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The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Genomic DNA Clean & Concentrator™

Catalog Nos. **D4010 & D4011**

Highlights

- Quick (5 minute) spin column recovery of large-sized DNA (e.g., genomic, mitochondrial, plasmid (BAC/PAC), viral, phage, (wga)DNA, etc.) from any enzymatic reaction or impure preparation (e.g., Proteinase K digestion). *No messy precipitations!*
- Unique spin column for low volume (≥ 10 μ l) elution of ultra-pure, high-yield DNA.
- Eluted DNA is ideal for PCR, restriction endonuclease digestion, Sanger and Next-Gen sequencing, etc.

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

Product Contents

Genomic DNA Clean & Concentrator™ (Kit Size)	D4010 (25 Preps.)	D4011 (100 Preps.)	Storage Temperature
ChIP DNA Binding Buffer	50 ml	2 x 50 ml	Room Temp.
DNA Wash Buffer¹	6 ml	24 ml	Room Temp.
DNA Elution Buffer	1 ml	4 ml	Room Temp.
Zymo-Spin™ IC-XL Columns	25	100	Room Temp.
Collection Tubes	50	100	Room Temp.
Instruction Manual	1	1	-

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.

¹ Ethanol must be added prior to use as indicated on **DNA Wash Buffer** label.

Specifications

- **DNA Purity** – High-quality ($A_{(260/280)} \geq 1.8$) high molecular weight DNA ideal for ligation, sequencing, labeling, PCR, microarray, transfection, transformation, and restriction digestion procedures.
- **DNA Size Limits** – Capable of purifying small DNA fragments >50 bp and large sized DNAs >200 kb.
- **DNA Recovery** – Typically, up to 10 µg total DNA per column can be eluted into as ≥10 µl of low salt **DNA Elution Buffer** or water. Recovery of DNA ranges from 70-95%.
- **Sample Sources** – DNA from impure preparations of genomic DNA (e.g., Proteinase K digestions), plasmid DNA (including BAC), viral DNA, and *whole genome amplified* (wga) DNA. Can also be used for the purification of low molecular weight DNA (50 bp to 10 kb) from PCR, endonuclease digestion, post-RT cDNA synthesis, *etc.*
- **Product Detergent Tolerance** – ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 1% SDS.

Note: ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended 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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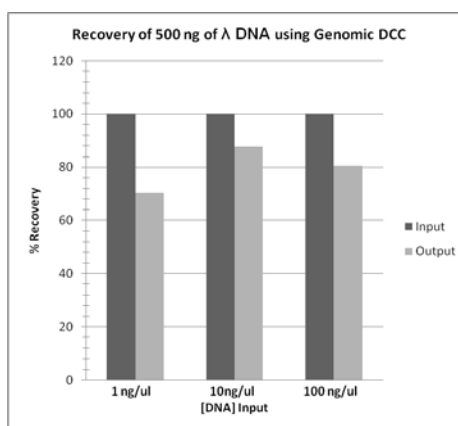
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Product Description

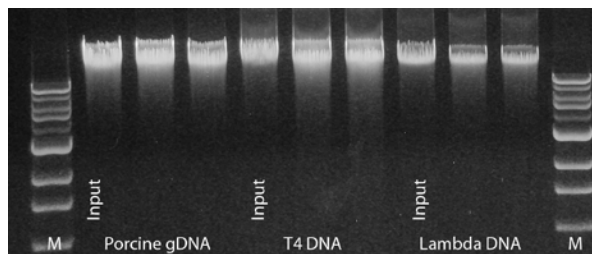
The **Genomic DNA Clean & Concentrator™ (DCC™)** is for the quick (5 minute) recovery of ultra-pure, large-sized DNA (e.g., genomic, mitochondrial, plasmid (BAC/PAC), viral, phage, (wga)DNA, etc.) from any enzymatic reaction or impure preparation (e.g., Proteinase K digestion). There is no need for organic denaturants, chloroform, or messy precipitations: simply add the specially formulated **ChIP DNA Binding Buffer** to a sample and then transfer the mixture to the supplied **Zymo-Spin™ Column**. Eluted DNA is suitable for sequencing, PCR, endonuclease digestion, and other enzymatic procedures. The product is also compatible with smaller DNAs (50 bp to 10 kb) from PCR, digestions, crude plasmid preparations, cDNA synthesis, etc.



Five minute **Genomic DCC™** procedure.









Lambda phage DNA (48.5 kb) is effectively recovered from various concentrations of starting material using the **Genomic DCC™**.



High molecular weight DNA is efficiently purified using the Genomic DCC™. Porcine gDNA (~35-50 kb), T4 phage DNA (170 kb), and lambda phage DNA (48.5 kb) were purified (in duplicate) from input material using the Genomic DCC™. Eluted DNAs were analyzed in a 0.8% (w/v) TAE/agarose/EtBr gel (shown above). The size marker "M" is a 1 kb ladder (Zymo Research).

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Available Formats

	DCC™-5	DCC™-25	DCC™-100	DCC™-500	Genomic DCC™	ZR-96 DCC™-5
						
Name	Zymo-Spin™ I & IC	Zymo-Spin™ II & IIC	Zymo-Spin™ V	Zymo-Spin™ VI	Zymo-Spin™ IC-XL	Zymo-Spin™ I-96
Capacity	5 µg/ prep.	25 µg/ prep.	100 µg/ prep.	500 µg/ prep.	10 µg/ prep.	5 µg/ prep.
Elution Vol.	≥ 6 µl	≥ 25 µl	≥ 150 µl	≥ 2 ml	≥ 10 µl	≥ 10 µl
Cat. Nos.	D4003, D4013	D4005, D4033	D4029, D4030	D4031, D4032	D4010, D4011	D4023, D4024

Typical DCC™ Applications

Post-PCR DNA Clean-up	Efficient desalting of DNA with the removal of DNA polymerases, primers and free dNTPs.
DNA Clean-up From Enzymatic Reactions	Efficient desalting of DNA with the removal of modifying enzymes, RNA polymerases, ligases, kinases, nucleases, phosphatases, endonucleases, <i>etc.</i>
Post-Reverse Transcription (RT) & cDNA Clean-up	Efficiently purifies DNA following RT, either as a DNA/RNA complex or as single stranded cDNA following chemical hydrolysis of the RNA template.
Plasmid DNA Clean-up	Efficiently purifies plasmid DNA from “home-made” preparations of cell free lysates or from commercial kits. Plasmid DNA purified and concentrated using the DCC™ has proven an excellent substrate for high quality DNA sequencing.
Isotope and Dye Removal	Efficiently removes unincorporated fluorescent (<i>i.e.</i> , AMCA, FITC, BIO, DIG, Cy3, Cy5, FAM, <i>etc.</i>) and radiolabeled dNTP derivatives from DNA following <i>in vitro</i> labeling reactions.
Purification of M13 ssDNA	The DCC™ can be used for the rapid isolation of single stranded M13 phage DNA directly from phage-infected <i>E. coli</i> culture supernatant.

- ✓ For purification of short DNA or RNA oligonucleotides ≥16 nt, use the **Oligo Clean & Concentrator (D4060, D4061)**.
- ✓ For ChIP (Chromatin Immunoprecipitation) sample cleanup, use **the ChIP DNA Clean & Concentrator (D5201, D5205)** for high quality DNA from any step in a standard ChIP protocol.
- ✓ For post-cycle sequencing samples, use the **ZR Sequencing DNA Clean-up Kit (D4050, D4051)** for dye blob elimination.
- ✓ For samples containing PCR inhibitors, use the **OneStep™ PCR Inhibitor Removal Kit (D6030, D6035)**.

Selected Citations

- Marandel, L. et al. (2012). Evolutionary history of *c-myc* in teleosts and characterization of the duplicated *c-myca* genes in goldfish embryos. *Molecular Reproduction and Development*, 79: 85–96.
- Depledge, D.P. et al. (2011). Specific capture and whole-genome sequencing of viruses from clinical samples. *PLoS ONE* 6(11): e27805.
- Furst, R.W. et al. (2012). Is DNA methylation an epigenetic contribution to transcriptional regulation of the bovine endometrium during the estrous cycle and early pregnancy? *Molecular and Cell Endocrinology*, 348 (1), 67-77.

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Buffer Preparation

- ✓ ***Before starting:*** Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **DNA Wash Buffer** concentrate. Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA Wash Buffer** concentrate.

Protocol

Note: All centrifugation steps should be performed between 10,000 - 16,000 x g.

1. In a 1.5 ml microcentrifuge tube, add 2-5 volumes of **ChIP DNA Binding Buffer** to each volume of DNA sample¹ (see table below). Mix thoroughly.

Application	DNA Binding Buffer : Sample	Example
Plasmid, genomic DNA (>2 kb)	2 : 1	200 µl : 100 µl
PCR product, DNA fragment	5 : 1	500 µl : 100 µl

2. Transfer mixture to a provided **Zymo-Spin™ IC-XL Column²** in a **Collection Tube**.
3. Centrifuge for 30 seconds. Discard the flow-through.
4. Add 200 µl **DNA Wash Buffer** to the column. Centrifuge for 1 minute. Repeat the wash step.
5. Add ≥ 10 µl **DNA Elution Buffer³** or water⁴ directly to the column matrix and incubate at room temperature for one minute. Transfer the column to a 1.5 ml microcentrifuge tube and centrifuge at for 30 seconds to elute the DNA.

Ultra-pure DNA is now ready for use.

For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

Notes:

¹ It may be necessary to add RNase A to cell lysates prior to performing the procedure to ensure RNA-free DNA will be recovered in Step 5.

² The sample capacity of the column is 1 ml. It may be necessary to load and spin a column multiple times if a sample has a volume larger than 1 ml.

³ **DNA Elution Buffer:** 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA

⁴ Elution of DNA from the column is dependent on pH and temperature. If water is used, make sure the pH is >6.0. The total yield may be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**.

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Troubleshooting

Low Recovery

- **Improperly Prepared/Stored DNA Wash Buffer**

Make sure ethanol has been added to the **DNA Wash Buffer** concentrate. Cap the bottle tightly to prevent evaporation over time.

- **Addition of DNA Elution Buffer**

Add elution buffer directly to the column matrix and not to the walls of the column. Elution buffer requires contact with the matrix for at least 1 minute for large DNA ≥ 10 kb.

- **Incomplete Elution**

1. DNA elution is dependent on pH, temperature, and time. For large genomic DNA (≥ 50 kb), apply heated elution buffer (60-70 °C) and incubate for several minutes prior to elution.
2. Sequential elutions may be performed for quantitatively higher recovery but lower final DNA concentration. This is recommended for DNA ≥ 10 kb.

Low A_{260}/A_{230} Ratios

- **Column Tip Contaminated**

When removing the column from the collection tube, be careful that the tip of the column does not come into contact with the flowthrough. Trace amounts of salt from the flowthrough can contaminate a sample resulting in low A_{260}/A_{230} ratios. Ethanol contamination from the flowthrough can also interfere with DNA elution. Zymo-Spin™ columns are designed for complete elution with no buffer retention or carryover.

Following Clean-up with the DCC™, Multiple Bands Appear in an Agarose Gel

- **Acidification of DNA Loading Dye**

Most loading dyes do not contain EDTA and will acidify ($\text{pH} \leq 4$) over time due to some microbial growth. This low pH is enough to cause DNA degradation. Therefore, if water is used to elute the DNA, 6X Loading Dye containing 1 mM EDTA is recommended.

Ordering Information

Product Description	Catalog No.	Kit Size (Preps.)
DNA Clean & Concentrator™-5 (for purification of up to 5 µg DNA per prep.) <i>Supplied with uncapped columns</i>	D4003 D4004	50 200
DNA Clean & Concentrator™-5 (for purification of up to 5 µg DNA per prep.) <i>Supplied with capped columns</i>	D4013 D4014	50 200
ZR-96 DNA Clean & Concentrator™-5 (for 96-well purification of up to 5 µg DNA per well)	D4023 D4024	2 x 96 4 x 96
DNA Clean & Concentrator™-25 (for purification of up to 25 µg DNA per prep.) <i>Supplied with uncapped columns</i>	D4005 D4006	50 200
DNA Clean & Concentrator™-25 (for purification of up to 25 µg DNA per prep.) <i>Supplied with capped columns</i>	D4033 D4034	50 200
DNA Clean & Concentrator™-100 (for purification of up to 100 µg DNA per prep.)	D4029 D4030	25 50
DNA Clean & Concentrator™-500 (for purification of up to 500 µg DNA per prep.)	D4031 D4032	10 20
Oligo Clean & Concentrator™ (for purification of up to 5 µg of oligonucleotides per prep.)	D4060 D4061	50 200
Genomic DNA Clean & Concentrator™ (for purification of up to 10 µg genomic DNA per prep.)	D4010 D4011	25 100

Refer to Page 3 for column design specifics in each kit.

For Individual Sale	Catalog No.	Size
DNA Binding Buffer	D4003-1-L	50 ml
	D4004-1-L	100 ml
DNA Wash Buffer (concentrate)	D4003-2-6	6 ml
	D4003-2-24	24 ml
DNA Elution Buffer	D3004-4-1	1 ml
	D3004-4-4	4 ml
Collection Tubes	C1001-50	50 tubes
	C1001-500	500 tubes
	C1001-1000	1000 tubes

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Popular Products From Zymo Research

Product	Description	Kit Size (Preps.)	Catalog No. (Format)
DNA Clean-up, Concentration & Recovery			
DNA Clean & Concentrator™-5	Clean and concentrate up to 5 µg DNA into ≥6 µl elution volume in as little as 2 minutes with no wash residue carryover.	50 200 50 200	D4003 (uncapped) D4004 (uncapped) D4013 (capped) D4014 (capped)
DNA Clean & Concentrator™-25	Clean and concentrate 25 µg of DNA into ≥25 µl elution volume in as little as 2 minutes with no wash residue carryover.	50 200 50 200	D4005 (uncapped) D4006 (uncapped) D4033 (capped) D4034 (capped)
ZR-96 DNA Clean & Concentrator™-5	Quick (30 minute), high throughput recovery of up to 5 µg pure DNA into 10-15 µl minimum elution volume allows for highly concentrated DNA.	2 x 96 4 x 96	D4023 D4024
Genomic DNA Clean & Concentrator™	Quick (5 minute) clean-up of up to 10 µg high molecular weight DNA (≥ 20kb - 200 kb) from any enzymatic reaction or impure preparation without precipitations.	25 100	D4010 D4011
Zymoclean™ Gel DNA Recovery Kit	Purify DNA from high and low-melting agarose gels in minutes.	50 200 50 200	D4001 (uncapped) D4002 (uncapped) D4007 (capped) D4008 (capped)
ZR-96 Zymoclean™ Gel DNA Recovery Kit	High-throughput DNA purification from high and low-melting agarose gels.	2 x 96 4 x 96	D4021 D4022
Zymoclean™ Large Fragment DNA Recovery Kit	Purify high molecular weight DNA (≥ 20 kb - 200 kb) from high and low-melting agarose gels in minutes.	25 100	D4045 D4046
OneStep™ PCR Inhibitor Removal Kit	Fast, one step procedure for removal of PCR inhibitors such as polyphenolics, humic/fulvic acids, melanin, etc. for successful PCR and other downstream applications.	50 2 x 96	D6030 D6035

Plasmid DNA Purification			
Zyppy™ Plasmid Miniprep Kit	Pellet-Free™ plasmid DNA purification in less than 10 minutes. Recover up to 25 µg DNA in as low as 30 µl.	50 100 400	D4036 D4019 D4020
Zyppy™-96 Plasmid Miniprep	The fastest and simplest high-throughput method for plasmid purification. Magnetic bead format available for automated liquid handling platforms.	2 x 96 4 x 96 8 x 96 2 x 96 4 x 96 8 x 96	D4041 (spin plate) D4042 (spin plate) D4043 (spin plate) D4100 (magnetic bead) D4101 (magnetic bead) D4102 (magnetic bead)
Zyppy™ Plasmid Midiprep Kit	Pellet-Free™ plasmid DNA purification in 15 minutes in a 150 µl minimum elution volume.	25 50	D4025 D4026
ZR Plasmid MiniPrep™-Classic	Plasmid DNA purification in minutes: (alkaline lysis/spin column format for low 30 µl elution volume).	100 400 800	D4015 D4016 D4054

Genomic DNA Purification			
Quick-gDNA™ MiniPrep	Easy purification from whole blood, plasma, serum, body fluids, buffy coat, tissue, swabs or cultured cells ≥15 minutes <u>without</u> the use of Proteinase K or organic denaturants.	50/200 50/200	D3006/D3007 (uncapped) D3024/D3025 (capped)
ZR Genomic DNA™-Tissue MiniPrep	High quality DNA purification from <u>solid tissues</u> (e.g., tail snips, ear punches, adipose tissue, etc.), body fluids, cultured cells, buccal cells, FFPE tissues, hair, and other biological sources using Proteinase K and Fast.	50/200 50/200	D3050 D3051
Environmental DNA Purification Kits	Unique BashingBead™ technology allows isolation of DNA from samples refractory to conventional lysis procedures including tough-to-lyse tissues, soil samples, feces, plants, seeds, insects, bacteria, yeast, filamentous fungi, unicellular and filamentous algae, and protozoa	Spin Column & 96-well Plate	Visit website for a comprehensive list

RNA Purification			
RNA Clean & Concentrator™-5	Clean and concentrate up to 5 µg RNA into ≥6 µl elution volume in as little as 5 minutes with no wash residue carryover.	50 200	R1015 R1016
Direct-Zol™ RNA MiniPrep	Quick, spin column purification of high-quality (DNA-free) total RNA directly from TRI-Reagent® or similar acid-guanidinium-phenol based reagents (TRIzol®, RNAzol®, QIAzol®, TriPure, RNA-Bee etc.).	50 200	R2051 R2053
ZR RNA MiniPrep	Quick (15 minute) RNA isolation from a variety of sources using <i>Fast-Spin</i> column technology without the use of organic denaturants..	50 200	R1064 R1065

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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 automated liquid handling platforms.	50/200 Rxns. 2 x 96 Rxns. 2 x 96 Rxns. 4 x 96 Rxns.	D5001/D5002 (column) D5003 (shallow-well plate) D5004 (deep-well plate) D5040 (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 automated liquid handling platforms.	50/200 Rxns. 2 x 96 Rxns. 2 x 96 Rxns. 4 x 96 Rxns.	D5005/D5006 (column) D5007 (shallow-well plate) D5008 (deep-well plate) D5042 (magnetic bead)
EZ DNA Methylation-Direct™ Kit	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.	50/200 Rxns. 2 x 96 Rxns. 2 x 96 Rxns. 4 x 96 Rxns.	D5020/D5021 (column) D5022 (shallow-well plate) D5023 (deep-well plate) D5044 (magnetic bead)
EZ DNA Methylation-Lightning™ Kit	Complete bisulfite conversion in about an hour using a unique liquid format conversion reagent. <i>Fast-Spin</i> technology ensures ultra-pure, converted DNA for subsequent DNA methylation analysis. Magnetic bead format for automated liquid handling platforms.	50/200 Rxns. 2 x 96 Rxns. 2 x 96 Rxns. 4 x 96 Rxns.	D5030/D5031 (column) D5032 (shallow-well plate) D5033 (deep-well plate) D5046 (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
Region-Specific DNA Methylation Screening			
OneStep qMethyl™ Kit	Single step real-time PCR procedure for bisulfite-free determination of DNA methylation status. Available without fluorescent dye for probe-based detection (Lite).	1 x 96 Rxns. 1 x 96 Rxns.	D5310 D5311 (Lite)
OneStep qMethyl™ Array	Premade 96-well assay for bisulfite-free determination of region-specific DNA methylation assessment in the promoter region of any one of the following prominent tumor suppressor genes: RASSF1, RARB, CDKN2A (p16), MGMT, or CCND2.	1 x 96 Rxns.	D5312
Epigenetics Services			
For more information, visit http://www.zymoresearch.com/services or inquire at services@zymoresearch.com .			
Services for Methylated DNA Analysis			
Simplify biomarker discovery with our 5-mC Analysis platforms that combine Zymo's well-established bisulfite technologies with next-generation sequencing for the most comprehensive DNA methylation analysis services available.			
Services for Hydroxymethylated DNA Analysis			
Novel genome-wide 5-hmC analysis platform featuring cutting-edge 5-hmC DNA enrichment, library prep, and next-generation sequencing technologies to ensure the sensitivity of 5-hmC detection in genome-wide context.			
Hydroxymethylation Detection			
Quest 5-hmC™ DNA Enrichment Kit	Featuring J-base binding protein (JBP) for the specific enrichment of 5-hmC containing DNA, the consolidated workflow makes the procedure reliable for robust analysis of multiple samples.	25 Rxns. 50 Rxns.	D5420 D5421
Quest 5-hmC™ DNA ELISA Kit	Streamlined workflow for both the direct and relative quantitation of 5-hmC, in a global genomic context, with a robust colorimetric readout.	1 x 96 Rxns. 2 x 96 Rxns.	D5425 D5426
Anti-5-Hydroxymethylcytosine Polyclonal Antibody	Polyclonal antibody has been engineered to maximize sensitivity to low amounts of hydroxymethylated gDNA while minimizing crossreactivity with unmodified or methylated cytosine residues. The antibody is suitable for use in ELISA, IP, and immunohistochemical labeling.	50 µg 200 µg	A4001-50 A4001-200
DNA Degradase™ DNA Degradase Plus™	Whole genomic DNA can be treated with these enzyme cocktails for processing to individual nucleotides (Degradase™) or nucleosides (Degradase Plus™) for interrogation in chromatographic and spectroscopic methods including TLC, LC/MS, MALDI-TOF, and more.	500 U 2000 U 250 U 1000 U	E2016 E2017 E2020 E2021
Other...			
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.	50 Rxns. 200 Rxns	E2001/E2001 (system) E2003/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

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