



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR Plasmid Gigaprep Kit

Catalog Nos. **D4056 & D4057**

Highlights

- 2 - 10 mg of high quality, *endotoxin free* (for transfection) plasmid in less than 60 minutes.
- Innovative chemistry and streamlined procedure for consistent high concentration plasmid recovery directly in water or low salt buffer.
- Colored buffers for visualization of complete bacterial cell lysis and neutralization.

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

Product Contents:

ZR Plasmid Gigaprep Kit (Kit Size)	D4056 (5 preps.)	D4057 (10 preps.)	Storage Temperature
RNase A	24 mg	2 x 24 mg	Room Temp.
P1 Buffer¹ (Red)	500 ml	2 x 500 ml	4 - 8°C after adding RNase A.
P2 Buffer*² (Green)	500 ml	2 x 500 ml	Room Temp.
P3 Buffer* (Yellow)	2 x 500 ml	4 x 500 ml	Room Temp.
ZymoBeads™	20 ml	2 x 20 ml	Room Temp
Endo-Wash Buffer²	60 ml	120 ml	Room Temp.
Plasmid Wash Buffer (concentrate)³	24 ml	48 ml	Room Temp.
DNA Elution Buffer	50 ml	50 ml	Room Temp.
Midi Filter	5	10	Room Temp.
Instruction Manual	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 Add the RNase A to P1 Buffer and store at 4-8°C. The final concentration of RNase A will be 48 µg/ml (see page 7).

² P2 Buffer and Endo-Wash Buffer may have precipitated during shipping. To completely resuspend the buffers, incubate the bottles at 30 – 37 °C for 10-20 minutes and mix by inversion. DO NOT MICROWAVE.

³ Add 96 ml of 100% ethanol or 104 ml of 95% ethanol to the 24 ml Plasmid Wash Buffer concentrate (D4027-4-24), or add 192 ml of 100% ethanol or 208 ml of 95% ethanol to the 48 ml Plasmid Wash Buffer concentrate (D40207-4-48) before use.

*Caution: P2 Buffer contains NaOH and Buffer P3 Contains chaotropic reagents. Please use proper safety precautions with these reagents.

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended 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.

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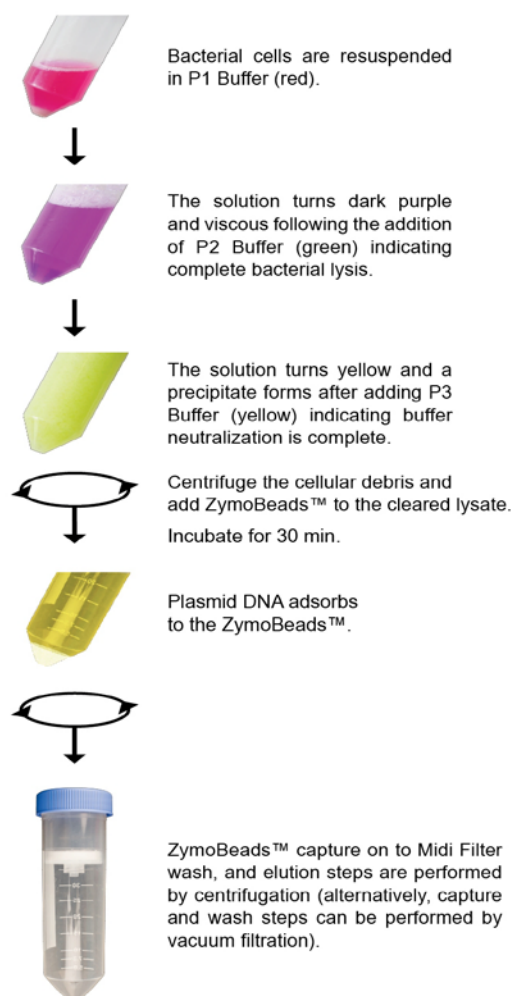
Phone: (949) 679-1190 ▪ Toll Free: (888) 882-9682 ▪ Fax: (949) 266-9452 ▪ info@zymoresearch.com ▪ www.zymoresearch.com

Product Description

The **ZR Plasmid Gigaprep Kit** employs a modified alkaline lysis method in conjunction with DNA binding beads (ZymoBeads™) to isolate high quality *endotoxin-free* (for transfection) plasmid DNA in less than an hour. The purified DNA is suitable for use in transfection, restriction endonuclease digestion, ligation, bacterial transformation, PCR amplification, DNA sequencing and other sensitive molecular biology applications.

The innovative patented colored buffers included in the kit permit error-free visualization of both complete bacterial cell lysis and neutralization. Additionally, the uniquely designed **Midi Filter** allows the capture of ZymoBeads™ either by centrifugation or vacuum. The unique design of the filter also allows for low elution volumes of 2-3 ml directly in supplied **Elution buffer** or water, thus eliminating the need for plasmid DNA precipitation and resuspension steps common to other column-based gigaprep procedures.

The **ZR Plasmid Gigaprep Kit** is designed for use with a combination of both centrifuge, and vacuum manifold, or a centrifuge alone, therefore providing flexibility in large scale plasmid DNA purification from *E. coli*. An overview of the purification procedure is shown below.



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

Specifications:

- **DNA Purity:** Plasmid DNA is *endotoxin free* (for transfection) and is well suited for transfection, ligation, sequencing, restriction endonuclease digestion, *in vitro* transcription, and other sensitive applications requiring pure DNA. Typical $Abs_{260/280}$ index is ≥ 1.8 .
- **Plasmid DNA Yield:** ≥ 2 mg per preparation (1000 ml culture), depending on the plasmid copy number, culture growth conditions, and strain of *E. coli* utilized.
Can be scaled up to 10 mg by increasing culture volume and the amount of Zymobeads used
- **Plasmid DNA Size:** Up to 25 kb.
- **Recovery Volume:** ≤ 3 ml.
- **Procedure:** Performed at room temperature (15-30°C) using a vacuum manifold and/or a centrifuge.

Buffer Preparation:

1. Add the **RNase A** to **P1 Buffer**: aliquot one ml of **P1 Buffer** into the tube containing the lyophilized **RNase A**, mix, and transfer the solution back into the **P1 Buffer** bottle. The final concentration of **RNase A** will be 48 $\mu\text{g/ml}$. Store at 4-8 °C.
2. Add 96 ml of 100% ethanol or 104 ml of 95% ethanol to the 24 ml **Plasmid Wash Buffer** concentrate (D4027-4-24), or add 192 ml of 100% ethanol or 208 ml of 95% ethanol to the 48 ml **Plasmid Wash Buffer** concentrate (D4027-4-48) before use.
3. **P2 Buffer** and **Endo-Wash Buffer** may have precipitated during shipping. To completely resuspend the buffers, incubate the bottles at 30 – 37 °C for 10-20 minutes and mix by inversion. **DO NOT MICROWAVE.**

Vacuum Pump and Manifold:

- The vacuum pump should be a single- or double-staged unit capable of producing approximately 650 mm Hg pressure at the vacuum manifold.
- This product is compatible with any conventional vacuum-based manifold.

Recommended Protocol:

Isolation of Plasmid DNA Using a Centrifuge

For Combined Vacuum/Centrifuge Based Procedure Please see Page 5

The following procedure is performed at room temperature. P2 and Endo-Wash Buffers may have precipitated during shipping. To completely resuspend the buffers, incubate the bottles at 30-37 °C for 10-20 minutes and mix by inversion. DO NOT MICROWAVE.

1. Centrifuge up to 1000 ml of fresh bacterial culture¹ at $\geq 3,400 \times g$ for 10 minutes using spin bottles. Discard supernatant.
2. Add 100 ml of cold **P1 Buffer (Red)** to the bacterial cell pellet(s) and resuspend completely by vortexing or pipetting and pooling samples if necessary.
3. Add 100 ml of **P2 Buffer (Green)** and mix *immediately* by inverting the spin bottle 4-6 times. Let stand for 1-2 minutes to lyse the cells completely.
4. Add 200 ml of **P3 Buffer (Yellow)** and mix gently but thoroughly. Incubate on ice for 5 minutes. *The sample will turn yellow when the neutralization is complete and a greenish precipitate will form.*
5. Centrifuge at $\geq 3,400 \times g$ for 10 minutes to pellet the bacterial cell debris.
6. Transfer the supernatant (~ 350 ml) into a new spin bottle and add 4 ml of **ZymoBeads™** to the cleared lysate. Incubate at RT for 30 minutes while shaking the container every 3-5 minutes. Alternatively, a rocker can be used
7. Centrifuge at $\geq 3,400 \times g$ for 5 minutes to pellet the beads. Carefully decant the supernatant (during the centrifugation, place the **Midi Filter** into a 50 ml conical tube).
8. Add 10 ml **Endo-Wash Buffer** to the **ZymoBeads™** and resuspend by pipeting. Transfer the resuspended beads to the **Midi Filter** and centrifuge² at $\geq 3,400 \times g$ for 3 minutes. Discard the flow-through.

Use a Swing Bucket rotor for this and all subsequent steps.

9. Add 10 ml of **Plasmid™ Wash Buffer** to the **Midi Filter** and centrifuge at $\geq 3,400 \times g$ for 3 minutes in a swing bucket rotor. Discard the flow-through.
10. Transfer the **Midi Filter** into a clean 50 ml conical tube then add 2-3 ml of **DNA Elution Buffer**² (or water) to the column. Incubate at RT for one minute, then centrifuge³ at $\geq 3,400 \times g$ for 3 minutes in a swing bucket rotor to elute the plasmid DNA.

Notes:

¹ Adequate aeration is essential for efficient plasmid recovery. See page 6 for growth conditions

² The **DNA Elution Buffer** contains 0.1 mM EDTA. If required, pure water can also be used to elute the DNA

³ Second elution can yield an additional 5-10 % more plasmid if needed

Alternate Protocol:

Isolation of Plasmid DNA Using Combined Vacuum/Centrifuge based Procedure

Notes:

¹ Adequate aeration is essential for efficient plasmid recovery. See page 6 for growth conditions

² The **DNA Elution Buffer** contains 0.1 mM EDTA. If required, pure water can also be used to elute the DNA

³ Second elution can yield an additional 5-10 % more plasmid if needed

The following procedure is performed at room temperature. P2 and Endo-Wash Buffers may have precipitated during shipping. To completely resuspend the buffers, incubate the bottles at 30-37 °C for 10-20 minutes and mix by inversion. DO NOT MICROWAVE.

1. Centrifuge up to 1000 ml of fresh bacterial culture¹ at $\geq 3,400 \times g$ for 10 minutes using spin bottles. Discard supernatant.
2. Add 100 ml of cold **P1 Buffer (Red)** to the bacterial cell pellet(s) and resuspend completely by vortexing or pipetting and pooling samples if necessary.
3. Add 100 ml of **P2 Buffer (Green)** and mix *immediately* by inverting the spin bottle 4-6 times. Let stand for 1-2 minutes to lyse the cells completely.
4. Add 200 ml of **P3 Buffer (Yellow)** and mix gently but thoroughly. Incubate on ice for 5 minutes. *The sample will turn yellow when the neutralization is complete and a greenish precipitate will form.*
5. Centrifuge at $\geq 3,400 \times g$ for 10 minutes to pellet the bacterial cell debris.
6. Transfer the supernatant (~ 350 ml) into a new spin bottle and add 4 ml of **ZymoBeads™** to the cleared lysate. Mix well and incubate at RT for 30 minutes while shaking the container every 3-5 minutes. Alternatively, a rocker can be used
7. Centrifuge at $\geq 3,400 \times g$ for 5 minutes to pellet the beads. Carefully decant the supernatant

*During the centrifugation, place the **Midi Filter** onto a vacuum manifold.*

8. Add 10 ml **Endo-Wash Buffer** to the **ZymoBeads™** and resuspend by pipeting. Transfer the resuspended beads to the **Midi Filter** and apply vacuum
9. After the **Endo-Wash Buffer** has completely passed through the Midi Filter, add 10 ml of **Plasmid Wash Buffer** and turn on the vacuum. Leave the vacuum for an additional 10 minutes to remove all residual **Plasmid Wash Buffer**.
10. Transfer the **Midi Filter** into a clean 50 ml conical tube then add 2-3 ml of **DNA Elution Buffer**² (or water) to the column. Incubate at RT for one minute, then centrifuge³ at $\geq 3,400 \times g$ for 3 minutes in a swing bucket rotor to elute the plasmid DNA.

Troubleshooting Guide:

Problem	Possible Causes and Suggested Solutions
Low DNA Yield	
<i>Culture growth conditions</i>	<ul style="list-style-type: none"> • Poor aeration of culture. The optimal culture volume to air volume ratio is 1:4 or less (20% culture, 80% air). For best aeration, use baffled culture flasks, or a vented or gas-permeable seal on the culture vessel. • The culture was overgrown, undergrown or contaminated. Use a fresh culture for optimal performance. • Antibiotics were omitted from the growth medium.
<i>Procedural errors</i>	<ul style="list-style-type: none"> • Incomplete lysis: After addition of P2 Buffer, the solution should change from opaque pink to clear, viscous purple, indicating complete lysis. Different <i>E. coli</i> strains often require different growth conditions and may vary in their susceptibility to alkaline lysis. • Incomplete neutralization: Cell debris will float to the surface after centrifugation and the pellet may appear “puffy”. Make sure the neutralization is complete prior to centrifugation. Invert the tube an additional 2 – 3 times after the sample turns yellow following the addition of P3 Buffer. • Too much culture used. Lysis and Neutralization will be incomplete resulting in low yields. • Insufficient centrifugation: make sure that all centrifugation steps are performed $\geq 3,400 \times g$. If a lower centrifuge speed is used, then extend the centrifugation time to compensate.
<i>P2 Buffer and Endo-Wash Buffer precipitation</i>	<ul style="list-style-type: none"> • Both buffers may have precipitated during shipping. To completely resuspend the buffers, incubate the bottles at 30 – 37 °C for 10 – 20 minutes and mix by inversion. DO NOT MICROWAVE.
<i>Wash buffer</i>	<ul style="list-style-type: none"> • Ensure that ethanol has been added to the Plasmid Wash Buffer.
<i>DNA elution</i>	<ul style="list-style-type: none"> • Incomplete elution: For large size plasmids (> 10 kb), add DNA Elution Buffer and incubate the column for 5–10 minutes before centrifugation. Also, pre-warm the DNA Elution Buffer to 50 °C prior to elution. • Ethanol contamination in eluate. Vacuum or centrifuge the Midi Filter to dryness as indicated in the protocol prior to adding the DNA Elution Buffer.
Low DNA Quality	
<i>DNA does not perform well</i>	<ul style="list-style-type: none"> • Incomplete neutralization: Incomplete neutralization generates poor quality supernatant. Ensure that neutralization is complete by inverting the sample an additional 2 – 3 times after the addition of P3 Buffer. • Centrifuge procedure: The Midi Filter tip is contaminated with plasmid wash buffer flow-through. Empty the collection tube when recommended in the protocol.
<i>RNA in eluate</i>	<ul style="list-style-type: none"> • Ensure that RNase A has been added to the P1 Buffer (store at 4 – 8 °C).
<i>Genomic DNA in eluate</i>	<ul style="list-style-type: none"> • 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. Older cultures may contain more genomic DNA contamination than fresh cultures.

Ordering Information:

Product Description	Catalog No.	Kit Size
ZR Plasmid Gigaprep Kit	D4056 D4057	5 preps 10 preps.

For Individual Sale	Catalog No.	Amount
P1 Buffer (Red)	D4027-1-500	500 ml
P2 Buffer (Green)	D4027-2-500	500 ml
P3 Buffer (Yellow)	D4027-3-500	500 ml
Endo-Wash Buffer	D4036-3-60 D4036-3-120	60 ml 120 ml
Plasmid Wash Buffer (concentrate)	D4027-4-24 D4027-4-48	24 ml 48 ml
DNA Elution Buffer	D3004-4-50	50 ml
RNase A	E1008-2 E1008-8 E1008-24	2 mg 8 mg 24 mg
Midi Filter	C1015-2-5 C1015-2-10	5 10
ZymoBeads™	D3004-3-1 D3004-3-20	1 ml 20 ml

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Popular DNA Purification Products from Zymo Research

Product	Format	Kit Size	Cat No.
Fragment DNA Clean-up, Concentration & Recovery			
DNA Clean & Concentrator™-5	Spin Column Format (up to 5 µg/prep.)	50 preps. 200 preps.	D4003*, D4013 D4004*, D4014
DNA Clean & Concentrator™-25	Spin Column Format (up to 25 µg/prep.)	50 preps. 200 preps.	D4005*, D4033 D4006*, D4034
DNA Clean & Concentrator™-100	Spin Column Format (up to 100 µg/prep.)	25 preps. 50 preps.	D4029 D4030
DNA Clean & Concentrator™-500	Spin Column Format (up to 500 µg/prep.)	10 preps. 20 preps.	D4031 D4032
ZR-96 DNA Clean & Concentrator™-5	96-Well Format (up to 5 µg/well; deep well)	2x96 preps. 4x96 preps.	D4023 D4024
Genomic DNA Clean & Concentrator™	Spin Column Format (up to 10 µg/prep.)	25 preps. 100 preps.	D4010 D4011
ZR-96 DNA Clean-up Kit™	96-Well Format (up to 5 µg/well; shallow well)	2x96 preps. 4x96 preps.	D4017 D4018
ZR DNA Sequencing Clean-up Kit™	Spin Column Format (up to 5 µg/prep.)	50 preps. 200 preps.	D4050 D4051
ZR-96 DNA Sequencing Clean-up Kit™	96-Well Format (up to 5 µg/well)	2x96 preps. 4x96 preps.	D4052 D4053
OneStep™ PCR Inhibitor Removal Kit	Spin Column Format (up to 25 µg/prep.)	50 preps.	D6030
OneStep-96™ PCR Inhibitor Removal Kit	96-Well Format (up to 5 µg/well)	2x96 preps.	D6035
Zymoclean™ Gel DNA Recovery Kit	Spin Column Format (up to 5 µg/prep.)	50 preps. 200 preps.	D4001 D4002
ZR-96 Zymoclean™ Gel DNA Recovery Kit	96-Well Format (up to 5 µg/well)	2x96 preps. 4x96 preps.	D4021 D4022
Zymoclean™ Large Fragment DNA Recovery Kit	Spin Column Format (up to 10 µg/prep.)	25 preps. 100 preps.	D4045 D4046
Plasmid DNA Isolation			
Zyppy™ Plasmid Miniprep Kit	Pellet Free, Spin Column Format	50 preps. 100 preps. 400 preps. 800 preps.	D4036 D4019 D4020 D4037
Zyppy™ Plasmid Midiprep Kit	Pellet Free, Spin Column Format	25 preps. 50 preps.	D4025 D4026
Zyppy™ Plasmid Maxiprep Kit	Spin/Vacuum Column Format	10 preps. 20 preps.	D4027 D4028
ZR Plasmid Miniprep™-Classic	Spin Column Format	100 preps. 400 preps. 800 preps.	D4015 D4016 D4054
ZR BAC DNA Miniprep Kit	BAC/PAC plasmid DNA Isolation. Spin Column Format	25 preps. 100 preps.	D4048 D4049
Environmental DNA Isolation			
ZR Soil Microbe DNA MicroPrep™ ZR Soil Microbe DNA MiniPrep™ ZR Soil Microbe DNA MidiPrep™ ZR-96 Soil Microbe DNA Kit™	Bead Bashing, Spin Column Format (up to 5 µg/prep.) Bead Bashing, Spin Column Format (up to 25 µg/prep.) Bead Bashing, Spin Column Format (up to 125 µg/prep.) Bead Bashing, 96-Well Format (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	D6003 D6001 D6101 D6002
ZR Fungal/Bacterial DNA MicroPrep™ ZR Fungal/Bacterial DNA MiniPrep™ ZR Fungal/Bacterial DNA MidiPrep™ ZR-96 Fungal/Bacterial DNA Kit™	Bead Bashing, Spin Column Format (up to 5 µg/prep.) Bead Bashing, Spin Column Format (up to 25 µg/prep.) Bead Bashing, Spin Column Format (up to 125 µg/prep.) Bead Bashing, 96-Well Format (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	D6007 D6005 D6105 D6006
ZR Fecal DNA MicroPrep™ ZR Fecal DNA MiniPrep™ ZR Fecal DNA MidiPrep™ ZR-96 Fecal DNA Kit™	Bead Bashing, Spin Column Format (up to 5 µg/prep.) Bead Bashing, Spin Column Format (up to 25 µg/prep.) Bead Bashing, Spin Column Format (up to 125 µg/prep.) Bead Bashing, 96-Well Format (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	D6012 D6010 D6110 D6011
ZR Tissue & Insect DNA MicroPrep™ ZR Tissue & Insect DNA MiniPrep™ ZR Tissue & Insect DNA MidiPrep™ ZR-96 Tissue & Insect DNA Kit™	Bead Bashing, Spin Column Format (up to 5 µg/prep.) Bead Bashing, Spin Column Format (up to 25 µg/prep.) Bead Bashing, Spin Column Format (up to 125 µg/prep.) Bead Bashing, 96-Well Format (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	D6015 D6016 D6115 D6017
ZR Plant/Seed DNA MicroPrep™ ZR Plant/Seed DNA MiniPrep™ ZR Plant/Seed DNA MidiPrep™ ZR-96 Plant/Seed DNA Kit™	Bead Bashing, Spin Column Format (up to 5 µg/prep.) Bead Bashing, Spin Column Format (up to 25 µg/prep.) Bead Bashing, Spin Column Format (up to 125 µg/prep.) Bead Bashing, 96-Well Format (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	D6022 D6020 D6120 D6021

* Uncapped Spin Column Format

* Bulk quantities are available upon request. Please contact: busdev@zymoresearch.com or call 1-888-882-9682 for assistance.

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