ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZymoPURE™ Plasmid Miniprep Kit

Catalog Nos. **D4209, D4210, D4211 & D4212** (Patent Pending)

Highlights

- Fast and reliable purification of up to 100 µg of transfection-grade plasmid DNA using a spin-column.
- Innovative ZymoPURE™ technology enables elution of ultra-pure endotoxin-free plasmid DNA in as little as 25 µl.

Contents

ZYMO RESEARCH CORP.

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

Product Contents:

ZymoPURE™ Plasmid Miniprep Kit Size	D4209 50 preps.	D4210 100 preps.	D4211 400 preps.	D4212 800 preps.	Storage Temperature
ZymoPURE™ P1[1] (Red)	13 ml	2x 13 ml	100 ml	210 ml	4°C
ZymoPURE™ P2[2,3] (Green)	13 ml	2x 13 ml	100 ml	210 ml	Room Temp.
ZymoPURE™ P3 (Yellow)	13 ml	2x 13 ml	100 ml	210 ml	Room Temp.
ZymoPURE™ Binding Buffer[3]	14 ml	2x 14 ml	110 ml	2x 110 ml	Room Temp.
ZymoPURE™ Wash 1	2x 20 ml	4x 20 ml	320 ml	2x 320 ml	Room Temp.
ZymoPURE™ Wash 2[4]	12 ml	23 ml	3x 28 ml	6x 28 ml	Room Temp.
ZymoPURE™ Elution Buffer	2x 1 ml	6 ml	12 ml	30 ml	Room Temp.
Zymo-Spin™ II-P Columns	50	100	400	800	Room Temp.
Collection Tubes	50	100	400	800	Room Temp.
Instruction Manual	1	1	1	1	-

Note - 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 maximal performance and reliability.

[1] ZymoPURE™ P1 contains RNase A (100 µg/ml) and is stable at room temperature without loss in RNase activity, however, for long-term storage the product should be stored at 4-8° C.

[2] Caution: ZymoPURE™ P2 Buffer contains NaOH. Please use proper safety precautions.

[3] The ZymoPURE™ P2 and ZymoPURE™ Binding Buffer may have precipitated. If this occurs, dissolve the precipitate by incubating the bottles at 30-37 ºC for 10-20 minutes and mix by inversion. Do not microwave!

[4] ZymoPURE™ Wash 2 included with D4208S is supplied ready-to-use and does not require the addition of ethanol prior to use. ZymoPURE™ Wash 2 included with D4209, D4210, D4211, and D4212 are supplied as a concentrate and require the addition of ethanol prior to use. See Buffer Preparation (page 4) for instructions.

Specifications:

- **DNA Purity:** Eluted DNA is ultrapure, endotoxin-free, and well suited for transfection, transformation, sequencing, restriction endonuclease digestion, *in vitro* transcription, and other sensitive applications.
    - Typical $Abs_{260/280} \geq 1.8$ and $Abs_{260/230} \geq 2.0$
- **Plasmid DNA Yield:** Up to 100 µg per preparation *(Actual yield is dependent on the plasmid copy number, culture growth conditions, and strain of E. coli utilized)*
- **Plasmid DNA Size:** Up to 25 kb
- **Recovery Volume:** ≥ 25 µl of ZymoPURE™ Elution Buffer or DNase free water
- **Required Equipment:** Microcentrifuge and/or vacuum manifold (recommended).
- **Processing Time:** 15 min

Notes:

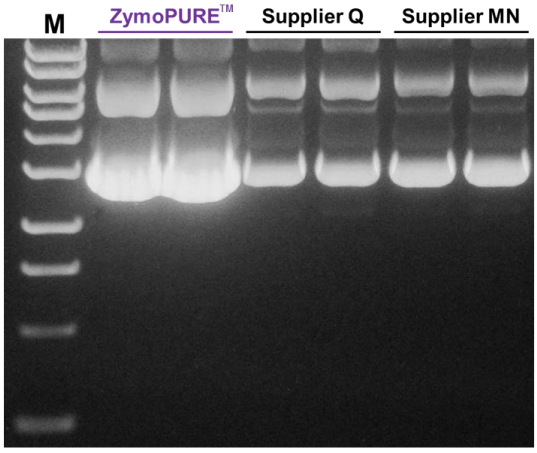
This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

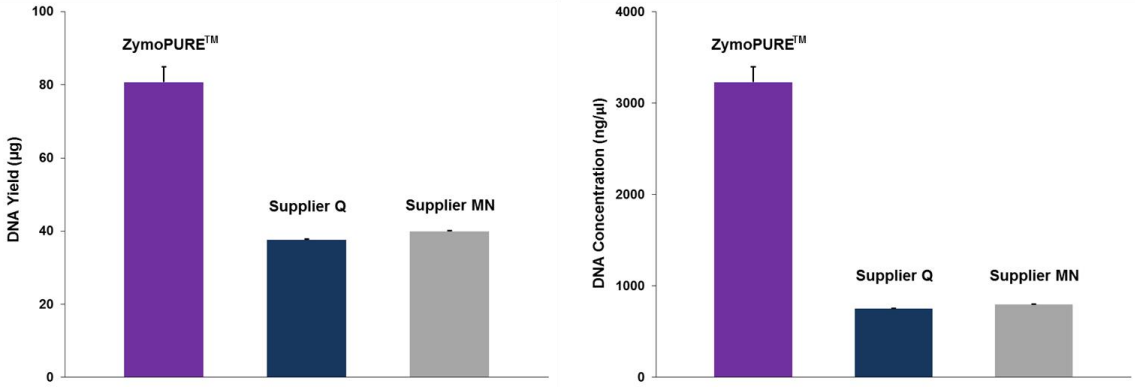
Product Description

The **ZymoPURE™ Plasmid Miniprep Kit** features a spin column-based method for the purification of up to 100 µg of ultra-pure endotoxin-free plasmid DNA in less than 15 minutes. The unique spin-column design also provides zero buffer retention and a low elution volume.

ZymoPURE™ technology uses a modified alkaline lysis method and features novel binding chemistry that yields highly concentrated plasmid DNA (up to 3 µg/µl). In addition, the wash regimen has been optimized to ensure the plasmid DNA is free of endotoxins, salt, protein, and RNA. The result is plasmid DNA suitable for transfection, restriction endonuclease digestion, bacterial transformation, PCR amplification, DNA sequencing, and other sensitive downstream applications.

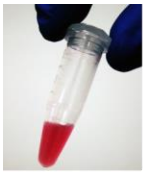
As an added convenience, the **ZymoPURE™ Plasmid Miniprep Kit** contains colored buffers that permit error-free visualization and identification of complete bacterial cell lysis and neutralization.





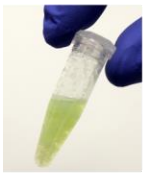
Plasmid DNA yield and concentration from the ZymoPure™ Miniprep Kit compared to other major suppliers. Plasmid DNA (pGL3®) was isolated from 5 ml of JM109 *E. coli* culture grown overnight following the manufacturer's suggested protocol (in duplicate). One (1) µl of eluted plasmid DNA was visualized post agarose gel electrophoresis. M, ZR 1 kb DNA Marker (Zymo Research).

Procedure Overview:



Bacterial cells are resuspended in **ZymoPURE™ P1** (red).

The solution will turn dark purple and viscous following the addition of **ZymoPURE™ P2** (green) indicating bacterial lysis is complete.



The solution will turn yellow and a precipitate will form after adding **ZymoPURE™ P3** (yellow) indicating neutralization is complete.

ZymoPURE™ Binding Buffer is added to the cleared lysate and mixed thoroughly.

The mixture is loaded into the **Zymo-Spin™ II-P Column** using a vacuum manifold or microcentrifuge.

The **Zymo-Spin™ II-P Column** is washed using a vacuum manifold or microcentrifuge.

Ultra-pure plasmid DNA is eluted from the **Zymo-Spin™ II-P Column** using a microcentrifuge.

Buffer Preparation:

- ✓ Add 46 ml of 95% ethanol to the **12 ml ZymoPURE™ Wash 2 (Concentrate)** (D4209), 88 ml of 95% ethanol to the **23 ml ZymoPURE™ Wash 2 (Concentrate)** (D4210), or 107 ml of 95% ethanol to the **28 ml ZymoPURE™ Wash 2 (Concentrate)** (D4211 & D4212) before use.
- ✓ The **ZymoPURE™ P2** and **ZymoPURE™ Binding Buffer** may have precipitated. If this occurs, dissolve the precipitate by incubating the bottles at 30-37 ºC for 10-20 minutes and mix by inversion. Do not microwave!
- ✓ *Before Starting:* Incubate **ZymoPURE™ P3** on ice for 30 minutes before use.

Protocol:

The following procedure should be performed at room temperature (15-30°C).

1. Centrifuge 0.5-5 ml[1] of bacterial culture in a clear 1.5 ml tube at full speed for 15-20 seconds in a microcentrifuge. Discard supernatant.
2. Add 250 µl of **ZymoPURE™ P1 (Red)** to the bacterial cell pellet and resuspend completely by vortexing or pipetting.
3. Add 250 µl of **ZymoPURE™ P2 (Green)** and immediately mix by gently inverting the tube 6-8 times. Do not vortex! Let sit at room temperature for 2-3 minutes[2]. *Cells are completely lysed when the solution appears clear, purple, and viscous.*
4. Add 250 µl of ice cold **ZymoPURE™ P3 (Yellow)** and mix thoroughly by inversion. Do not vortex! Invert the tube an additional 3-4 times after the sample turns completely yellow. *The sample will turn yellow when the neutralization is complete and a yellowish precipitate will form.*
5. Incubate the neutralized lysate on ice for 5 minutes.
6. Centrifuge the neutralized lysate for 5 minutes at 16,000 *x g*.
7. Transfer 600 µl of supernatant from step 6 into a clean 1.5 ml microcentrifuge tube. *Be careful not to disturb the yellow pellet and avoid transferring any cellular debris to the new tube.*
8. Add 275 µl of **ZymoPURE™ Binding Buffer** to the cleared lysate from step 7 and mix thoroughly by inverting the capped tube 8 times.

   To continue processing the lysate using the recommended vacuum protocol, proceed to the next page. If a vacuum is not available, proceed to page 6 for an alternative centrifugation method.

For **Technical Assistance**, please contact us at 1-888-882-9682 or E-mail tech@zymoresearch.com.

Notes:

[1] Depending on the volume of bacterial culture it may be necessary to repeat Step 1 several times.

[2] Do not allow the lysis reaction to proceed for more than 3 minutes. Excessive lysis can result in denatured plasmid DNA. When processing a large number of samples, work with groups of ≤ 10 at a time.

Notes:

[1] To achieve optimal performance, the vacuum pump should be able to apply at least 400 mm Hg pressure. If less pressure is applied, centrifuge the column prior to washing to remove any residual lysate remaining in the matrix.

[2] The **ZymoPURE™ Elution Buffer** contains 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.

[3] The DNA yield can be increased by pre-warming the **Zymo PURE™ Elution Buffer** to 50 ºC and/or increasing the incubation period up to 5 minutes prior to centrifugation.

Vacuum Protocol: *(Recommended)*

This product is compatible with any conventional vacuum-based manifold. The vacuum pump should be a single or double-staged unit capable of producing up to 400 mm Hg pressure at the vacuum manifold[1].

9. Place the **Zymo-Spin™ II-P Column** onto a vacuum manifold. (If vacuum is not available, see page 6 for the centrifugation protocol.)

10. With the vacuum off, add the entire mixture from step 8 into the Zymo-Spin™ II-P Column. Turn on the vacuum until all of the liquid has passed completely through the column.

11. With the vacuum off, add 800 µl of **ZymoPURE™ Wash 1** to the Zymo-Spin™ II-P Column**.** Turn on the vacuum until all of the liquid has passed completely through the column.

12. With the vacuum off, add 800 µl of **ZymoPURE™ Wash 2** to the Zymo-Spin™ II-P Column. Turn on the vacuum until all of the liquid has passed completely through the column.

13. With the vacuum off, add 200 µl of **ZymoPURE™ Wash 2** to the Zymo-Spin™ II-P Column. Turn on the vacuum until all of the liquid has passed completely through the column.

14. Place the Zymo-Spin™ II-P Column in a **Collection Tube** and transfer to a microcentrifuge. Centrifuge at ≥ 10,000 x *g* for 1 minute in order to remove any residual wash buffer.

15. Transfer the Zymo-Spin™ II-P Column into a clean 1.5 ml tube and add 25 µl of **ZymoPURE™ Elution Buffer**[2,3] directly to the column matrix. Incubate at room temperature for 2 minutes, and then centrifuge at ≥ 10,000 x *g* for 1 minute in a microcentrifuge. Store the eluted plasmid DNA at ≤ -20°C.

Centrifugation Protocol: *(Alternative)*

Perform steps 1-8 as indicated in the general protocol, see page 4.

9. Place a **Zymo-Spin™ II-P Column** in a Collection Tube and transfer the entire mixture from step 8 into the Zymo-Spin™ II-P Column.

10. Incubate the **Zymo-Spin™ II-P/Collection Tube** assembly at room temperature for 2 minutes and then centrifuge at 5,000 *x g* for 1 min. Discard the flow through[1].

11. Add 800 µl of **ZymoPURE™ Wash 1** to the Zymo-Spin™ II-P Column and centrifuge at 5,000 *x g* for 1 min. Discard the flow through.

12. Add 800 µl of **ZymoPURE™ Wash 2** to the Zymo-Spin™ II-P Column and centrifuge at 5,000 *x g* for 1 min. Discard the flow through.

13. Add 200 µl of **ZymoPURE™ Wash 2** to the Zymo-Spin™ II-P Column and centrifuge at 5,000 *x g* for 1 min. Discard the flow through.

14. Centrifuge the Zymo-Spin™ II-P Column at ≥ 10,000 x *g* for 1 minute in order to remove any residual wash buffer.

15. Transfer the Zymo-Spin™ II-P Column into a clean 1.5 ml tube and add 25 µl of **ZymoPURE™ Elution Buffer**[2,3] directly to the column matrix. Incubate at room temperature for 2 minutes, and then centrifuge at ≥ 10,000 x *g* for 1 minute in a microcentrifuge. Store the eluted plasmid DNA at ≤ -20°C.

Notes:

[1] The capacity of the collection tube with the column inserted is 900 µl. Empty the collection tube whenever necessary to prevent contamination of the spin-column with the flow through.

[2] The **ZymoPURE™ Elution Buffer** contains 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.

[3] The DNA yield can be increased by pre-warming the **Zymo PURE™ Elution Buffer** to 50 ºC and/or increasing the incubation period up to 5 minutes prior to centrifugation.

Troubleshooting Guide:

Problem	Possible Causes and Suggested Solutions
Low DNA Yield	
Culture growth conditions	• **Poor aeration of culture.** The optimal culture volume to air volume ratio is 1:5 or less. For best aeration, use baffled culture flasks, or a vented or gas-permeable seal on the culture vessel. • **The culture was overgrown, undergrown, contaminated, or antibiotics were omitted from the growth medium.** Use a fresh culture for optimal performance. An OD_{600} of 0.2-0.35 is the optimal optical density of a tenfold dilution of the culture.
Cell density is too high	• **Too much culture used.** Lysis and neutralization will be incomplete resulting in poor lysate clarification. More culture does not always equal more plasmid. Incomplete lysis and neutralization are two of the most common causes of failed plasmid preps and both are caused by too much culture being used. • **Incomplete lysis:** After addition of ZymoPURE™ P2, the solution should change from opaque pink to a clear viscous purple, indicating complete lysis. Different *E. coli* strains often require different growth conditions and may vary in their susceptibility to alkaline lysis. • **Incomplete neutralization:** The solution should not be viscous following neutralization and the yellowish precipitate should appear fluffy and readily float to the surface. Make sure the neutralization is complete prior to centrifugation. Invert the tube an additional 3-4 times after the sample turns yellow following the addition of ZymoPURE™ P3.
Lysate clarification	• **Less than 600 µl of supernatant was recovered after pelleting the lysate debris.** For optimal performance, add 275 µl of ZymoPURE™ Binding Buffer to 600 µl of clarified lysate.
ZymoPURE P2 and ZymoPURE Binding Buffer precipitated	• **Both buffers may have precipitated during shipping.** To completely resuspend the buffers, incubate the bottles at 30-37 ºC for 10 minutes and mix by inversion. DO NOT MICROWAVE.
Wash buffer	• **Ensure that the correct volume of ethanol was added** to the ZymoPURE™ Wash 2. • **Ensure that the bottle cap is screwed on tightly** after each use to prevent evaporation of the ethanol.
DNA elution	• **Incomplete elution:** For large size plasmids (> 10 kb), add ZymoPURE™ Elution Buffer and incubate the column for 5-10 minutes before centrifugation. Also, pre-warm the ZymoPURE™ Elution Buffer to 50 ºC prior to elution.
Low DNA Quality	
DNA does not perform well	• **Incomplete neutralization:** Incomplete neutralization generates poor quality supernatant. Ensure that neutralization is complete by inverting the sample an additional 3-4 times after the sample turns yellow following the addition of ZymoPURE™ P3. • **Insufficient centrifugation:** Make sure that all centrifugation steps are performed at the indicated speed and time. If a lower centrifuge speed is used, then extend the centrifugation time to compensate.
RNA in eluate	• Ensure that ZymoPURE™ P1 has been stored at 4°C. RNase A can be purchased separately if necessary.
Genomic DNA in eluate	• **Improper handling** (Sample was vortexed or handled too roughly). Genomic DNA contamination is usually caused by excessive mechanical shearing during the lysis and neutralization steps. Also, prolonged lysis or incomplete mixing of lysis or neutralization buffers may contribute to genomic DNA contamination in your sample. • **Overgrown culture.** Overgrown or old cultures may contain more genomic DNA contamination than fresh cultures.

Ordering Information

Product Description	Kit Size	Catalog No.
ZymoPURE™ Plasmid Miniprep Kit	50 preps.	D4209
ZymoPURE™ Plasmid Miniprep Kit	100 preps.	D4210
ZymoPURE™ Plasmid Miniprep Kit	400 preps.	D4211
ZymoPURE™ Plasmid Miniprep Kit	800 preps.	D4212

For Individual Sale	Amount	Catalog No.
ZymoPURE™ P1 (Red)	3 ml 13 ml 100 ml 210 ml	D4200-1-3 D4200-1-13 D4200-1-100 D4200-1-210
ZymoPURE™ P2 (Green)	3 ml 13 ml 100 ml 210 ml	D4200-2-3 D4200-2-13 D4200-2-100 D4200-2-210
ZymoPURE™ P3 (Yellow)	3 ml 13 ml 100 ml 210 ml	D4200-3-3 D4200-3-13 D4200-3-100 D4200-3-210
ZymoPURE™ Binding Buffer	3 ml 14 ml 110 ml	D4200-4-3 D4200-4-14 D4200-4-110
ZymoPURE™ Wash 1	12 ml 20 ml 320 ml	D4200-5-12-S D4200-5-20 D4200-5-320
ZymoPURE™ Wash 2 (Concentrate)	12 ml 23 ml 28 ml	D4200-6-12 D4200-6-23 D4200-6-28
ZymoPURE™ Elution Buffer	1 ml 6 ml 12 ml 30 ml	D4200-7-1 D4200-7-6 D4200-7-12 D4200-7-30
Zymo-Spin™ II-P	10 50	C1055-10 C1055-50
Collection Tubes	50 500 1000	C1001-50 C1001-500 C1001-1000

DNA PURIFICATION

What is Clean-Spin™ Technology?

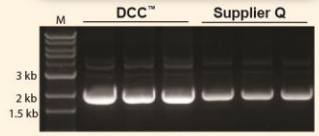
The spin columns from Zymo Research have been designed to ensure complete elution with no binding/wash buffer carryover. The result is ultra-pure inhibitor-free DNA and RNA.

Purify DNA from PCR & other sources

DNA Clean & Concentrator™ (DCC™)

- ✓ Recovery of ultra-pure DNA that is free of salts and contaminants.
- ✓ Small (≥6 µl) elution volume.
- ✓ DNA is ideal for ligation, PCR, Next-Gen sequencing, etc.

Product	Size (Cat. No.)
DNA Clean & Concentrator™-5	50 Preps. (D4013) 200 Preps. (D4014)
ZR-96 DNA Clean & Concentrator™-5	2 x 96 Preps. (D4023) 4 x 96 Preps. (D4024)
Genomic DNA Clean & Concentrator™	25 Preps. (D4010) 100 Preps. (D4011)



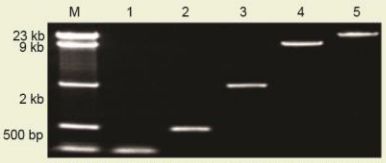
High efficiency DNA recovery with the DCC™-5 compared to Supplier Q .

Boost DNA recoveries from agarose gels to >80%

Zymoclean™ Gel DNA Recovery

- ✓ Rapid (15 min.) recovery of ultra-pure DNA from agarose gels in ≥6 µl.
- ✓ Ultra-pure DNA ideal for DNA ligation, sequencing, etc.
- ✓ Format also available for large DNA >20 kb.

Product	Size (Cat. No.)
Zymoclean™ Gel DNA Recovery Kit	50 Preps. (D4001) 200 Preps. (D4002)
Zymoclean™ Large Fragment DNA Recovery Kit	25 Preps. (D4045) 100 Preps. (D4046)



DNA fragments recovered from an agarose gel using the Zymoclean™ Gel DNA Recovery Kit. Lanes: M: DNA Ladder; 1-5: individual ladder DNA fragments.

Recover transfection-quality plasmid DNA directly from culture

Zyppy™ Plasmid Prep Kits

- ✓ The fastest, simplest method available for purifying high quality plasmid DNA from *E. coli.*
- ✓ Pellet-Free™ procedure omits conventional cell-pelleting and resuspension steps.
- ✓ Transfection quality plasmid DNA directly from culture in under 15 minutes.

Easy, Pellet-free Procedure: Add Lysis Buffer ***Directly*** to Bacterial Culture

Product	Size (Cat. No.)
Zyppy™ Plasmid Miniprep Kit	50 Preps. (D4036) 100 Preps. (D4019) 400 Preps. (D4020) 800 Preps. (D4037)

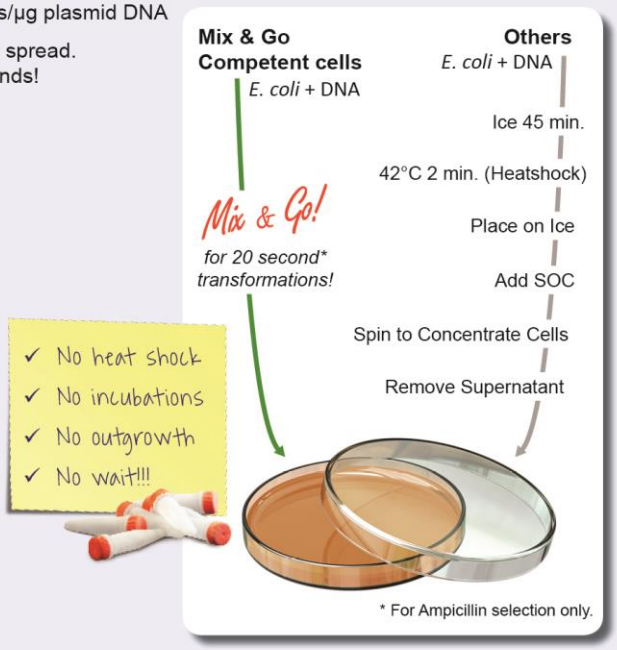
OTHER INNOVATIVE PRODUCTS FROM ZYMO RESEARCH...

Competent cells for transformations *without* heat shock!

Mix & Go! Pre-made Competent *E. Coli*

- ✓ High efficiency: 10^8-10^9 transformants/µg plasmid DNA
- ✓ Just *Mix & Go!* Simply add DNA then spread. Transformation in as little as 20 seconds!

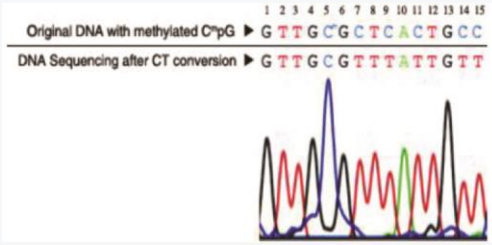
Product	Size (Cat. No.)
Zymo 5α (Same as DH5α)	10 x 100 µl aliquots (T3007) 96 x 50 µl aliquots (T3009) 96 x 50 µl aliquots PCR-plate (T3010)
Zymo 10B (Same as DH10B)	10 x 100 µl aliquots (T3019) 96 x 50 µl aliquots (T3020)
JM109	10 x 100 µl aliquots (T3003) 96 x 50 µl aliquots (T3005)
HB101	10 x 100 µl aliquots (T3011) 96 x 50 µl aliquots (T3013)
C600	10 x 100 µl aliquots (T3015)
TG1	10 x 100 µl aliquots (T3017)



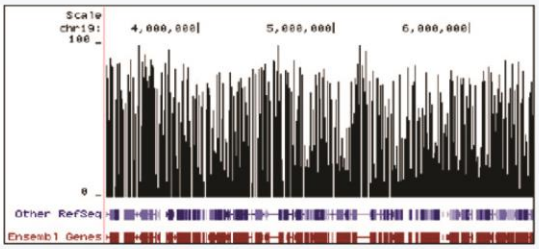
The fastest method for complete bisulfite conversion of DNA

EZ DNA Methylation-Lightning™ Kits

- ✓ The next generation of bisulfite conversion technology by the most cited provider in the industry
- ✓ Guarantees high conversion efficiencies of cytosine (>99.5%)
- ✓ Maintains the highest template integrity following bisulfite conversion
- ✓ Recovered DNA is ideal for PCR, MSP, array, bisulfite, and next-generation sequencing.



DNA Sequencing Results Following Bisulfite Treatment



Methylation Plot From Reduced Representation Bisulfite Sequencing (RRBS)

Product		Size (Cat. No.)
EZ DNA Methylation-Lightning™ Kit		50 rxns. (D5030) 200 rxns. (D5031)
EZ-96 DNA Methylation-Lightning™ Kit	Shallow-Well Deep-Well	2 x 96 rxns. (D5032) 2 x 96 rxns. (D5033)
EZ-96 DNA Methylation-Lightning™ MagPrep		4 x 96 rxns. (D5046) 8 x 96 rxns. (D5047)

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