ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Zyppy™ Plasmid Miniprep Kit

Catalog Nos. **D4036, D4019, D4020 & D4037** (Patent Pending)

Highlights

- Pellet-Free™ procedure* omits conventional cell-pelleting and resuspension steps.
- The fastest, simplest procedure for purifying the highest quality endotoxin-free plasmid DNA.
- Innovative colored buffers* permit error-free visual identification of complete bacterial cell lysis and neutralization.

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* Patent pending; For Research Use Only

Ver. 1.2.6

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:

Zyppy™ Plasmid Miniprep Kit	D4036 50 preps.	D4019 100 preps.	D4020 400 preps.	D4037 800 preps.	Storage Temperature
7X Lysis Buffer*[1] (Blue)	6 ml	12 ml	48 ml	2 x 48 ml	Room Temp.
Neutralization Buffer*[2] (Yellow)	20 ml	40 ml	160 ml	2 x 160 ml	4-8 ºC
Endo-Wash Buffer	15 ml	30 ml	120 ml	2 x 120 ml	Room Temp.
Zyppy™ Wash Buffer (concentrate)[3]	6 ml	12 ml	48 ml	2 x 48 ml	Room Temp.
Zyppy™ Elution Buffer	5 ml	5 ml	20 ml	2 x 20 ml	Room Temp.
Zymo-Spin™ IIN Columns	50	2 x 50	8 x 50	16 x 50	Room Temp.
Collection Tubes	50	2 x 50	8 x 50	16 x 50	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] The 7X Lysis Buffer may have precipitated during shipping. To completely resuspend the buffer, incubate the bottle at 30 – 37 ºC for 30 minutes and mix by inversion. DO NOT MICROWAVE.

[2] Neutralization Buffer contains RNase A at a concentration of 200 µg/ml.

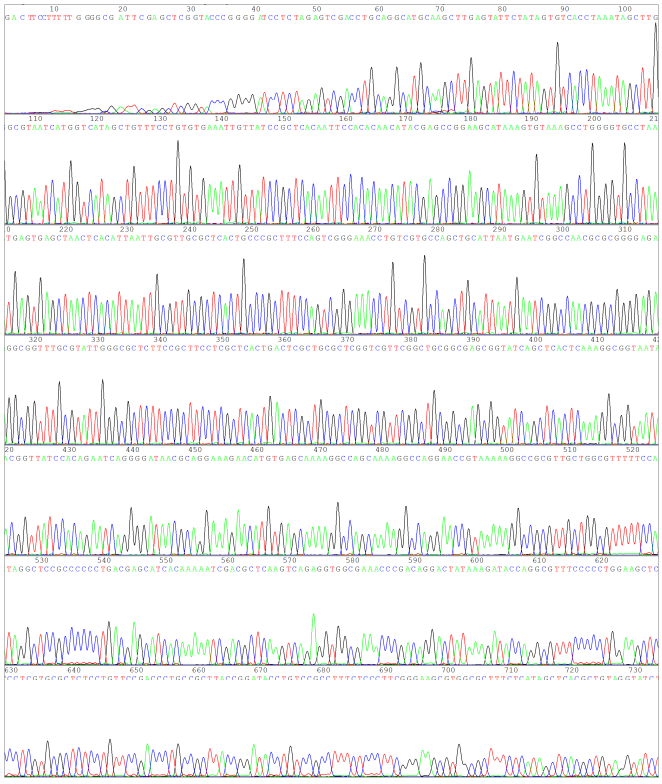
[3] Add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml Zyppy™ Wash Buffer concentrate (D4036), 48 ml of 100% ethanol (52 ml of 95% ethanol) to the 12 ml Zyppy™ Wash Buffer concentrate (D4019), or 192 ml of 100% ethanol (208 ml of 95% ethanol) to the 48 ml Zyppy™ Wash Buffer concentrate (D4020 & D4037) before use.

* Caution: 7X Lysis Buffer contains NaOH and Neutralization Buffer contains chaotropic reagents. Please use proper safety precautions with these reagents.

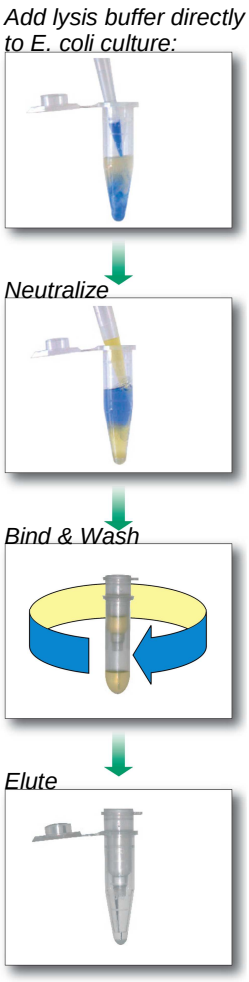
Product Description:

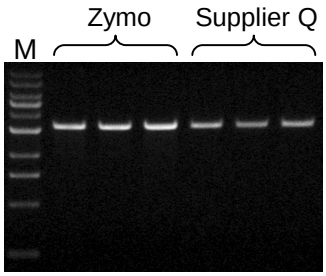
The **Zyppy™ Plasmid Miniprep Kit** features a **Pellet-Free™** modified alkaline lysis method that bypasses bacterial culture centrifugation and resuspension steps common to classical plasmid preparation procedures. Simply add the uniquely formulated **7X Lysis Buffer** *directly to your bacterial culture*, neutralize, then purify using our *Fast-Spin* column technology (alternatively, the samples may be processed by the classical centrifugation method). Additionally, the innovative colored buffers included in the kit permit error-free visualization identification of complete bacterial cell lysis and neutralization.

Our **Zyppy™ Plasmid Miniprep Kit** is the *fastest* and *simplest* method available to efficiently separate plasmid DNA from *E. coli*. The plasmid DNA is of the *highest quality*, is endotoxin-free, and is well suited for use in bacterial transformation, restriction endonuclease digestion (below right), DNA ligation, PCR, transcription, sequencing (below), and other sensitive downstream applications. An overview of the purification procedure is shown to the right.



Sequencing chromatogram of plasmid DNA pGEM® purified using the **Zyppy™ Plasmid Miniprep Kit**. The DNA was labeled with ABI BigDye v3.1 terminators, cleaned using the **ZR DNA Sequencing Clean-up Kit™ (D4050, D4051)** and sequenced on an ABI 3730*xl* DNA analyzer.





*Eco*RI digestion of plasmid DNA (pGEM®) isolated from a 600 µl *E. coli* culture using the **Zyppy™ Plasmid Miniprep Kit** or a kit from Supplier Q. Performed in triplicate. M, ZR 1 kb DNA Marker.

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

Note:

pGEM® is a registered trademark of Promega Corporation.

Specifications:

- **DNA Purity:** Plasmid DNA is well suited for 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:** Up to 25 µg per preparation, depending on the plasmid copy number, culture growth conditions, and strain of *E. coli* utilized.
- **Plasmid DNA Size:** Up to 25 kb.
- **Recovery Volume:** ≥ 30 µl.
- **Procedure:** Performed at room temperature (15-30℃).

Buffer Preparation:

1. Add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml **Zyppy™ Wash Buffer** concentrate (D4036), 48 ml of 100% ethanol (52 ml of 95% ethanol) to the 12 ml **Zyppy™ Wash Buffer** concentrate (D4019), or 192 ml of 100% ethanol (208 ml of 95% ethanol) to the 48 ml **Zyppy™ Wash Buffer** concentrate (D4020 & D4037) before use.
2. The **7X Lysis Buffer** may have precipitated during shipping. To completely resuspend the buffer, incubate the bottle at 30 – 37 ºC for 30 minutes and mix by inversion. DO NOT MICROWAVE.

Protocol:

The following procedure is performed at room temperature.
Ensure that buffers have been prepared according to the instructions on *page 3*.

1. Add 600 µl of bacterial culture grown in LB medium to a 1.5 ml microcentrifuge tube.

   *The **Zyppy™ Plasmid Miniprep Kit** may also be used with the classical centrifuge-based procedure for processing up to 3 ml of bacterial culture. The procedure should be modified as follows: 1A) Centrifuge 1.5 ml of bacterial culture for 30 seconds at maximum speed. 1B) Discard the supernatant. 1C) Repeat steps 1A and 1B as needed. 1D) Add 600 µl of TE or water to the bacterial cell pellet and resuspend completely.*

2. Add 100 µl of **7X Lysis Buffer (Blue)**[1] and mix by inverting the tube 4-6 times. Proceed to step 3 within 2 minutes.

   After addition of 7X Lysis Buffer the solution should change from opaque to clear blue, indicating complete lysis.

3. Add 350 µl of cold **Neutralization Buffer (Yellow)** and mix thoroughly. *The sample will turn yellow when the neutralization is complete and a yellowish precipitate will form.* **Invert the sample an additional 2-3 times** to ensure complete neutralization.

4. Centrifuge at 11,000 – 16,000 x *g* for 2-4 minutes.

5. Transfer the supernatant (~900 µl) into the provided **Zymo-Spin™ IIN** column. Avoid disturbing the cell debris pellet.

6. Place the column into a **Collection Tube** and centrifuge for 15 seconds.

7. Discard the flow-through and place the column back into the same **Collection Tube**.

8. Add 200 µl of **Endo-Wash Buffer** to the column. Centrifuge for 30 seconds. *It is not necessary to empty the collection tube.*

9. Add 400 µl of **Zyppy™ Wash Buffer** to the column. Centrifuge for 1 minute.

10. Transfer the column into a clean 1.5 ml microcentrifuge tube then add 30 µl of **Zyppy™ Elution Buffer**[2] directly to the column matrix and let stand for one minute at room temperature.

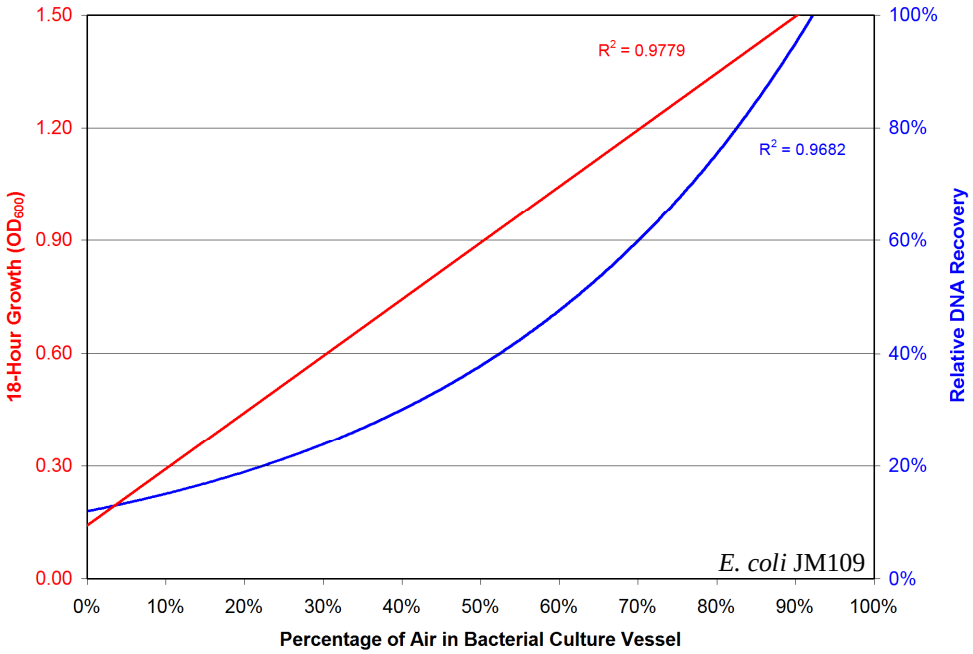
11. Centrifuge for 30 seconds to elute the plasmid DNA.

Notes:

[1] Excessive lysis can result in denatured plasmid DNA. If processing a large number of samples, we recommend working with groups of ten or less at a time. Continue with the next set of ten samples after the first set has been neutralized and mixed thoroughly.

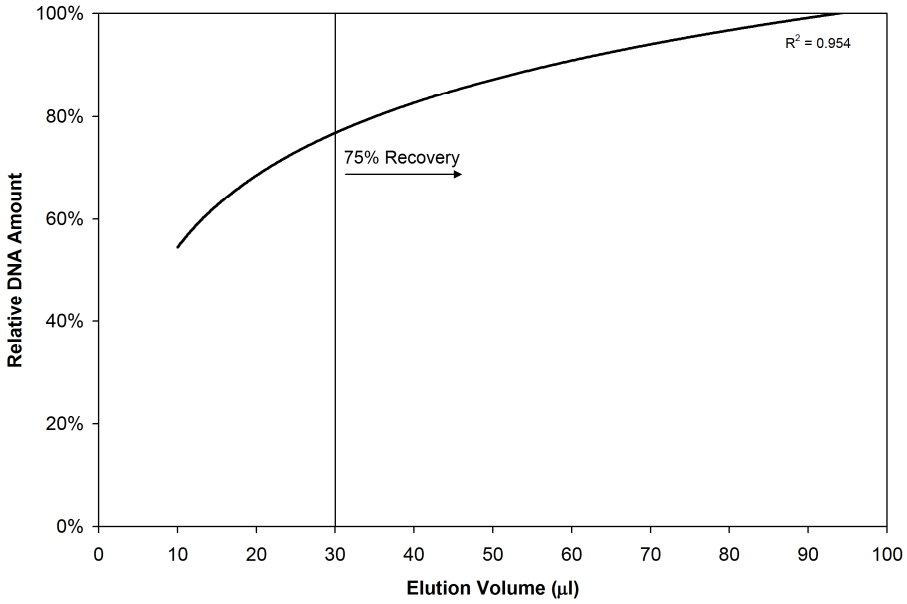
[2] The Zyppy™ Elution Buffer contains 10 mM Tris-HCl, pH 8.5 and 0.1 mM EDTA. If required, pure water (neutral pH) can also be used to elute the DNA.

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.
	
	• Incorrect culture medium. LB medium is recommended for use with the Direct Culture Lysis method. Other culture media are not recommended for direct lysis, but can be used with the classical pellet-based procedure. • 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 7X Lysis Buffer the solution should change from opaque to clear blue, 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 Neutralization Buffer.
7X Lysis Buffer precipitation	• The 7X Lysis Buffer may have precipitated during shipping. To completely resuspend the buffer, incubate the bottle at 30 – 37 ºC for 30 minutes and mix by inversion. DO NOT MICROWAVE.
Zyppy™ 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 Zyppy™ 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: 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 Neutralization 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

- Ensure that P3 Buffer is stored at 4 - 8 ºC.

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. Older cultures may contain more genomic DNA contamination than fresh cultures.

Ordering Information:

Product Description	Kit Size	Catalog No.
Zyppy™ Plasmid Miniprep Kit	50 preps. 100 preps. 400 preps. 800 preps.	D4036 D4019 D4020 D4037

For Individual Sale	Amount	Catalog No.
7X Lysis Buffer (Blue)	6 ml 12 ml 30 ml 48 ml 60 ml	D4036-1-6 D4036-1-12 D4036-1-30 D4036-1-48 D4036-1-60
Neutralization Buffer (Yellow)	20 ml 40 ml 100 ml 160 ml 200 ml	D4036-2-20 D4036-2-40 D4036-2-100 D4036-2-160 D4036-2-200
Endo-Wash Buffer	15 ml 30 ml 60 ml 120 ml 240 ml	D4036-3-15 D4036-3-30 D4036-3-60 D4036-3-120 D4036-3-240
Zyppy™ Wash Buffer (concentrate)	6 ml 12 ml 24 ml 48 ml	D4036-4-6 D4036-4-12 D4036-4-24 D4036-4-48
Zyppy™ Elution Buffer	5 ml 10 ml 20 ml 30 ml 60 ml	D4036-5-5 D4036-5-10 D4036-5-20 D4036-5-30 D4036-5-60
Zymo-Spin™ IIN Columns	50 250	C1019-50 C1019-250
Collection Tubes	50 500 1000	C1001-50 C1001-500 C1001-1000

Popular DNA Purification & Analysis Products from Zymo

Product	Description	Kit Size (Preps)	Catalog No. (Column Format)
DNA Clean & Concentrator™-5	Clean & concentrate DNA from any reaction or "crude" preparation in 2 minutes. A 6 µl minimum elution volume allows for highly concentrated DNA. Designed for samples containing up to 5 µg of DNA.	50 200 50 200	**D4003** (uncapped) **D4004** (uncapped) **D4013** (capped) **D4014** (capped)
DNA Clean & Concentrator™-25	Clean & concentrate DNA in minutes. 25 µl minimum elution volume allows for highly concentrated DNA. Designed for purifying up to 25 µg of DNA.	50 200	**D4005** **D4006**
DNA Clean & Concentrator™-100	Clean & concentrate DNA in minutes. 100 µl minimum elution volume allows for highly concentrated DNA. Designed for purifying up to 100 µg of DNA.	25 50	**D4029** **D4030**
DNA Clean & Concentrator™-500	Clean & concentrate DNA in minutes. 1 ml minimum elution volume allows for highly concentrated DNA. Designed for samples containing up to 500 µg of DNA.	10 20	**D4031** **D4032**
ZR-96 DNA Clean & Concentrator™-5	Quick (15 minute), high-output recovery of pure DNA from PCR, endonuclease digestions, plasmid preparations, etc. 10-15 µl minimum elution volume allows for highly concentrated DNA. Designed for samples containing up to 5 µg of DNA.	2x96 4x96	**D4023** **D4024**
Zymoclean™ Gel DNA Recovery Kit	Purify DNA from high and low-melting agarose gels in minutes	50 200	**D4001** **D4002**
ZR-96 Zymoclean™ Gel DNA Recovery Kit	High-throughput DNA purification from high and low-melting agarose gels.	2x96 4x96	**D4021** **D4022**
Pinpoint™ Slide DNA Isolation System	Recover genomic DNA from paraffin-embedded or fresh tissue sections for PCR. Ideal for isolating DNA from clinical tissue samples.	50	**D3001**
Zyppy™ Plasmid Miniprep Kit	Pellet-Free™ plasmid DNA purification in minutes: (alkaline lysis/spin column format for low 30 µl elution volume).	50 100 400 800	**D4036** **D4019** **D4020** **D4037**
Zyppy™ Plasmid Midiprep Kit	Pellet-Free™ plasmid DNA purification in minutes: (alkaline lysis/spin column format and 150 µl minimum elution volume).	25 50	**D4025** **D4026**
Zyppy™ Plasmid Maxiprep Kit	High-purity plasmid DNA purification in minutes: (alkaline lysis/spin column format and 2 ml minimum elution volume).	10 20	**D4027** **D4028**
ZR Genomic DNA I Kit™	Genomic DNA isolation from whole blood, tissue culture cells, solid tissue and liquid samples. (Silica bead format is scalable to fit your requirements).	100 400	**D3004** **D3005**
ZR Genomic DNA II Kit™	Genomic DNA purification from whole blood, tissue culture cells, solid tissue and liquid samples. No requirement for beads or phenol chloroform.	50 200 50 200	**D3006** (uncapped) **D3007** (uncapped) **D3024** (capped) **D3025** (capped)
ZR-96 Genomic DNA Kit™	High-output genomic DNA purification from whole blood, tissue culture cells, solid tissue and liquid samples. No requirement for beads or phenol chloroform.	2x96 4x96	**D3010** **D3011**
ZR Soil microbe DNA Kit™	Simple, rapid isolation of humic-free, PCR-quality genomic DNA from soil microbes.	50	**D6001**
ZR Fungal/Bacterial DNA Kit™	Simple, rapid isolation of PCR-quality genomic DNA from fungi.	50	**D6005**
ZR Fecal DNA Kit™	Simple, rapid isolation of PCR-quality genomic DNA from feces.	50	**D6010**
ZR Viral DNA Kit™	Isolation of viral DNA from cell-free body fluids or sample mixtures containing cells at concentrations less than 10^5 cells per ml.	50 200	**D3015** **D3016**
ZR-96 Viral DNA Kit™	High-output (96-well) isolation of viral DNA from cell-free body fluids or sample mixtures containing cells at concentrations less than 10^5 cells per ml.	2x96 4x96	**D3017** **D3018**
EZ DNA Methylation™ Kit	For the conversion of unmethylated cytosines in DNA to uracil via the chemical-denaturation of DNA and a specially designed CT Conversion Reagent. *Fast-Spin* technology ensures ultra-pure, converted DNA for subsequent DNA methylation analysis.	50 200 2x96 2x96	**D5001** **D5002** **D5003** Shallow **D5004** Deep Well
EZ DNA Methylation-Gold™ Kit	For the fast (3 hr.) conversion of unmethylated cytosines in DNA to uracil via heat/chemical-denaturation of DNA and a specially designed CT Conversion Reagent. *Fast-Spin* technology ensures ultra-pure, converted DNA for subsequent DNA methylation analysis.	50 200 2x96 2x96	**D5005** **D5006** **D5007** Shallow **D5008** Deep Well
EZ DNA Methylation-Direct™ Kit	Features simple and reliable DNA bisulfite conversion directly from blood, tissue (FFPE/LCM), and cells without the prerequisite for DNA purification in as little as 4-6 hrs. The increased sensitivity of this kit makes it possible to amplify bisulfite converted DNA from as few as 10 cells or 50 pg DNA.	50 200 2x96 2x96	**D5020** **D5021** **D5022** Shallow **D5023** Deep Well

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