ZYMO RESEARCH

Epigenetics
Made Simple™

EZ DNA Methylation-Lightning™ Kit

The fastest method for complete bisulfite conversion of DNA for methylation analysis

Highlights

- 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
D5030-E, D5031-E

CE IVD

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Product Contents

EZ DNA Methylation Lightning™ Kit	D5030-E (50 prep)	D5031-E (200 prep)
Lightning Conversion Reagent[1]	5 tubes	20 tubes
M-Binding Buffer	30 ml	125 ml
M-Wash Buffer[2]	6 ml	24 ml
L-Desulphonation Buffer	10 ml	40 ml
M-Elution Buffer	1 ml	4 ml
Zymo-Spin™ IC Columns	50	200
Collection Tubes	50	200
Instruction Manual	1 pc	1 pc

Specification

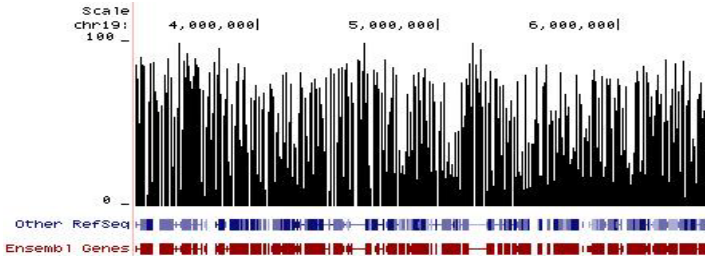
- **DNA Input –** Samples containing between 100 pg to 2 µg of DNA. For optimal results, the amount of input DNA should be from 200 to 500 ng.
- **Conversion Efficiency –** > 99.5% of non-methylated C residues are converted to U; > 99.5% protection of methylated cytosines.
- **Required Additional Equipment, but not included –** 95-100% ethanol
- **Storage Temperature and Stability**
  - ✓ Store all components (i.e., buffers/reagents, columns) at room temperature (15-30°C).
  - ✓ Expiration dates for each of the unopened components are indicated on the individual component labels. These storage conditions apply to both opened and unopened components.
  - ✓ Eluted DNA/RNA can be used immediately or stored frozen (-20/-80°C).

Storage Temperature - Store all kit components (*i.e.,* buffers, columns) at room temperature (15-30°C).

[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.
[2]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.

Product Description

The **EZ DNA Methylation-Lightning™ Kit** features rapid and reliable bisulfite treatment and conversion of DNA for methylation analysis[1]. 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 Clean & Concentrator™** (D4010, D4011 – Zymo Research) and bisulfite converted using **EZ DNA Methylation™** technology prior to Next-Gen sequencing.

Zymo-Spin™ Columns Ensure *No Buffer Retention*

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.

Zymo-Spin™ Column
Q Spin Column

[1]96-Well spin-plate formats are available for processing larger numbers of samples. Also, MagPrep kits are available for adaptation to liquid handling robots (e.g., Tecan – Freedom EVO®) and automated sample prep.

General Laboratory Warnings/Precautions

This assay is for *in vitro* diagnostic use. Bisulfite conversion kits are designed for procedures of molecular diagnostic and can only be handled by personal trained in molecular biology methods.

- ✓ This product is intended for professional use only. DO NOT use if the product is visibly damaged.
- ✓ Wear gloves when handling specimens or reagents.
- ✓ Do not pipette by mouth.
- ✓ Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- ✓ Clean and disinfect spills of specimens by including the use of soap and water (i.e., 20% aqueous solution of Sodium Dodecyl Sulfate disinfectant (SDS)).
- ✓ Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and European regulations.
- ✓ Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the relevant regulatory authority in which the user and/or the patient is established.

  Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet. Safety Data Sheets are available from Zymo Research Corp. Inquire directly.

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.

Bisulfite Conversion

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

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[2]

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

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**[4] 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.

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[1]If the volume of DNA is less than 20 µl, compensate with water. 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]The 4°C storage step is optional.
[3]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]Alternatively, water or TE (pH ≥ 6.0) can be used for elution if required for your experiments.

Appendices

Bisulfite Conversion of Double Stranded DNA Templates.

The following illustrates what occurs to a DNA template during bisulfite conversion. Methylated "C" is underlined in the example.

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

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

Following bisulfite conversion the strands are no longer complementary.

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′
Forward: 5′-GATYGTTTTAGGT-3′

R= G/A
Y= C/T

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

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.

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 such as **ZymoTaq™** is strongly recommended for the amplification of bisulfite-treated DNA. **ZymoTaq™** is specifically designed for the amplification of bisulfite treated DNA.

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.

Safety Data Sheet

Request the product Safety Data Sheet (SDS) by contacting SDS@zymoresearch.com, or visit the product web page at www.zymoresearch.com

Ordering Information

Product Description	Catalog No.	Size
EZ DNA Methylation-Lightning™ Kit	D5030-E D5031-E	50 preps. 200 preps.
EZ-96 DNA Methylation-Lightning™ Kit (Shallow-Well)	D5032-E	2 x 96 rxns.
EZ-96 DNA Methylation-Lightning™ Kit (Deep-Well)	D5033-E	2 x 96 rxns.
EZ-96 DNA Methylation-Lightning™ MagPrep[1]	D5046 D5047	4 x 96 rxns. 8 x 96 rxns.

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 columns 250 columns
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 tubes 500 tubes 1,000 tubes

Product Description	Catalog No.	Size
MagBinding Beads	D4100-5-3 D4100-5-4 D4100-5-8 D4100-5-16	3 ml 4 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

[1]**MagPrep** kits are adaptable to liquid handling robots (e.g., Tecan – Freedom EVO®) making them ideal for automated sample prep.

Symbols

	Authorized representative in the European community/European Union
	Warning
	CE IVD vertical
	CE IVD horizontal
	Contains sufficient for <n> preps or reactions
	*In vitro* diagnostic medical device
	Lot number
	Manufacturer
	Reference **or** Catalogue number
	Consult instructions for use
	Temperature limit
	Use-by-date

Complete Your Workflow

✓ For sample DNA purification from cells, tissue, blood, biological fluids and more, use Quick-DNA and Quick-DNA Plus purification kits:

Quick-DNA Kits	
Quick-DNA Kits #D3020, D3024, D3010 Quick-DNA Plus Kits #D4074, D4068, D4075, D4070, D4081	For cells, whole blood, plasma, biological fluids, solid tissue, etc.
Quick-DNA FFPE Kit #D3067	For FFPE tissue and sections
Quick-cfDNA Serum & Plasma Kit #D4076	For cfDNA from serum, plasma, amniotic fluid, CSF and saliva

✓ For NGS library preparation for DNA methylation analysis:

NGS Library Prep Kits	
Zymo-Seq RRBS Library Kit #5460, D5461	For preparing reduced representation bisulfite sequencing libraries
Pico Methyl-Seq Library Prep Kit #5455, D5456	For preparing whole genome bisulfite sequencing libraries

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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 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.

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.

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