SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

ZYMO RESEARCH

Epigenetics Made Simple™

EZ DNA Methylation-Lightning™ Kit

The fastest bisulfite conversion

Highlights

- Fastest method for complete bisulfite conversion of DNA for methylation analysis.
- Ready-to-use conversion reagent is added directly to DNA.
- High-yield, converted DNA is ideal for PCR, MSP, array, bisulfite and Next-Gen sequencing.

Catalog Numbers:
D5030T, D5030, D5031

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

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

Table of Contents

Product Contents

EZ DNA Methylation-Lightning™ Kit	D5030T (10 Rxns.)	D5030 (50 Rxns.)	D5031 (200 Rxns.)	Storage Temperature
Lightning Conversion Reagent*	1 Tube	5 Tubes	20 Tubes	Room Temp.
M-Binding Buffer	7 ml	30 ml	125 ml	Room Temp.
M-Wash Buffer**	6 ml	6 ml	24 ml	Room Temp.
L-Desulphonation Buffer	2 ml	10 ml	40 ml	Room Temp.
M-Elution Buffer	500 µl	1 ml	4 ml	Room Temp.
Zymo-Spin™ IC Columns	10	50	200	Room Temp.
Collection Tubes	10	50	200	Room Temp.
Instruction Manual	1	1	1	-

* The **Lightning Conversion Reagent** is in a ready-to-use liquid format. The reagent should be stored tightly capped at room temperature with minimum exposure to light.
** Add 24 ml of 100% ethanol to the 6 ml **M-Wash Buffer** concentrate (D5030) or 96 ml of 100% ethanol to the 24 ml **M-Wash Buffer** concentrate (D5031) before use. **M-DNA Wash Buffer** included with D5030T is supplied ready-to-use and does not require the addition of ethanol prior to use.

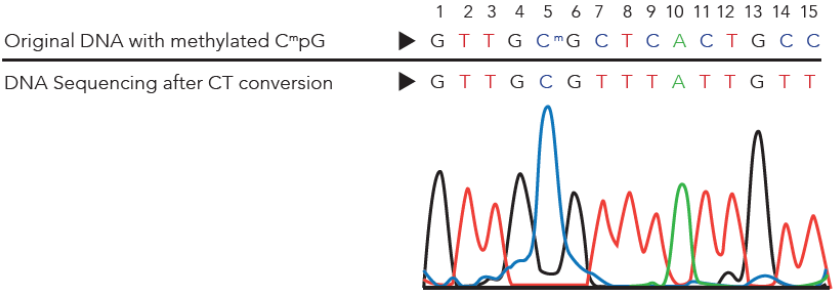
Introduction to DNA Methylation

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 patterns 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:

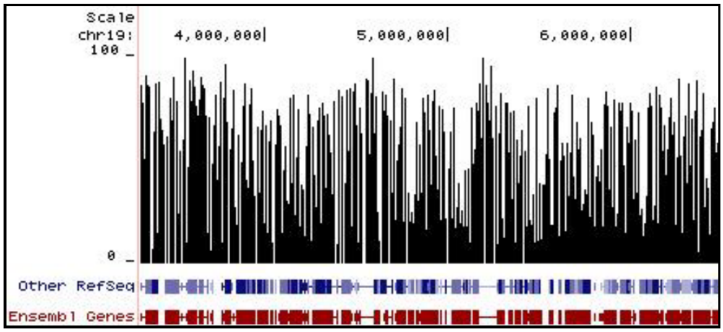
1. Adams RL. Bioessays. 1995; 17(2): 139-145.
2. Costello JF, Plass CJ. Med. Genet. 2001; 38(5): 285-303.
3. Stirzaker C. Cancer Res. 1997; 57(11): 2229-2237.
4. Fraga MF, et al. Electrophoresis. 2000; 21(14): 2990-2994.
5. Gonzalgo ML. Cancer Res. 1997; 57(4): 594-599.
6. Frommer M. Proc. Natl. Acad.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

Specifications

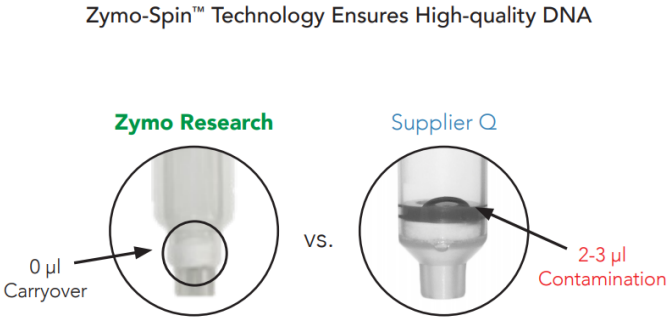
- **DNA Input –** Samples containing between 100 pg to 2 µg of DNA. For optimal results, the amount of input DNA should be from 200 to 500 ng.
- **Conversion Efficiency –** > 99.5% of non-methylated C residues are converted to U; > 99.5% protection of methylated cytosines.
- **DNA Recovery –** >80%

Product Description

The **EZ DNA Methylation-Lightning™ Kit** features rapid and reliable bisulfite treatment and conversion of DNA for methylation analysis. Key to the fast workflow is the ready-to-use **Lightning Conversion Reagent**. No preparation is necessary, simply add this unique reagent to a DNA sample, wait about an hour, and let the reaction proceed to completion. DNA denaturation and bisulfite conversion processes are combined with added heat to facilitate rapid denaturation. Desulphonation and clean-up of the converted DNA is performed using a unique low-elution spin column. High yield, converted DNA is ideal for PCR, array, bisulfite and next generation sequencing, etc.

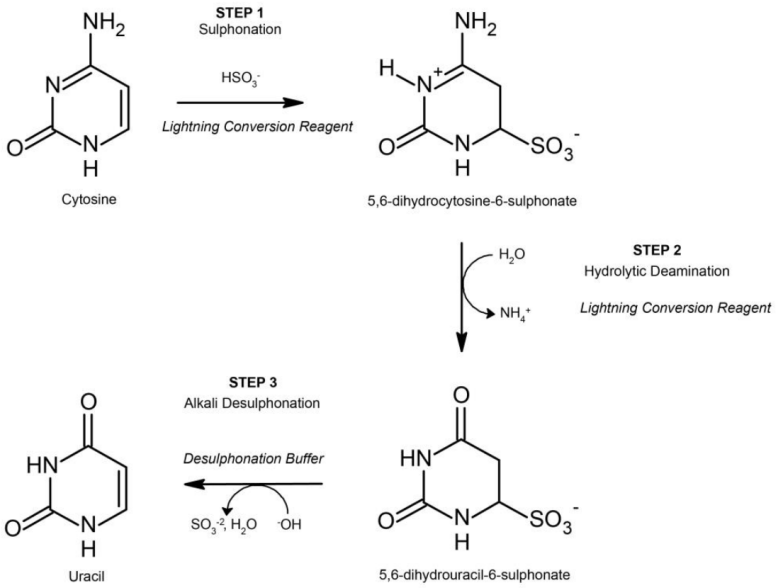


Methylation Plot From Reduced Representation Bisulfite Sequencing (RRBS). Data shows the relative percentage of methylation at individual CpG sites in mouse DNA. Methylation percentage is shown across a ~3 Mb region of mouse chromosome 19. Bisulfite sequencing libraries were prepared using mouse genomic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



Zymo-Spin™ IC Design Characteristics. The image above shows the unique design of the Zymo column that facilitates extremely small elution volumes (≥10 µl) without buffer carryover. This is unlike other columns that can retain liquid (binding/wash buffer residue) leading to carryover into the eluate.

96-Well spin-plate formats are available for processing larger numbers of samples. Also, MagPrep kits are available (p. 12) for adaptation to liquid handling robots (*e.g.*, Tecan – Freedom EVO®) and automated sample prep.



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.

Protocol

Buffer Preparation

- ✓ Add 24 ml of 100% ethanol to the 6 ml **M-Wash Buffer** concentrate (D5030) or 96 ml of 100% ethanol to the 24 ml **M-Wash Buffer** concentrate (D5031) before use.

- ✓ **M-Wash Buffer** included with D5030S & D5030T is supplied ready-to-use and does not require the addition of ethanol prior to use.

Sample Processing

1. Add 130 µl of **Lightning Conversion Reagent** to 20 µl of a DNA sample in a PCR tube. Mix, then centrifuge briefly to ensure there are no droplets in the cap or sides of the tube.

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

   Note: Samples >20 µl must be processed using multiple conversion reactions. Replicate reactions can be cleaned using the same column for each by repeating steps 3-5.

2. Place the PCR tube in a thermal cycler and perform the following steps:

   1. 98°C for 8 minutes
   2. 54°C for 60 minutes
   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** to a **Zymo-Spin™ IC Column** and place the column into a provided **Collection Tube**.

   Note: 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.

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 **L-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. Repeat this wash step.

9. Place the column into a 1.5 ml microcentrifuge tube and add 10 µl of **M-Elution Buffer** directly to the column matrix. Centrifuge for 30 seconds at full speed to elute the DNA.

   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 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 higher DNA concentrations.

Appendix

Appendix I: 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**

   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.

   Zymo Research provides primer design assistance with its Bisulfite Primer Seeker Program, available at:

   www.zymoresearch.com/tools/bisulfite-primer-seeker

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?

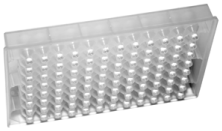
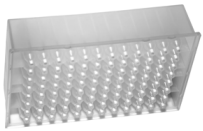
A: *Water, TE or modified TE buffers can be used to dissolve the DNA and do not interfere with the conversion process.*

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- Lightning™ 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	**D5032**	**D5033**

Ordering Information

Product Description	Catalog No.	Size
EZ DNA Methylation-Lightning™ Kit	D5030T D5030 D5031	10 Rxns. 50 Rxns. 200 Rxns.
EZ-96 DNA Methylation-Lightning™ Kit (Shallow-Well)	D5032	2 x 96 Rxns.
EZ-96 DNA Methylation-Lightning™ Kit (Deep-Well)	D5033	2 x 96 Rxns.
EZ-96 DNA Methylation-Lightning™ MagPrep*	D5046 D5047	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.

Individual Kit Components	Catalog No.	Amount
Lightning Conversion Reagent	D5030-1 D5032-1	1 Tube 1 Bottle
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
L-Desulphonation Buffer	D5030-5 D5031-5 D5046-5	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
Zymo-Spin™ IC Columns (capped)	C1004-50 C1004-250	50 Pack 250 Pack
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 Pack 500 Pack 1000 Pack
MagBinding Beads	D4100-5-3 D4100-5-8 D4100-5-16	6 ml 8 ml 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

100% satisfaction guarantee on all Zymo Research products, or your money back.

Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

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.

EZ DNA Methylation-Lightning™ Kit technologies are patent pending.

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.

™ Trademarks of Zymo Research Corporation
Freedom EVO® is a registered trademark of Tecan Group Ltd. Pyrosequencing® is a registered trademark of Biotage.

ZYMO RESEARCH

The **BEAUTY** *of* **SCIENCE** *is to Make Things* **SIMPLE**®

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682