ZYMO RESEARCH

Epigenetics Made Simple™

EZ-96 DNA Methylation-Lightning™ Kit

(Shallow-Well Format)

The fastest method for complete high-throughput 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:
D5032

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

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Table of Contents

Product Contents

EZ-96 DNA Methylation Lightning™ Kit	D5032 2 x 96 reactions
Lightning Conversion Reagent[1]	2 bottles
M-Binding Buffer	125 ml
M-Wash Buffer[2]	2 x 36 ml
L-Desulphonation Buffer	40 ml
M-Elution Buffer	8 ml
Silicon-A™ Binding Plates	2 plates
Conversion Plates w/ Pierceable Cover Film	2 plates/films
Collection Plates	2 plates
Elution Plates	2 plates
Instruction Manual	1 pc

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

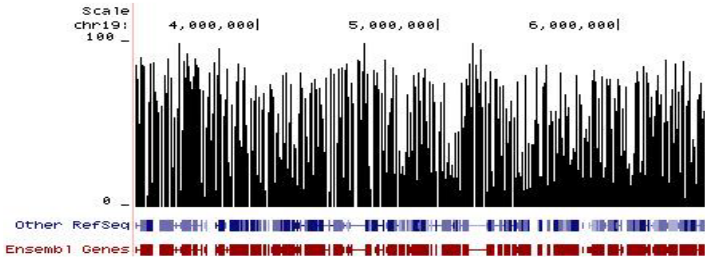
[1]The **Lightning Conversion Reagent** is in a ready-to-use liquid format. The reagent should be stored tightly capped at room temperature with minimum exposure to light.
[2]Add 144 ml of 100% ethanol to the 36 ml **M-Wash Buffer** concentrate before use.

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-96 DNA Methylation-Lightning™ Kit** features high-throughput[1] (96-well) 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 96-well spin-plate. 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.

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

Protocol

Buffer Preparation

✓ Add 144 ml of 100% ethanol to the 36 ml **M-Wash Buffer** concentrate before use.

Bisulfite Conversion

1. Add 130 µl of **Lightning Conversion Reagent** to 20 µl of a DNA sample[1] in a **Conversion plate**. Mix by pipetting up and down.

2. Seal the plate with the provided film. Transfer the **Conversion Plate** to 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 400 µl of **M-Binding Buffer** to the wells of a **Silicon-A™ Binding Plate** mounted on a **Collection Plate**[3]**.**

4. Transfer the samples from the **Conversion Plate** (Step 2) to the wells of the **Silicon-A™ Binding Plate**. Mix by pipetting up and down.

5. Centrifuge at ≥ 3,000 x g (5,000 x g max.) for 5 minutes. Discard the flow-through.

6. Add 400 µl of **M-Wash Buffer** to each well of the plate. Centrifuge at ≥ 3,000 x g for 5 minutes.

7. Add 200 µl of **L-Desulphonation Buffer** to each well and allow the plate to stand at room temperature (20-30°C) for 15-20 minutes. After the incubation, centrifuge at ≥ 3,000 x g for 5 minutes. Discard the flow-through.

8. Add 400 µl of **M-Wash Buffer** to each well of the plate. Centrifuge at ≥ 3,000 x g for 5 minutes. Discard the flow-through. Add another 400 µl of **M-Wash Buffer** and centrifuge for 10 minutes.

9. Place the **Silicon-A™ Binding Plate** onto an **Elution Plate**. Add 30 µl of **M-Elution Buffer**[4] directly to each well. Wait 5 minutes, then centrifuge at ≥ 3,000 x g for 3 minutes 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] Samples >20 µl must be processed using multiple conversion reactions. Replicate reactions can be cleaned using the same well by repeating steps 3-5. If the volume is less than 20 µl, compensate with water.
[2] The 4°C storage step is optional.
[3] The capacity of each well of the **Binding Plate** is 600 µl. The capacity of each well of the **Collection Plate** is 800 µl. Empty the **Collection Plate** whenever necessary to prevent contamination of the **Binding Plate** 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 Ab260 = 1.0 when determining the concentration of the recovered bisulfite-treated DNA.

Ordering Information

Product Description	Catalog No.	Size
EZ DNA Methylation-Lightning™ Kit	D5030T D5030 D5031	10 preps. 50 preps. 200 preps.
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[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.

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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 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.

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