ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR Plasmid Miniprep™-*Classic*

Catalog Nos. **D4015, D4016, & D4054**

Highlights

- For purification of high quality, endotoxin-free plasmid DNA for restriction endonuclease digestion, DNA sequencing, transformation, cloning, transfection, *in vitro* transcription reactions, etc.
- Innovative colored buffers* for rapid identification of complete bacterial cell lysis and neutralization steps.
- Unique column design: zero buffer retention and low volume (30 µl) elution.

Contents

* Patent Pending; For Research Use Only

Ver. 1.0.4

Satisfaction of all Zymo Research products is guaranteed. If you are not satisfied with this product please call 1-888-882-9682.

Product Contents

ZR Plasmid Miniprep™-*Classic* (Kit Size)	D4015 (100 preps.)	D4016 (400 preps.)	D4054 (800 preps.)	Storage Temperature
P1 Buffer (Red)	20 ml	80 ml	160 ml	Room Temp.
P2 Buffer[1] (Green)	20 ml	80 ml	160 ml	Room Temp.
P3 Buffer[2] (Yellow)	50 ml	220 ml	2 x 220 ml	4° after opening.
Endo-Wash Buffer	30 ml	2 x 60 ml	3 x 60 ml	Room Temp.
Plasmid Wash Buffer (concentrate)[2]	24 ml	48 ml	2 x 48 ml	Room Temp.
DNA Elution Buffer	4 ml	16 ml	2 x 16 ml	Room Temp.
Zymo-Spin™ IIN Columns	100	400	800	Room Temp.
Collection Tubes	100	400	800	Room Temp.
Instruction Manual	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] Caution: **P2 Buffer** contains NaOH and **P3 Buffer** contains chaotropic reagents. Please use proper safety precautions with these reagents.

[2] Add ethanol to **Plasmid Wash Buffer** (concentrate) prior to use. See **Buffer Preparation** (page 3) for instructions.

Specifications:

- **DNA Purity:** High purity, endotoxin-free (<50 EU/µg) plasmid DNA eluted in low salt buffer or water; typical $A_{(260/280)}$ ≥1.8. DNA is suitable for restriction endonuclease digestion, sequencing, transfection, ligation, *in vitro* transcription, labeling, and other reactions requiring highly purified DNA.
- **Recovery Volume:** ≥30 µl
- **Plasmid DNA Size:** Up to 25 kb
- **Plasmid DNA yield:** Up to 25 µg per preparation depending on the plasmid copy number, input volume of *E. coli* culture, and culture growth conditions.
- **Procedure:** Can be conducted at room temperature, between 15 - 30°C.

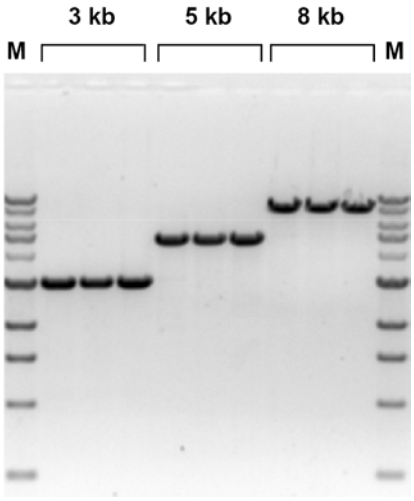
Note: ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety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

Several ZR Plasmid Miniprep™-*Classic* product technologies are subject to U.S. and foreign patents or are patent pending.

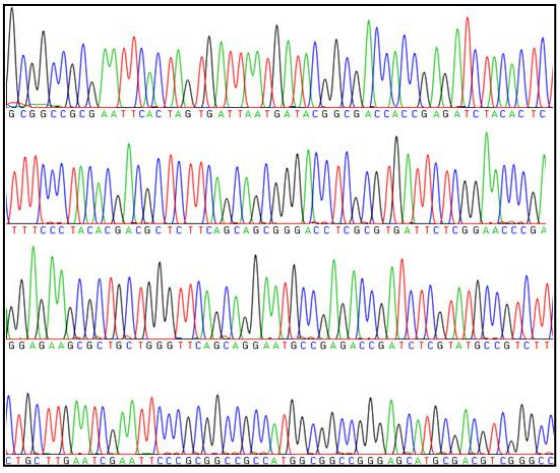
Product Description

The **ZR Plasmid Miniprep™-*Classic*** kit is designed for efficient isolation of plasmid DNA from *E. coli* cell lysates using a procedure that is simple, rapid, user-friendly, and reliable compared to the products offered by the competition. It features a modified alkaline lysis protocol together with a unique *Fast Spin* column to yield high quality plasmid DNA in minutes. The ZR Plasmid Miniprep™-*Classic* features color-coded (red, green, yellow) reagents for easy determination of complete cell lysis. The innovative **Zymo-Spin™ IIN** columns facilitate high yield plasmid DNA that is endotoxin-free. Plasmid DNA purified using the ZR Plasmid Miniprep™-*Classic* kit is well suited for use in restriction endonuclease digestion, sequencing, DNA ligation, cloning, PCR, bacterial transformation, transfection, etc.

Visualize complete bacterial cell lysis with unique colored **P1**, **P2**, and **P3** buffers.



Endonuclease digestion of three different DNA plasmids prepared using the **ZR Plasmid Miniprep™-*Classic*** (performed in triplicate). **M:** ZR 1 kb DNA Marker.



DNA sequencing chromatogram of plasmid DNA prepared using the **ZR Plasmid Miniprep™-*Classic*.**

The Zymo-Spin™ IIN Column: binds up to 25 µg DNA, 900 µl capacity, and 30 µl elution volume.

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

Buffer Preparation:

1. Add ethanol to the **Plasmid Wash Buffer** at a 4:1 volume ratio of ethanol to buffer.
   - For product **D4015**, add 96 ml 95 % ethanol to 24 ml **Plasmid Wash Buffer**.
   - For product **D4016**, add 192 ml 95 % ethanol to 48 ml **Plasmid Wash Buffer**.
   - For product **D4054**, add 192 ml 95 % ethanol to each 48 ml **Plasmid Wash Buffer**.

Protocol[1]

1. Centrifuge 0.5 - 5 ml[1,2] of bacterial culture in a clear 1.5 ml tube at full speed for 15 - 20 seconds in a microcentrifuge. Discard supernatant.
2. Add 200 µl of **P1 Buffer** (Red) to the tube and resuspend pellet completely (i.e., by vortexing or pipeting).
3. Add 200 µl of **P2 Buffer** (Green)[3] and mix by inverting the tube 2 - 4 times. Cells are completely lysed when the solution appears clear, purple, and viscous. Proceed to the next step within 1-2 minutes.
4. Add 400 µl of **P3 Buffer** (Yellow) and mix gently but thoroughly. Do not vortex. The sample will turn yellow when the neutralization is complete[4]. Allow the lysate to incubate at room temperature for 1-2 minutes.
5. Centrifuge sample(s) for 2 minutes.
6. Place a **Zymo-Spin™ IIN** column in a **Collection Tube** and transfer the supernatant from Step 5 into the **Zymo-Spin™ IIN** column. When pipeting the supernatant be careful not to disturb the green pellet to avoid transferring any cellular debris to the column.
7. Centrifuge the **Zymo-Spin™ IIN/Collection Tube** assembly for 30 seconds.
8. Discard the flow-through in the **Collection Tube**, making sure the flow-through does not touch the bottom of the column. Return the **Zymo-Spin™ IIN** column to the **Collection Tube**[5].
9. Add 200 µl of **Endo-Wash Buffer** to the column and centrifuge for 30 seconds.
10. Add 400 µl of **Plasmid Wash Buffer**[6] to the column**.** Centrifuge for 1 minute.
11. Transfer the column into a clean 1.5 ml microcentrifuge tube and then add 30 µl (of **DNA Elution Buffer**[7] to the column. Centrifuge for 30 seconds to elute the plasmid DNA.

Notes:

[1] The following procedures are carried out at a room temperature. All centrifugation steps should be performed between 11,000 - 16,000 x *g*.

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

[3] Excessive lysis can result in denatured plasmid DNA formation. When processing a large number of samples, work with groups of ≤ 10 at a time.

[4] A green precipitate consisting of K·SDS and cell debris will form. A good way to mix is to shake the tube gently several times while it is inverted.

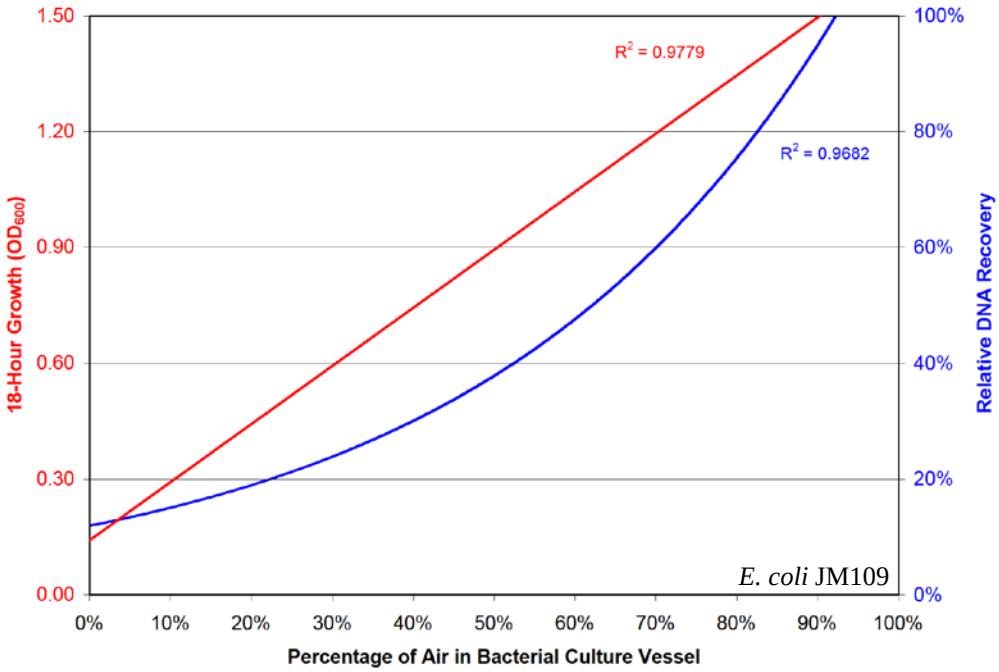
[5] The capacity of the collection tube with the column inserted is 800 µl. Empty the collection tube whenever necessary to prevent contamination of the spin column with the flow-through.

[6] Ensure that ethanol has been added to the concentrated **Plasmid Wash Buffer** prior to use.

[7] The **DNA Elution Buffer** contains 10 mM Tris·HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can be used to elute the DNA. Add the **DNA Elution Buffer** directly to the center of the **Zymo-Spin™ IIN** column matrix to ensure optimal DNA elution.

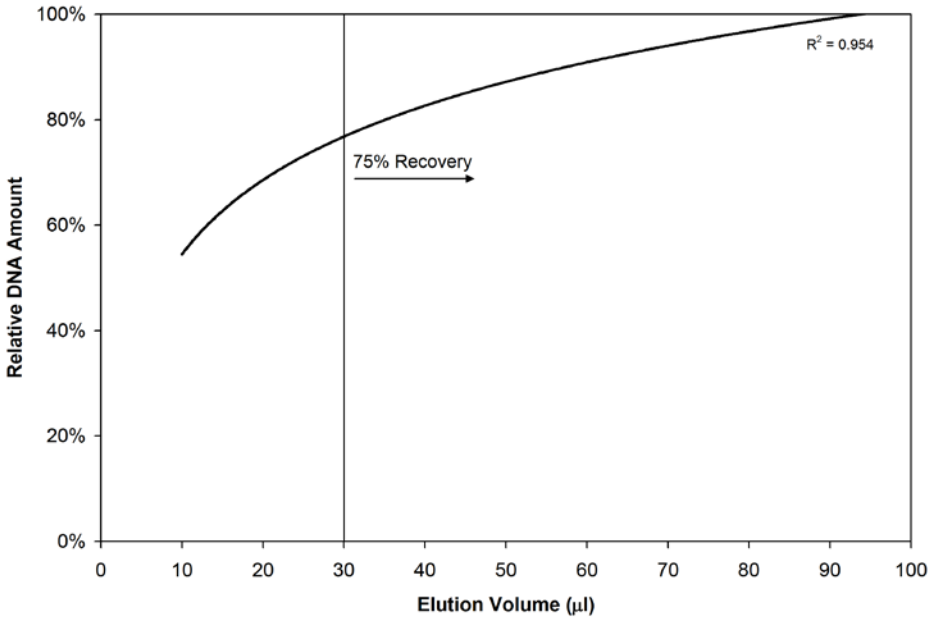
Troubleshooting Guide:

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 1:4 or less (20% culture, 80% air). For best aeration, use baffled culture flasks, a vented or gas-permeable seal on the culture vessel, and incubate with vigorous shaking.
	• Other Possible reasons may include: An overgrown/undergrown or contaminated culture, or omission of antibiotics from the growth medium. Use a fresh culture for optimal performance. Grow the culture to an $O.D._{600}$ >1.0.
Procedural errors	• Incomplete lysis: After addition of P2 Buffer the solution should change from opaque red to clear purple, indicating complete lysis. Different *E. coli* 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.
Plasmid Wash Buffer	• Ensure that ethanol has been added to the wash buffer.



DNA elution

- Incomplete elution: For large size plasmids (> 10 kb), incubate the column for 5 - 10 minutes before centrifugation. Also, pre-warm the DNA Elution Buffer to 50 ºC prior to elution and increase the elution volume to ≥ 50 µl.



Low DNA Quality

DNA does not perform well

- Incomplete neutralization generates poor quality supernatant and results in loading too much cell debris onto the column. Ensure that neutralization is complete by inverting the sample an additional 2 - 3 times after the addition of P3 Buffer.
- The spin column tip is contaminated with wash buffer flowthrough. Avoid tilting the collection tube after the last wash step to ensure that the column tip does not contact the flowthrough. Empty the collection tube when recommended in the protocol.
- Insufficient centrifugation: make sure that all centrifugation steps are performed between 11,000 - 16,000 x *g*. If a lower centrifuge speed is used, then extend the centrifugation time to compensate.

RNA in eluate

- After neutralization, be sure to allow lysate to incubate 1-2 minutes before centrifugation.

Genomic DNA in eluate

- Improper handling (sample was vortexed or handled too roughly after the addition of P2 & P3 Buffer). 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	Kit Size	Catalog No.
ZR Plasmid Miniprep™-*Classic*	100 preps. 400 preps. 800 preps.	D4015 D4016 D4054

For Individual Sale	Amount	Catalog No.
P1 Buffer (Red)	20 ml 80 ml 160 ml	D4027-1-20 D4027-1-80 D4027-1-160
P2 Buffer (Green)	20 ml 80 ml 160 ml	D4027-2-20 D4027-2-80 D4027-2-160
P3 Buffer (Yellow)	50 ml 220 ml 440 ml	D4027-3-50 D4027-3-220 D4027-3-440
Endo-Wash Buffer	30 ml 60 ml	D4036-3-30 D4036-3-60
Plasmid Wash Buffer (concentrate)	24 ml 48 ml	D4027-4-24 D4027-4-48
DNA Elution Buffer	4 ml 10 ml 16 ml	D3004-4-4 D3004-4-10 D3004-4-16
Zymo-Spin IIN™ Columns	50 columns 250 columns	C1019-50 C1019-250
Collection Tubes	50 tubes 500 tubes 1000 tubes	C1001-50 C1001-500 C1001-1000

Other 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)	2 x 96 preps. 4 x 96 preps.	**D4023** **D4024**
ZR-96 DNA Clean-up Kit™	96-Well Format (up to 5 µg/well; shallow well)	2 x 96 preps. 4 x 96 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)	2 x 96 preps. 4 x 96 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)	2 x 96 preps. 4 x 96 preps.	**D4021** **D4022**
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**
Genomic DNA Isolation			
ZR Genomic DNA I Kit™	Silica Bead Format - Scaleable	100 preps. 400 preps.	**D3004** **D3005**
ZR Genomic DNA II Kit™	Spin Column Format (up to 25 µg/prep)	50 preps. 200 preps.	**D3006*, D3024** **D3007*, D3025**
ZR-96 Genomic DNA Kit™	96-Well Format (up to 5 µg/well)	2 x 96 preps. 4 x 96 preps. 10 x 96 preps.	**D3010** **D3011** **D3012**
ZR Genomic DNA™-Tissue MiniPrep	Spin Column Format (up to 25 µg/prep)	50 preps. 200 preps.	**D3050** **D3051**
ZR-96 Genomic DNA™-Tissue MiniPrep	96-Well Format (up to 5 µg/well)	2 x 96 preps. 4 x 96 preps. 10 x 96 preps.	**D3055** **D3056** **D3057**
Pinpoint™ Slide DNA Isolation System	For Archived Tissue Sections, Spin Column Format (up to 5 µg/prep)	50 preps.	**D3001**
ZR Serum DNA Kit™	Silica Bead Format - Scaleable	scaleable	**D3013**
ZR Urine DNA Isolation Kit™	Filtration, Spin Column Format (up to 5 µg/prep)	20 preps.	**D3060**
ZR Viral DNA Kit™	Spin Column Format (up to 5 µg/prep)	50 preps. 200 preps.	**D3015** **D3016**
ZR-96 Viral DNA Kit	96-Well Format (up to 5 µg/well)	2 x 96 preps. 4 x 96 preps.	**D3017** **D3018**
Environmental DNA Isolation			
ZR Soil Microbe DNA Kit™	Bead Bashing, Spin Column Format (up to 25 µg/prep)	50 preps.	**D6001**
ZR-96 Soil Microbe DNA Kit™	Bead Bashing, 96-Well Format (up to 5 µg/well)	2 x 96 preps.	**D6002**
ZR Fungal/Bacterial DNA Kit™	Bead Bashing, Spin Column Format (up to 25 µg/prep)	50 preps.	**D6005**
ZR-96 Fungal/Bacterial DNA Kit™	Bead Bashing, 96-Well Format (up to 5 µg/well)	2 x 96 preps.	**D6006**
ZR Fecal DNA Kit™	Bead Bashing, Spin Column Format (up to 25 µg/prep)	50 preps.	**D6010**
ZR-96 Fecal DNA Kit™	Bead Bashing, 96-Well Format (up to 5 µg/well)	2 x 96 preps.	**D6011**
ZR Tissue & Insect DNA Kit-5™	Bead Bashing, Spin Column Format (up to 5 µg/prep)	50 preps.	**D6015**
ZR Tissue & Insect DNA Kit-25™	Bead Bashing, Spin Column Format (up to 25 µg/prep)	50 preps.	**D6016**
ZR-96 Tissue & Insect DNA Kit™	Bead Bashing, 96-Well Format (up to 5 µg/well)	2 x 96 preps.	**D6017**
ZR Plant/Seed DNA Kit™	Bead Bashing, Spin Column Format (up to 25 µg/prep)	50 preps.	**D6020**
ZR-96 Plant/Seed DNA Kit™	Bead Bashing, 96-Well Format (up to 5 µg/well)	2 x 96 preps.	**D6021**

* Uncapped Spin Column Format