temp.
M-Desulphonation Buffer	10 ml	40 ml	Room Temp.
M-Elution Buffer	1 ml	4 ml	Room Temp.
Zymo-Spin™ IC Columns	50 columns	200 columns	Room Temp.
Collection Tubes	50 tubes	200 tubes	Room Temp.
Instruction Manual	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.

* Add 260 µl (1040 µl for D5021) **Proteinase K Storage Buffer** to the **Proteinase K** tube prior to use. The final concentration of **Proteinase K** after the addition of **Proteinase K Storage Buffer** is 20 mg/ml.

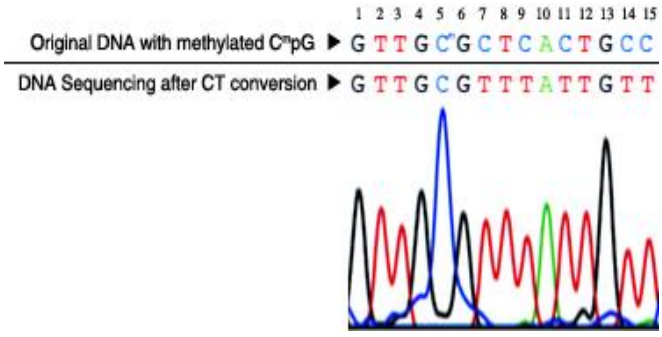
** 790 µl **M-Solubilization Buffer** and 300 µl **M-Dilution Buffer** are added per tube of **CT Conversion Reagent**, mixed, and then 160 µl **M-Reaction Buffer** is added prior to use.

*** Add 24 ml of 100% ethanol to the 6 ml **M-Wash Buffer** concentrate (D5020) or 96 ml of 100% ethanol to the 24 ml **M-Wash Buffer** concentrate (D5021) before use.

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 $C^{m}pG$ 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.

Note: 96-Well spin-plate formats are available for processing larger numbers of samples. Also, MagPrep kits are available (p. 10) 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.

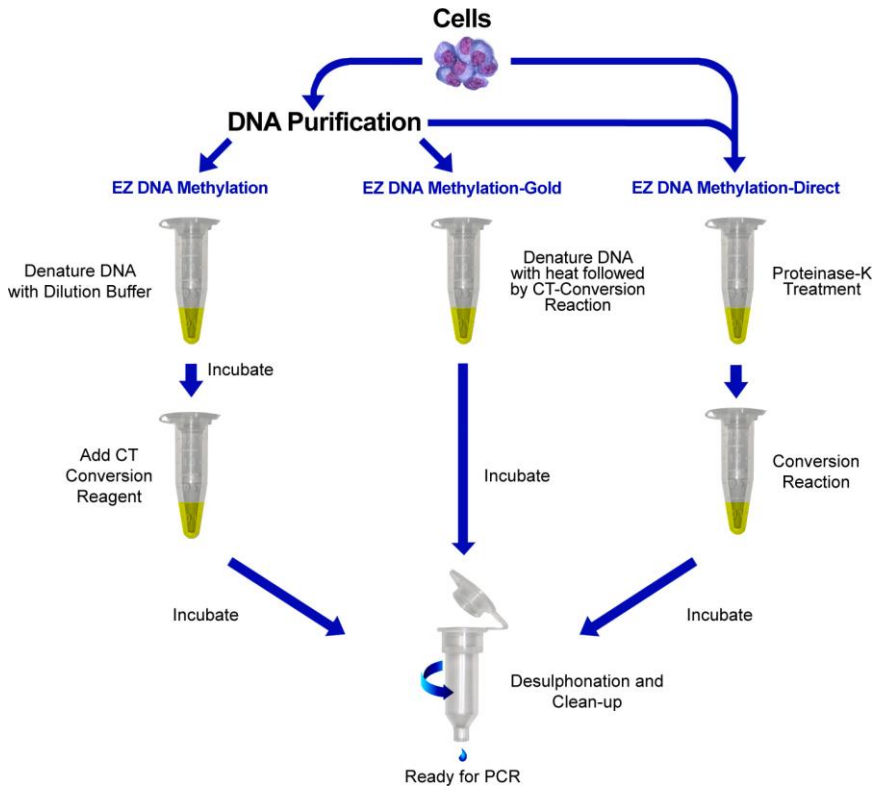
4. Oda M, *et al.* Genes & Dev. 2006; 20: 3382-3394.

5. England RPM, *et al.* Nature Meth. 2005; 2: 1-2.

Product Description:

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 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.

- **DNA Recovery:** > 80%

- **Sensitivity of Detection (Lower Limit):** 10 cells for successful PCR amplification.

Reagent Preparation:

- **Preparation of Proteinase K**

  Add 260 µl (D5020) or 1040 µl (D5021) of **Proteinase K Storage Buffer** to the tube containing **Proteinase K**. Dissolve completely and store at -20 °C.

- **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 790 µl of **M-Solubilization Buffer** and 300 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.
  3. Add 160 µl of **M-Reaction Buffer** and mix an additional 1 minute.

  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 (D5020) or 96 ml of 100% ethanol to the 24 ml **M-Wash Buffer** concentrate (D5021) 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 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 tube (e.g., PCR tube) using either 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. Incubate the sample(s) 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 of the reaction thoroughly then centrifuge for 5 minutes at 10,000 x *g.* Use 20 µl of the supernatant for bisulfite conversion as detailed in **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 20 µl of sample from Step 3 (**Section I**) to 130 µl of **CT Conversion Reagent** solution in a PCR tube. Mix the sample and then centrifuge briefly to ensure no droplets are in the cap or sides of the tube.

   Note: If purified DNA is used, add up to 20 µl of DNA to 130 µl of **CT Conversion Reagent** solution. If the volume of DNA is less than 20 µl, compensate with water.

2. Place the PCR tube(s) 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

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

3. Add 600 µl of **M-Binding Buffer** into a **Zymo-Spin™ IC Column** and place the column into a provided **Collection Tube**.

4. Load the sample (from Step 2) into the **Zymo-Spin™ IC Column** containing the **M-Binding Buffer**. Close the cap and mix by inverting the column several times.

5. Centrifuge at full speed (≥10,000 x *g*) for 30 seconds. Discard the flow-through.

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

7. 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.

8. 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.

9. 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.

If procedure **A** is used (p. 5), the CT-Conversion Reagent can be added directly to the samples in a PCR tube.

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 ≥ 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 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 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 µl 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 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 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 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.

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

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?

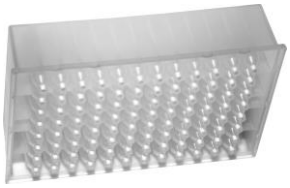
A: *We recommend a "hot start" DNA polymerase (e.g., ZymoTaq™, page 11).*

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	**D5022**	**D5023**

Ordering Information:

Product Description	Catalog No.	Kit Size
EZ DNA Methylation-Direct™ Kit	D5020 D5021	50 rxns. 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 D5045	4 x 96 rxns. 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)
CT Conversion Reagent	D5001-1 D5003-1	1 tube 1 bottle
M-Dilution Buffer	D5005-2 D5006-2	1.5 ml 7 ml
M-Binding Buffer	D5005-3 D5006-3 D5040-3	30 ml 125 ml 250 ml
M-Wash Buffer	D5001-4 D5002-4 D5007-4 D5040-4	6 ml 24 ml 36 ml 72 ml
M-Desulphonation Buffer	D5001-5 D5002-5 D5040-5	10 ml 40 ml 80 ml
M-Elution Buffer	D5001-6 D5002-6 D5007-6 D5041-6	1 ml 4 ml 8 ml 40 ml
M-Solubilization Buffer	D5020-7 D5021-7	4.5 ml 18 ml
M-Reaction Buffer	D5020-8 D5021-8	1 ml 4 ml
M-Digestion Buffer	D5020-9 D5021-9	4 ml 15 ml
Proteinase K and Storage Buffer	D3001-2-5 D3001-2-20	5 mg set 20 mg set
Zymo-Spin™ IC Columns (capped)	C1004-50 C1004-250	50 columns 250 columns
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 tubes 500 tubes 1,000 tubes
MagBinding Beads	D4100-2-6 D4100-2-8 D4100-2-12 D4100-2-16 D4100-2-24	6ml 8 ml 12 ml 16 ml 24 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

THE Epigenetics COMPANY™

Epigenetics Products From Zymo Research

Product	Description	Kit Size	Cat No. (Format)
Bisulfite Kits for DNA Methylation Detection			
EZ DNA Methylation™ Kit	For the conversion of unmethylated cytosines in DNA to uracil via the chemical-denaturation of DNA and a specially designed CT Conversion Reagent. *Fast-Spin* 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.	**D5001** (spin column) **D5002** (spin column) **D5003** (shallow-well plate) **D5004** (deep-well plate) **D5040** (magnetic bead) **D5041** (magnetic bead)
EZ DNA Methylation-Gold™ Kit	For the fast (3 hr.) conversion of unmethylated cytosines in DNA to uracil via heat/chemical-denaturation of DNA and a specially designed CT Conversion Reagent. *Fast-Spin* 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.	**D5005** (spin column) **D5006** (spin column) **D5007** (shallow-well plate) **D5008** (deep-well plate) **D5042** (magnetic bead) **D5043** (magnetic bead)
EZ DNA Methylation-Direct™ Kit	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.	**D5020** (spin column) **D5021** (spin column) **D5022** (shallow-well plate) **D5023** (deep-well plate) **D5044** (magnetic bead) **D5045** (magnetic bead)
EZ DNA Methylation-Lightning™ Kit	Complete bisulfite conversion in about an hour using a unique liquid format conversion reagent that requires no preparation. *Fast-Spin* 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.	**D5030** (spin column) **D5031** (spin column) **D5032** (shallow-well plate) **D5033** (deep-well plate) **D5046** (magnetic bead) **D5047** (magnetic bead)
EZ DNA Methylation-Startup™ Kit	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	**D5024**
Methylated DNA Standards			
Universal Methylated Human DNA Standard	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	**D5011**
Universal Methylated Mouse DNA Standard	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	**D5012**
Other…			
ChIP DNA Clean & Concentrator™	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.	**D5201** (uncapped column) **D5205** (capped column)
Genomic DNA Clean & Concentrator™	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.	**D4010** **D4011**
Zymo*Taq*™ DNA Polymerase	Zymo*Taq*™ "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.	**E2001** (system) **E2002** (system) **E2003** (premix) **E2004** (premix)
Methylated-DNA IP Kit	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.	**D5101**
Services			
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