



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

EZ-96 DNA Methylation™ MagPrep

Catalog Nos. **D5040 & D5041**

Highlights

- Proven procedure for *complete* bisulfite conversion of DNA.
- High throughput (96-well), automated desulphonation and recovery of bisulfite-treated DNA.
- Recovered DNA is ideal for downstream analyses including PCR, endonuclease digestion, sequencing, microarrays, etc.

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For Research Use Only

Ver. 1.0.4

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Product Contents:

EZ-96 DNA Methylation™ MagPrep	D5040	D5041	Storage Temperature
	4 x 96 rxns.	8 x 96 rxns.	
CT Conversion Reagent*	4 bottles	8 bottles	Room Temp.
M-Dilution Buffer	2 x 5.2 ml	4 x 5.2 ml	Room Temp.
M-Binding Buffer	250 ml	2 x 250 ml	Room Temp.
M-Wash Buffer**	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.
MagBinding Beads	8 ml	16 ml	Room Temp.
Conversion Plates w/ Pierceable Cover Film	4 plates/films	8 plates/films	Room Temp.
Collection Plates***	6 plates	10 plates	Room Temp.
Elution Plates	4 plates	8 plates	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.

* 7.5 ml water and 2.1 ml **M-Dilution Buffer** are added per bottle of **CT Conversion Reagent** and mixed prior to use.

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

***Two additional **Collection Plates** are provided as stands for the **Conversion Plates** during processing.

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. Freedom EVO® is a registered trademark and Te-Shake™ is a trademark of Tecan Group Ltd. Pyrosequencing® is a registered trademark of Biotage.

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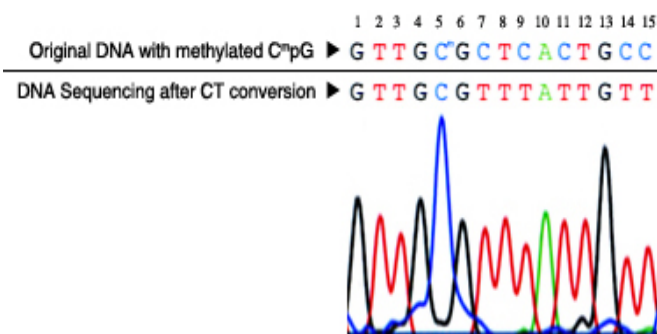
Introduction to DNA Methylation:

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).

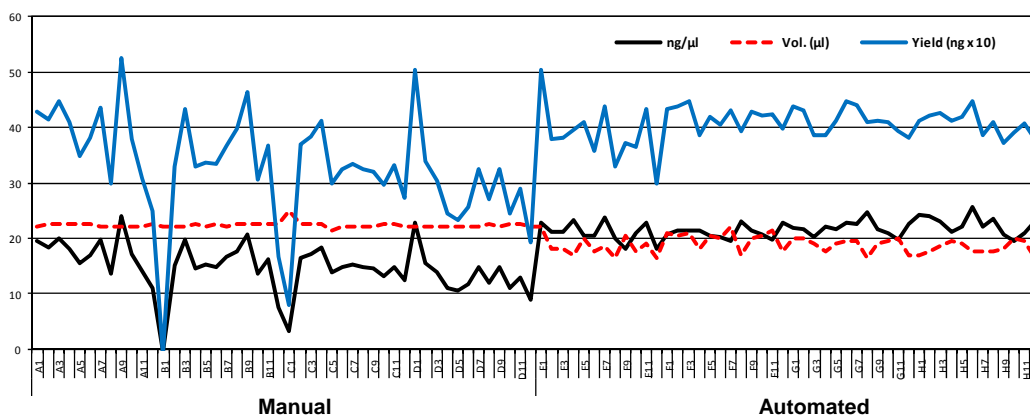
References:

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.

Note: Single spin-column and 96-Well spin-plate formats are available.

Product Description:

The **EZ-96 DNA Methylation™ MagPrep** features a high-throughput (96-well), 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. Desulphonation and clean-up of the converted DNA is performed while bound to the **MagBinding Beads**. 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.



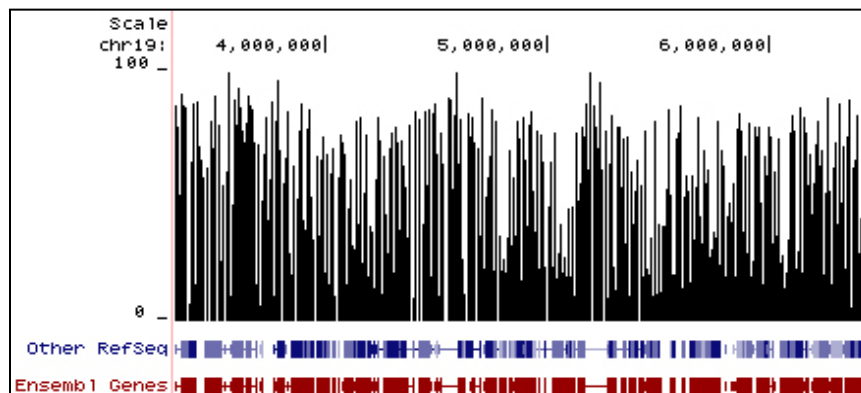
Manual

Automated

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.

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.

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Specifications:

- **DNA Input:** Samples containing between 500 pg to 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 cytosine residues are converted to uracil; > 99% protection of methylated cytosines.
- **Required Additional Equipment:** Magnetic Stand, Heating element for 96-well plate.

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

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:

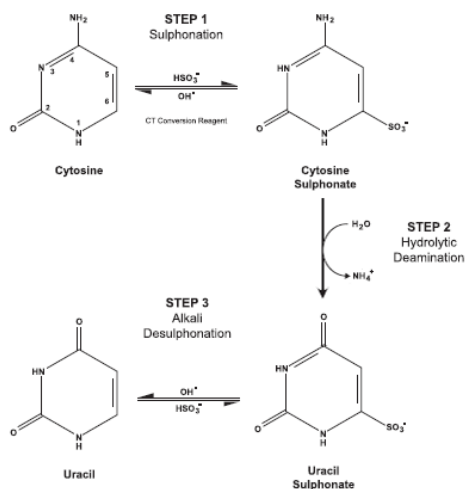
1. Add 7.5 ml water and 2.1 ml of **M-Dilution Buffer** to a bottle of **CT Conversion Reagent**.
2. Mix at room temperature with frequent vortexing or shaking for 10 minutes.

Note: It is normal to see trace amounts of undissolved reagent in the **CT Conversion Reagent**. Each 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.

Preparation of M-Wash Buffer

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



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.

Protocol:

1. Add 5 μ l of **M-Dilution Buffer** to each DNA sample in a **Conversion Plate** and adjust the total volume to 50 μ l with water. Mix each sample by pipetting up and down.

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

2. Incubate the **Conversion Plate** containing the samples at 37°C for 15 minutes.
3. After the above incubation, add 100 μ l of the prepared **CT Conversion Reagent** to each sample and mix.
4. Seal the plate with the provided film. Incubate the **Conversion Plate** in the dark at 50°C for 12-16 hours (e.g., using a thermal cycler).

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

5. Incubate the sample at 0-4°C (e.g., on ice or using a thermal cycler) for 10 minutes. Samples may be kept at 4°C for up to 20 hours.
6. 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 13.

7. Add 600 μ l of **M-Binding Buffer** and 10 μ l of **MagBinding Beads** to each well of a **Collection Plate**.

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

8. Transfer the samples from the **Conversion Plate** into the **Collection Plate** containing the **M-Binding Buffer** and **MagBinding Beads**. Mix by pipetting up and down 3-6 times and, if available, vortexing 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**.

9. 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.

10. Remove the **Collection Plate** from the magnetic stand for this and each subsequent buffer addition. Add 400 μ l of **M-Wash Buffer** to the beads. Re-suspend the beads by pipetting up and down or vortexing 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.

Protocol (continued):

11. Add 200 μ l of **M-Desulphonation Buffer** to the beads. Re-suspend the beads by pipetting up and down or vortexing for 30 seconds. Let plate stand at room temperature (20-30°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.

12. Add 400 μ l of **M-Wash Buffer** to the beads. Re-suspend the beads by pipetting up and down or vortexing 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.

13. 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.

14. Add 25 μ l of **M-Elution Buffer** directly to the dried beads and pipette or vortex 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.

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 μ l of eluted DNA for each PCR, however, up to 25 μ l can be used if necessary. The elution volume can be > 25 μ l 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.

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?

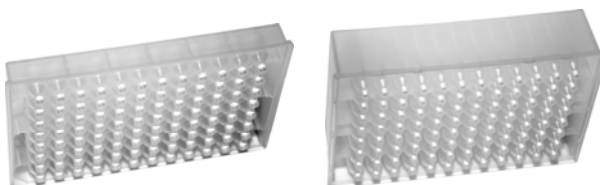
A: *Water, TE or modified TE buffers can be used to dissolve the DNA and do not interfere with the conversion process.*

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 10).*

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	D5003	D5004

Ordering Information:

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

For Individual Sale	Catalog No.	Amount(s)
CT Conversion Reagent	D5001-1	1 tube
	D5003-1	1 bottle
M-Dilution Buffer	D5001-2	1.3 ml
	D5002-2	5.2 ml
M-Binding Buffer	D5005-3	30 ml
	D5006-3	125 ml
	D5040-3	250 ml
M-Wash Buffer	D5001-4	6 ml
	D5002-4	24 ml
	D5007-4	36 ml
	D5040-4	72 ml
M-Desulphonation Buffer	D5001-5	10 ml
	D5002-5	40 ml
	D5040-5	80 ml
M-Elution Buffer	D5001-6	1 ml
	D5002-6	4 ml
	D5007-6	8 ml
	D5041-6	40 ml
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
MagBinding Beads	D4100-2-6	6ml
	D4100-2-8	8 ml
	D4100-2-12	12 ml
	D4100-2-16	16 ml
	D4100-2-24	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
ZR-96 MagStand	P1005	1 stand

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 <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.	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 <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.	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. <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.	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
ZymoTaq™ DNA Polymerase	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.	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			
Available for DNA Methylation and Hydroxymethylation at http://www.zymoresearch.com/services or inquire at services@zymoresearch.com ...powered by the latest Next-Gen Sequencing technologies!			

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