ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Zyppy™-96 Plasmid Miniprep

Catalog Nos. **D4041, D4042,** & **D4043**

Highlights

- Innovative centrifugation based procedure omits conventional cell pelleting and re-suspension steps.
- The fastest and simplest high-throughput procedure for purifying the highest quality endotoxin-free plasmid DNA.
- Patented colored buffer technology for visualization of complete bacterial cell lysis and neutralization.

Contents

ZYMO RESEARCH CORP.

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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:

Zyppy™-96 Plasmid Miniprep (Kit Size)	**D4041** (2x 96 preps)	**D4042** (4x 96 preps)	**D4043** (8x 96 preps)	**Storage Temperature**
Deep Blue Lysis Buffer†	30 ml	48 ml	2x 48 ml	Room Temp.
Neutralization/Clearing Buffer†* (yellow)	100 ml	200 ml	2x 200 ml	4-8 ºC
Endo-Wash Buffer	60 ml	120 ml	160 ml	Room Temp.
Zyppy™ Wash Buffer† (concentrate)	24 ml	48 ml	2x 48 ml	Room Temp.
Zyppy™ Elution Buffer	30 ml	60 ml	100 ml	Room Temp.
96-Well Block	2	4	8	-
Collection Plate	2	4	8	-
Zymo-Spin™ I-96 Plate	2	4	8	-
Elution Plate	2	4	8	-
Air-Permeable Sealing Cover	2	4	8	-
96-Well Plate Cover Foil	6	12	24	-
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 the highest performance and reliability.

†Buffers require preparation prior to use as described on page 3.

*Neutralization/Clearing Buffer contains RNase A at a concentration of 200 µg/ml.

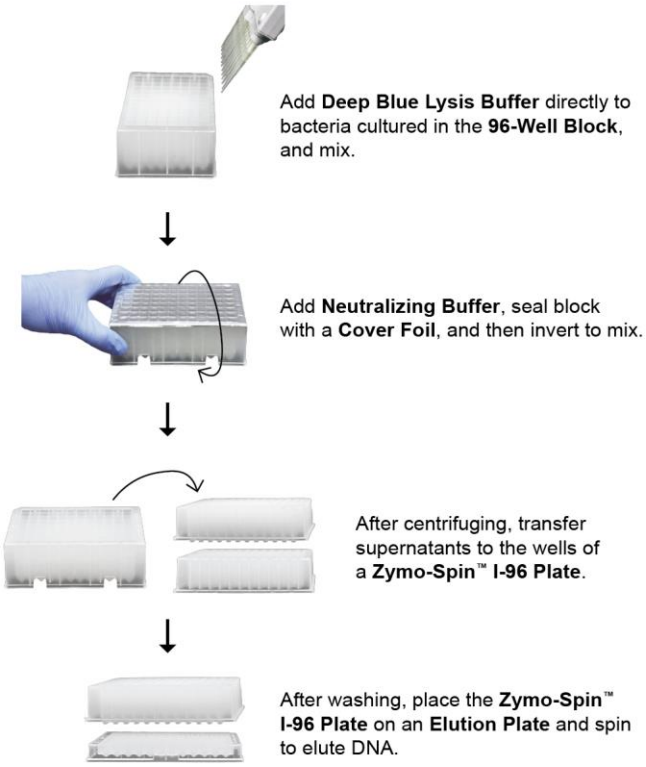
Specifications

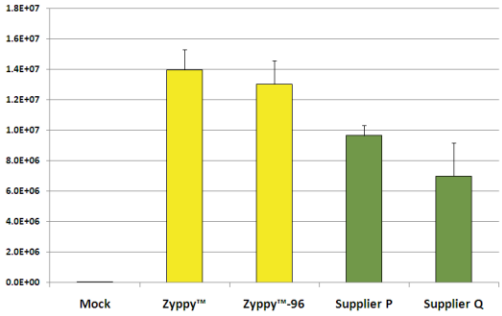
- **DNA Purity:** Eluted plasmid DNA is well suited for ligation, sequencing, restriction endonuclease digestion, transfection, *in vitro* transcription, and other sensitive applications requiring pure DNA. $Abs_{260/280}$ is ≥1.8
- **Plasmid DNA Yield:** Up to 10 µg per preparation, depending on the plasmid copy number, culture growth conditions, and the strain of *E. coli* processed.
- **Plasmid DNA Size:** Up to 25 kb.
- **Recovery Volume:** ≥30 µl per well.
- **Procedure:** Performed at room temperature (15-30°C) with a centrifuge with micro plate carriers.

Product Description

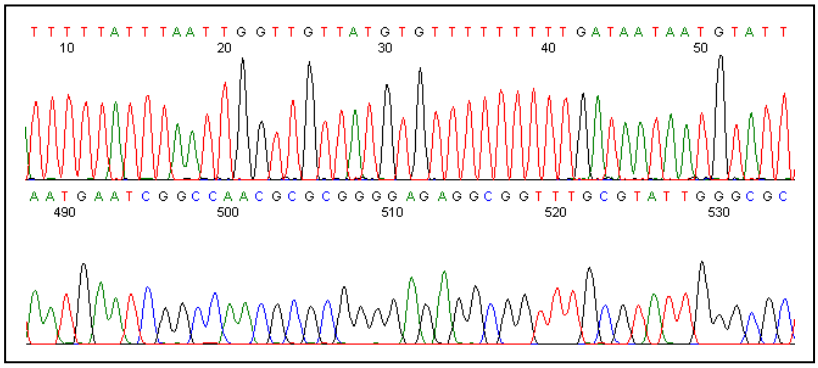
The **Zyppy™-96 Plasmid Miniprep Kit** is the fastest and simplest high-throughput method available for efficient isolation of plasmid DNA from *E. coli*. The kit features a modified, *Pellet-Free* alkaline lysis system that bypasses tedious centrifugation, pelleting, and re-suspension steps common to conventional procedures. Instead, the uniquely formulated **Deep Blue Lysis Buffer** is added *directly* to the bacterial cultures in a 96-well block. Buffer neutralization and lysate separation steps are expedited using a specially designed **Neutralization/Clearing Buffer**. The remaining DNA purification steps are straightforward and simple.

Eluted plasmid DNA is of the *highest quality*, endotoxin-free, and is well suited for use in restriction endonuclease digestion, DNA ligation, PCR, transcription, sequencing, and other sensitive downstream applications including transfection. An overview of the purification procedure is shown below.





Plasmid DNA isolated with Zyppy™ Miniprep Kits result in the highest transfection efficiencies. Luciferase activities were determined in lysates from cells transfected with DNA isolated by various methods. Plasmid DNA was purified from *E. coli* using the *Pellet-Free* Zyppy™ and Zyppy™-96 Kits or those Miniprep products from Suppliers P and Q.



Sequence chromatogram of plasmid DNA prepared with the Zyppy™-96 Plasmid Miniprep shows DNA is high quality and ideal for sequencing.

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

Buffer Preparation:

1. Add 104 ml of 95% ethanol to the 24 ml **Zyppy™ Wash Buffer** concentrate (D4041), or 208 ml of 95% ethanol to the 48 ml **Zyppy™ Wash Buffer** concentrate (D4042 & D4043) before use.

2. The **Deep Blue Lysis Buffer** may have precipitated during shipping. To completely re-suspend the buffer, incubate the bottle at 30-37 ºC for 30 minutes and mix by inversion. DO NOT MICROWAVE.

3. Completely re-suspend the **Neutralization/Clearing Buffer** prior to immediate use. Store buffer at 4-8 °C.

Protocol:

Culturing Bacteria in the 96-Well Block

1. Dispense 750 µl of LB medium (containing the appropriate antibiotic) into each well of a provided **96-Well Block**.
   *Note: Make sure to use a **96-Well Block** and not a **Collection Plate**.*

2. Inoculate each well from either a glycerol stock, culture plate, or pre-culture (2-3 µl) using a 96-pin device or other method.

3. Seal the block using one of the provided **Air-Permeable Sealing Covers**. Incubate cultures in an incubator/shaker for 24 hours at 37°C with constant shaking at 250-300 rpm.

Purification of Plasmid DNA

The following procedure should be done at room temperature and all centrifugation steps performed at 3,000-5,200 x g for 3-5 minutes.

Ensure that buffers have been prepared according to the instructions on page 3.

1. Remove the **96-Well Block** from the incubator and discard the **Air-Permeable Sealing Cover**.

2. Add 100 µl **Deep Blue Lysis Buffer** to each well of the block. Seal the block with a **96-Well Plate Cover Foil** (*the foil should be completely sealed on the sides of the block and the outline of each individual well clearly defined*). Invert 2-3 times and then incubate at room temperature for 1-2 minutes[1]. Proceed to *Step 3* within 3 minutes.
   *Note: After addition of **Deep Blue Lysis Buffer**, the solution should change from opaque to clear blue, indicating bacterial cell lysis is complete.*

3. Pierce foil to add 450 µl of cold **Neutralization/Clearing Buffer**[2] (yellow) to each well. Seal the block with a second **96-Well Plate Cover Foil** (*the foil should be completely sealed on the sides of the block and the outline of each individual well clearly defined*). Invert[1] gently 4-6 times until the lysate is completely neutralized.
   Note: The sample will turn yellow when neutralization is complete and a yellowish precipitate will form.

4. Centrifuge block. Pierce (or remove) foil and transfer the supernatants (~750 µl/well) to the wells of a **Zymo-Spin™ I-96 Plate** on a **Collection Plate**. Pipette only to a depth of ~75% of the volume of each well so as to not disturb the pelleted debris.

5. Centrifuge the **Zymo-Spin™ I-96/Collection Plate** combo[3]. Discard the flow through from the **Collection Plate**.

6. Re-place onto the **Collection Plate** and add 200 µl of **Endo-Wash Buffer** to each well of the **Zymo-Spin™ I-96 Plate**. Centrifuge[3].

7. Add 400 µl of **Zyppy™ Wash Buffer** to each well of the **Zymo-Spin™ I-96 Plate** and centrifuge[3]. Discard the flow through from the **Collection Plate** and centrifuge the combo again to remove any residual **Zyppy™ Wash Buffer**[3].

8. Add 30 µl of **Zyppy™ Elution Buffer**[4] directly to each well of the **Zymo-Spin™ I-96 Plate** on an **Elution Plate**. Let stand at room temperature 1-2 minutes and then centrifuge for 3 minutes to elute the plasmid DNA[3].
   *Note: DNA can be used immediately or the **Elution Plate** can be sealed with a provided **Cover Foil** for long term storage at -20°C.*

Notes:

[1] Inverting the block too many times may result in cross-contamination and/or genomic DNA inclusion in the eluted plasmid DNA.

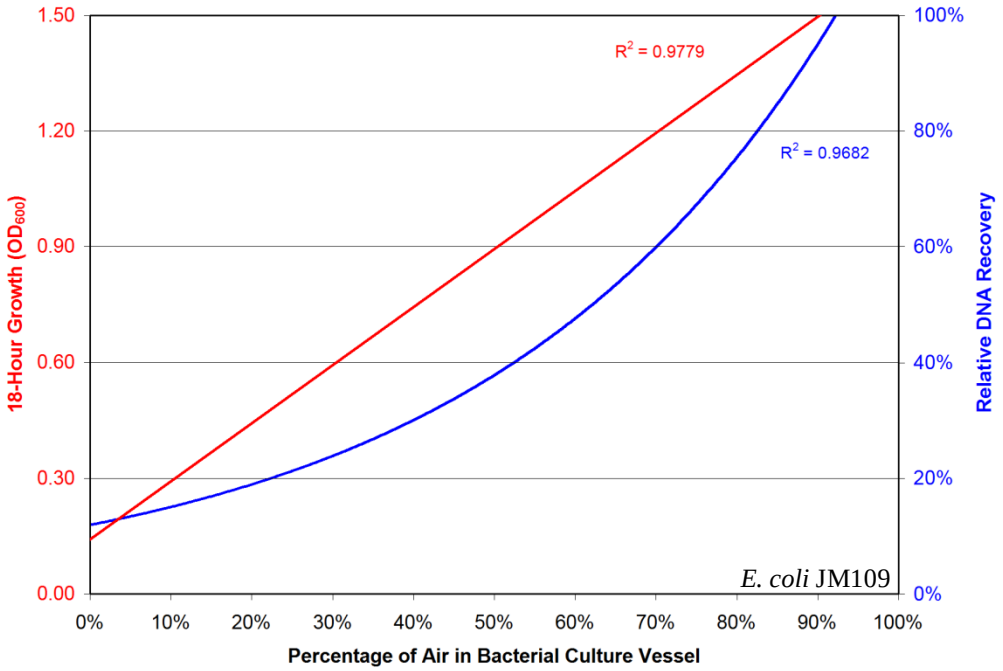
[2] The Neutralization/Clearing Buffer contains sediment. Completely re-suspend the buffer prior to use.

[3] It is not necessary to cover the Zymo-Spin I-96 Plate during centrifugation. If you desire to, please use an Air-Permeable Sealing Cover (Cat # C2011-8) to avoid damaging the plate.

[4] 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 for 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 (block), 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/under-grown 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 **Deep Blue 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 block an additional 2-3 times after the sample turns yellow following the addition of **Neutralization/Clearing Buffer**.
Deep Blue Lysis Buffer (precipitation)	• **Deep Blue Lysis Buffer** may have precipitated during shipping: To completely re-suspend the buffer, incubate the bottle at 30-37 ºC for 30 minutes and mix by inversion. DO NOT MICROWAVE.

DNA elution	• Incomplete elution: For large size plasmids (>10 kb), incubate the plate 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 into the wells of the plate. Ensure that neutralization is complete by inverting the sample an additional 2-3 times after the addition of **Neutralization/Clearing Buffer**.
	• The **Zymo-Spin™ I-96** plate tips are contaminated with wash buffer flow through: Avoid tilting the **Collection Plate** after the last wash step to ensure that the plate tips do not contact the flow through. Empty the **Collection Plate** when recommended in the protocol.
	• Insufficient centrifugation: make sure that all centrifugation steps are performed between 3,000-5,200 x *g*. If a lower centrifuge speed is used, extend the centrifugation time to compensate.
RNA in eluate	• Ensure **Neutralization/Clearing Buffer** is stored between 4-8 ºC.
	• After neutralization, allow lysate to sit 1-2 minutes prior to centrifugation.
Genomic DNA in eluate	• Improper handling (sample was vortexed or handled too roughly): Genomic DNA contamination is usually the result of 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 the sample.
Overgrown culture	• Older cultures may contain more genomic DNA contamination than fresh cultures.

Ordering Information:

Product Description	Kit Size	Catalog No.
Zyppy™-96 Plasmid Miniprep	2x 96 preps. 4x 96 preps. 8x 96 preps.	D4041 D4042 D4043

For Individual Sale	Amount	Catalog No.
Deep Blue Lysis Buffer	30 ml 48 ml	D4041-1-30 D4041-1-48
Neutralization/Clearing Buffer (yellow)	100 ml 200 ml	D4041-4-100 D4041-4-200
Endo-Wash Buffer	60 ml 120 ml 160ml	D4036-3-60 D4036-3-120 D4036-3-160
Zyppy™ Wash Buffer (concentrate)	24 ml 48 ml	D4036-4-24 D4036-4-48
Zyppy™ Elution Buffer	30 ml 60 ml 100 ml	D4036-5-30 D4036-5-60 D4036-5-100
96-Well Block	2 10	P1001-2 P1001-10
Collection Plate	2	C2002
Elution Plate	2	C2003
Air-Permeable Sealing Cover	2 4 8	C2011-2 C2011-4 C2011-8
96-Well Plate Cover Foil	6 12 24	C2007-6 C2007-12 C2007-24

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**
Genomic DNA Isolation			
***Quick-gDNA*™ Kits** (Total DNA from blood, cells, soft tissues, etc. w/o Proteinase K digestion in <10 min.)	MicroPrep. (up to 5 µg/prep.) MiniPrep. (up to 10 µg/prep.) MidiPrep. (up to 125 µg/prep.) 96-Well Format. (up to 125 µg/prep.)	50 preps. 50 preps. 25 preps. 2x96 preps.	**D3020** **D3024** **D3100** **D3010**
ZR Genomic DNA-Tissue Kits (Total DNA from blood, cells, solid & FFPE tissues, etc. w/ Proteinase K digestion)	MicroPrep. (up to 5 µg/prep.) MiniPrep. (up to 10 µg/prep.) MidiPrep. (up to 125 µg/prep.) 96-Well Format. (up to 125 µg/prep.)	50 preps. 50 preps. 25 preps. 2x96 preps.	**D3040** **D3050** **D3110** **D3055**
Environmental DNA Isolation			
ZR Soil Microbe DNA Kits™	MicroPrep. Bead Bashing, Spin Column Format (up to 5 µg/prep.) MiniPrep. Bead Bashing, Spin Column Format (up to 25 µg/prep.) MidiPrep. Bead Bashing, Spin Column Format (up to 125 µg/prep.) 96-Well Format. Bead Bashing (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	**D6003** **D6001** **D6101** **D6002**
ZR Fungal/Bacterial DNA Kits™	MicroPrep. Bead Bashing, Spin Column Format (up to 5 µg/prep.) MiniPrep. Bead Bashing, Spin Column Format (up to 25 µg/prep.) MidiPrep. Bead Bashing, Spin Column Format (up to 125 µg/prep.) 96-Well Format. Bead Bashing (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	**D6007** **D6005** **D6105** **D6006**
ZR Fecal DNA Kits™	MicroPrep. Bead Bashing, Spin Column Format (up to 5 µg/prep.) MiniPrep. Bead Bashing, Spin Column Format (up to 25 µg/prep.) MidiPrep. Bead Bashing, Spin Column Format (up to 125 µg/prep.) 96-Well Format. Bead Bashing (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	**D6012** **D6010** **D6110** **D6011**
ZR Tissue & Insect DNA Kits™	MicroPrep. Bead Bashing, Spin Column Format (up to 5 µg/prep.) MiniPrep. Bead Bashing, Spin Column Format (up to 25 µg/prep.) MidiPrep. Bead Bashing, Spin Column Format (up to 125 µg/prep.) 96-Well Format. Bead Bashing (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	**D6015** **D6016** **D6115** **D6017**
ZR Plant/Seed DNA Kits™	MicroPrep. Bead Bashing, Spin Column Format (up to 5 µg/prep.) MiniPrep. Bead Bashing, Spin Column Format (up to 25 µg/prep.) MidiPrep. Bead Bashing, Spin Column Format (up to 125 µg/prep.) 96-Well Format. Bead Bashing (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	**D6022** **D6020** **D6120** **D6021**

* Uncapped Spin Column Format (Also, see our website at: www.zymoresearch.com for additional kit sizes and formats)