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Zymo Plasmid Miniprep - Classic - CHEM 584

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ABSTRACT

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. 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 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.

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GUIDELINES

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

MATERIALS TEXT

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.

MINIPREP

1 Centrifuge **500 µl** - **5 mL** of bacterial culture in a clear 1.5 ml tube at full speed for 15 - 20 seconds in a microcentrifuge. Discard supernatant.

Depending on the volume of bacterial culture it may be necessary to repeat Step 1 several times. You can also centrifuge larger culture volumes (e.g., 5 mL) in a 15 mL centrifuge tube, then transfer to a microcentrifuge tube following step 2.

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) 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.

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 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. Allow the lysate to incubate at room temperature for 1-2 minutes **00:02:00** .

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.

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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 pipetting 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.

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.

9 Add **200 µl** of Endo-Wash Buffer to the column and centrifuge for 30 seconds.

10 Add **400 µl** of Plasmid Wash Buffer 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 to the column. Centrifuge for 30 seconds to elute the plasmid DNA.

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

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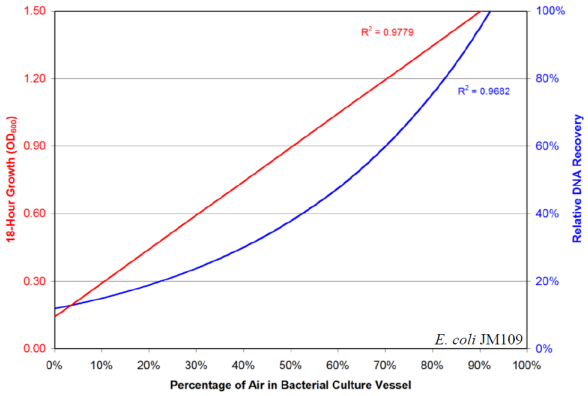
12 **Troubleshooting Guide:**

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.

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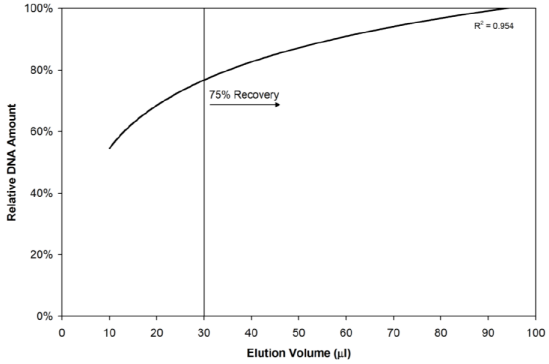
DNA elution

- 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.

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