ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR BAC DNA Miniprep Kit

Catalog Nos. **D4048** & **D4049**

Highlights

- For spin column purification of endotoxin-free BAC/PAC plasmid DNA (up to ~200 kb) for sequencing, PCR, endonuclease digestion, etc. *No messy precipitations!*
- Innovative colored buffers for rapid identification of complete bacterial cell lysis and neutralization steps.
- Unique column design: zero buffer retention and low-volume (≥10 µl) elution.

Contents

Product Contents

ZR BAC DNA Miniprep Kit (Kit Size)	D4048 (25 preps.)	D4049 (100 preps.)	Storage Temperature
P1 Buffer (Red)	10 ml	20 ml	Room Temp.
P2 Buffer[1] (Green)	10 ml	20 ml	Room Temp.
P3 Buffer[1,2] (Yellow)	12 ml	50 ml	0-4ºC
Endo-Wash Buffer	6 ml	30 ml	Room Temp.
Plasmid Wash Buffer (concentrate)[3]	6 ml	12 ml	Room Temp.
DNA Elution Buffer	1 ml	4 ml	Room Temp.
Zymo-Spin™ IC-XL Columns	25	100	Room Temp.
Collection Tubes	50	100	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] Caution: **P2 Buffer** contains NaOH and **P3 Buffer** contains chaotropic reagents. Please use proper safety precautions with these reagents.

[2] **P3 Buffer** contains RNase A (200 µg/ml).

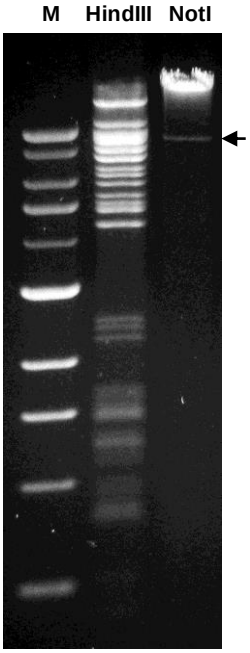
[3] Add ethanol to **Plasmid Wash Buffer** (concentrate) prior to use. See **Buffer Preparation** (page 3) for instructions.

Specifications:

- **DNA Purity:** High-purity and endotoxin-free (<50 EU/µg) plasmid DNA in low salt buffer or water; typical $A_{(260/280)}$ ≥1.8. Eluted DNA is suitable for sequencing, endonuclease digestion, PCR, and other reactions requiring highly purified DNA.
- **Recovery Volume:** ≥10 µl.
- **Plasmid DNA Size:** Up to 200 kb.
- **Plasmid DNA yield:** Up to 10 µg per preparation, depending on the plasmid copy number, initial volume of *E. coli* culture processed, and culture growth conditions. The typical yield is 1 µg per 3 ml of culture.
- **Procedure:** Can be conducted at room temperature, between 15-30°C.

Product Description

The **ZR BAC DNA Miniprep Kit** is for efficient isolation of BAC plasmid DNA or other large plasmids (e.g., PAC) from *E. coli* cell lysates using a procedure that is simple, rapid, user-friendly, and reliable compared to kits offered from other suppliers. It features a modified alkaline lysis protocol together with unique spin column matrix technology to yield high quality plasmid DNA in minutes. The **ZR BAC DNA Miniprep Kit** features color-coded reagents that allow easy visualization and assessment of complete bacterial cell lysis and buffer neutralization. The innovative **Zymo-Spin™ IC-XL** columns are optimized for high yield endotoxin-free plasmid DNA recovery. BAC DNA purified using the **ZR BAC DNA Miniprep Kit** is well suited for sequencing, PCR, endonuclease digestion, etc.



Digestion of BAC DNA with HindIII and NotI restriction endonucleases. A BAC clone (~160 kb) from a RPCI-11 human BAC library (CHORI) was purified from DH10B electrocompetent cells (Invitrogen) using the **ZR BAC DNA Miniprep Kit**. Digestion with NotI removed the insert (~148 kb) from the pBACe3.6 cloning vector[1] (11.6 kb) indicated by the (◄) in the gel image above. M is a 1 kb DNA ladder (Zymo Research). DNA was resolved in a 0.6% w/v agarose/TAE/EtBr gel for 12 hrs. at 40V.

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

Reference:

[1] Osoegawa, *et al.* (1998) Genomics 52, 1-8.

Buffer Preparation:

- Add 24 ml 100% ethanol to the 6 ml **Plasmid Wash Buffer** concentrate (48 ml ethanol to the 12 ml **Plasmid Wash Buffer** concentrate).

Protocol

Note: unless stated otherwise, all centrifugation steps should be performed between 10,000-16,000 x g.

1. Spin down 0.5 – 5 ml of bacterial culture to a pellet at 5,000-6,000 x *g* for 5 minutes in an appropriate tube and centrifuge. Discard supernatant.
2. Add 200 µl of **P1 Buffer** (Red) to the tube and resuspend pellet completely (i.e., by vortexing or pipetting). Transfer to a 1.5 ml microcentrifuge tube.
3. Add 200 µl of **P2 Buffer** (Green)[1] and mix by inverting the tube 4-6 times. Cells are completely lysed when the solution appears clear, purple, and viscous. Proceed to the next step within 3 minutes. Wait 1-3 minutes before proceeding to Step 4.
4. Add 400 µl of **P3 Buffer** (Yellow) and mix gently but thoroughly. **Do not vortex vigorously.** The sample will turn yellow when the neutralization is complete[2]. Wait 1-2 minutes before proceeding to Step 5.
5. Centrifuge sample(s) in a microcentrifuge for 3 minutes.
6. Place a **Zymo-Spin™ IC-XL** column into a **Collection Tube** and transfer the supernatant from Step 5 into the **Zymo-Spin™** column. When transferring the supernatant avoid carrying any cellular debris to the column and be careful not to disturb the green pellet.
7. Centrifuge the **Zymo-Spin™/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™** column to the **Collection Tube**[3].
9. Add 200 µl of **Endo-Wash Buffer** to the column and centrifuge for 15 seconds.
10. Add 400 µl of **Plasmid Wash Buffer**[4] to the column**.** Centrifuge for 30 seconds. Empty the **Collection Tube** and centrifuge for an additional minute.
11. Transfer the column into a clean 1.5 ml microcentrifuge tube and then add ≥10 µl of **DNA Elution Buffer**[6] to the column. Centrifuge for 30 seconds to elute the DNA.

Notes:

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

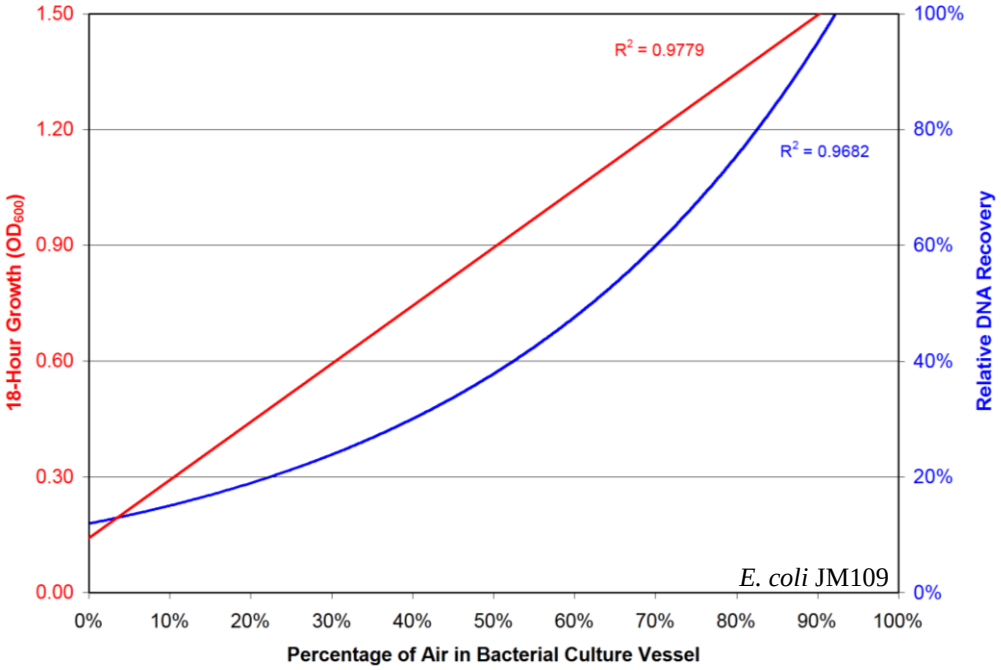
[2] A green precipitate consisting of K·SDS and cell debris will form. A good way to mix is to shake the tube gently several times while it is inverted.

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

[4] Ensure that ethanol has been added to the concentrated **Plasmid Wash Buffer**. See **Buffer Preparation** (above) for instructions.

[5] 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**™ column matrix for optimal plasmid 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 concentrate.

DNA elution

- Incomplete elution: pre-warming the DNA Elution Buffer to 50 ºC prior to elution can increase the yield of some DNAs.

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 10,000-16,000 *x g*. If a lower centrifuge speed is used, then extend the centrifugation time to compensate.

RNA in eluate

- P3 buffer contains RNase A. Prior to lysate filtration, ensure that RNase A has enough time to degrade RNA by allowing lysate to incubate at room temperature an additional 3-5 minutes after neutralization.
- Ensure that P3 buffer has been stored at 4-8 ºC.

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 BAC DNA Miniprep Kit	25 preps. 100 preps.	D4048 D4049

For Individual Sale	Amount	Catalog No.
P1 Buffer (Red)	10 ml 20 ml 80 ml 160 ml	D4027-1-10 D4027-1-20 D4027-1-80 D4027-1-160
P2 Buffer (Green)	10 ml 20 ml 80 ml 160 ml	D4027-2-10 D4027-2-20 D4027-2-80 D4027-2-160
P3 Buffer (Yellow)	12 ml 50 ml 220 ml 440 ml	D4027-3-12 D4027-3-50 D4027-3-220 D4027-3-440
Endo-Wash Buffer	6 ml 15 ml 30 ml 60 ml	D4036-3-6 D4036-3-15 D4036-3-30 D4036-3-60
Plasmid Wash Buffer (concentrate)	6 ml 12 ml 24 ml 48 ml	D4027-4-6 D4027-4-12 D4027-4-24 D4027-4-48
DNA Elution Buffer	4 ml 10 ml 16 ml	D3004-4-4 D3004-4-10 D3004-4-16
Collection Tubes	50 tubes 500 tubes 1000 tubes	C1001-50 C1001-500 C1001-1000

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**
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**
Plasmid DNA Isolation			
Zyppy™ Plasmid Miniprep Kit	Pellet Free, Spin Column Format	50 preps. 100 preps. 400 preps.	**D4036** **D4019** **D4020**
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)	2x96 preps. 4x96 preps. 10x96 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)	2x96 preps. 4x96 preps. 10x96 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)	2x96 preps. 4x96 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)	2x96 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)	2x96 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)	2x96 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)	2x96 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)	2x96 preps.	**D6021**

* Uncapped Spin Column Format