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siehe unsere Liefer- und Versandbedingungen

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ZYMO RESEARCH

Epigenetics
Made Simple™

EZ-96 DNA Methylation-Lightning™ MagPrep

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:
D5046-E, D5047-E

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

CE IVD

For *in vitro* diagnostic

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

Table of Contents

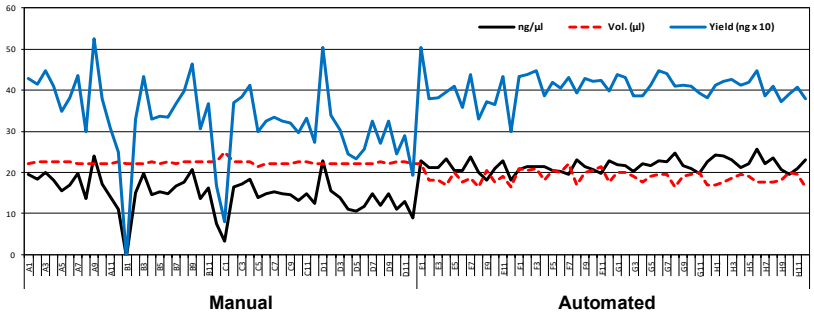
Product Contents

EZ-96 DNA Methylation Lightning™ MagPrep	D5046-E (4x96 rxns)	D5047-E (8x96 rxns)	Storage Temperature
Lightning Conversion Reagent[1]	4 bottles	8 bottles	Room Temp.
M-Binding Buffer	250 ml	2 x 250 ml	Room Temp.
M-Wash Buffer[2]	2 x 72 ml	4 x 72 ml	Room Temp.
L-Desulphonation Buffer	80 ml	2 x 80 ml	Room Temp.
M-Elution Buffer	2 x 8 ml	40 ml	Room Temp.
EZ-Methylation Magprep Beads	8 ml	16 ml	Room Temp.
Conversion Plates w/ Pierceable Cover Film	4 plates/films	8 plates/films	Room Temp.
Collection Plates[3]	6 plates	10 plates	Room Temp.
Elution Plates	4 plates	8 plates	Room Temp.
Instruction Manual	1	1	-

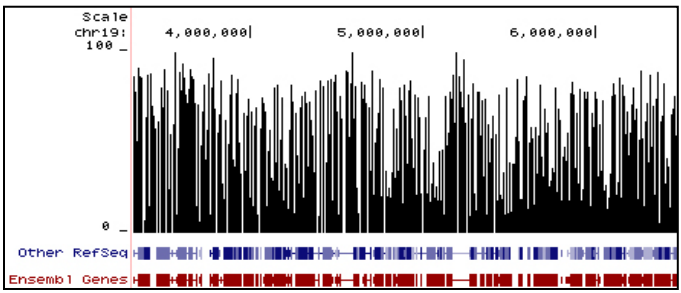
[1]The **Lightning Conversion Reagent** is in a ready-to-use liquid format. The reagent should be stored tightly capped at room temperature with minimum exposure to light.
[2]Add 288 mL of 100% ethanol to the 72 mL **M-Wash Buffer** concentrate prior to use.
[3]Two additional **Collection Plates** are provided as stands for the **Conversion Plates** during processing.

Product Description

The **EZ-96 DNA Methylation-Lightning™ MagPrep**[1] features rapid and reliable bisulfite treatment and conversion of DNA coupled to a magnetic bead-based clean-up for high-throughput 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 while bound to the **EZ-Methylation Magprep Beads**. High yield, converted DNA is ideal for PCR, array, bisulfite and next generation sequencing, etc.



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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[1] Single spin-column and 96-Well spin-plate formats are available. MagPrep kits can be adapted to liquid handling robots (e.g., Tecan – Freedom EVO®) and automated sample prep.

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.
- **Required Additional Equipment:** Magnetic stand[1], Heating element for 96- well plate.

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

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.

- ✓ 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.
- ✓ The following warning apply:

GHS07

Acute Tox. 4 H302 Harmful if swallowed.

Skin Irrit. 2 H315 Causes skin irritation.

Eye Irrit. 2B H320 Causes eye irritation.

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 288 ml of 95-100% ethanol to the 72 ml **M-Wash Buffer** concentrate before use.

Bisulfite Conversion

1. Add 130 µl of **Lightning Conversion Reagent** to 20 µl of a DNA sample in a **Conversion Plate**. Mix the samples by pipetting up and down.

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

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

   Note: The 4 °C storage step is *optional*.

3. 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 10.

4. Add 600 µl of **M-Binding Buffer** and 10 µl of **EZ-Methylation Magprep Beads** to each well of a **Collection Plate**.

   Note: EZ-Methylation Magprep Beads settle very quickly, ensure that beads are kept suspended in the reservoir while adding to the plate.

5. Transfer the samples from the **Conversion Plate** into the **Collection Plate** containing the **M-Binding Buffer** and **EZ-Methylation Magprep Beads**. Mix by pipetting up and down 3-6 times and, if available, shaking the plate 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 by using the tabs on the cover foil to prevent lifting of the **Conversion Plate**.

6. 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.

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

8. Add 200 µl of **L-Desulphonation Buffer** to the beads. Re-suspend the beads by pipetting up and down or shaking the plate for 30 seconds. Let plate stand at room temperature (20-30ºC) 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 **L-Desulphonation Buffer** for more than 20-25 minutes.

9. Add 400 µl of **M-Wash Buffer** to the beads, Re-suspend the beads by pipetting up and down or shaking the plate 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.

10. Transfer the plate to a heating element at 55ºC 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.

11. Add ≥ 25 µl of **M-Elution Buffer** directly to the dried beads and pipette or shake the plate for 30 seconds to re-suspend. Heat the elution at 55ºC 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.

    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 25 µl can be used if necessary. The elution volume can be > 25 µl 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.

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

Zymo Research provides primer design assistance with its Bisulfite Primer Seeker Program, available at: www.zymoresearch.com/tools/bisulfite-primer-seeker

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.

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.

✓ 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-96 DNA Methylation-Lightning™ MagPrep[1]	D5046-E D5047-E	4 x 96 rxns. 8 x 96 rxns.
EZ DNA Methylation-Lightning™ Kit	D5030-E D5031-E	50 rxns. 200 rxns.
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.

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

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

EZ-Methylation Magprep Beads	D4100-5-4 D4100-5-8 D4100-5-16	4ml 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
ZR-96 MagStand	P1005	1 stand

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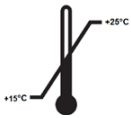

Symbols

	Authorized representative in the European community/European Union
	Caution
	CE IVD vertical
	CE IVD horizontal
	Contains sufficient for <n> tests
	*In vitro* diagnostic medical device
	Lot number
	Manufacturer
	Reference **or** Catalogue number
	Consult instructions for use
	Temperature limit
	Upper limit of temperature
	Lower limit of temperature
	Unique device identifier
	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

Notes

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Notes

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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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