ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Zyppy™-96 Plasmid MagBead Miniprep

Catalog Nos. **D4100, D4101,** & **D4102**

Highlights

- Innovative magnetic bead technology for automated and manual applications.
- Fastest, high-throughput (96-well), *Pellet-Free* procedure for purifying the highest quality endotoxin-free plasmid DNA without a centrifuge.
- Patented colored buffer technology for visualization of complete bacterial cell lysis and neutralization.

Contents

ZYMO RESEARCH CORP.

Toll Free: 1-888-882-9682 • Fax: 1-949-266-9452 • Web: www.zymoresearch.com • E-mail: info@zymoresearch.com

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 MagBead Miniprep (Kit Size)	**D4100** (2x 96 preps)	**D4101** (4x 96 preps)	**D4102** (8x 96 preps)	**Storage Temperature**
Deep Blue Lysis Buffer†	30 ml	48 ml	2x 48 ml	Room Temp.
Neutralization Buffer* (yellow)	100 ml	200 ml	2x 200 ml	4-8 ºC
Clearing Beads	10 ml	20 ml	40 ml	Room Temp.
Binding Beads	8 ml	16 ml	2x16 ml	Room Temp.
Endo-Wash Buffer	60 ml	120 ml	240 ml	Room Temp.
Zyppy™ Wash Buffer† (concentrate)	48 ml	2x 48 ml	3x 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	-
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 maximal performance and reliability.

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

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

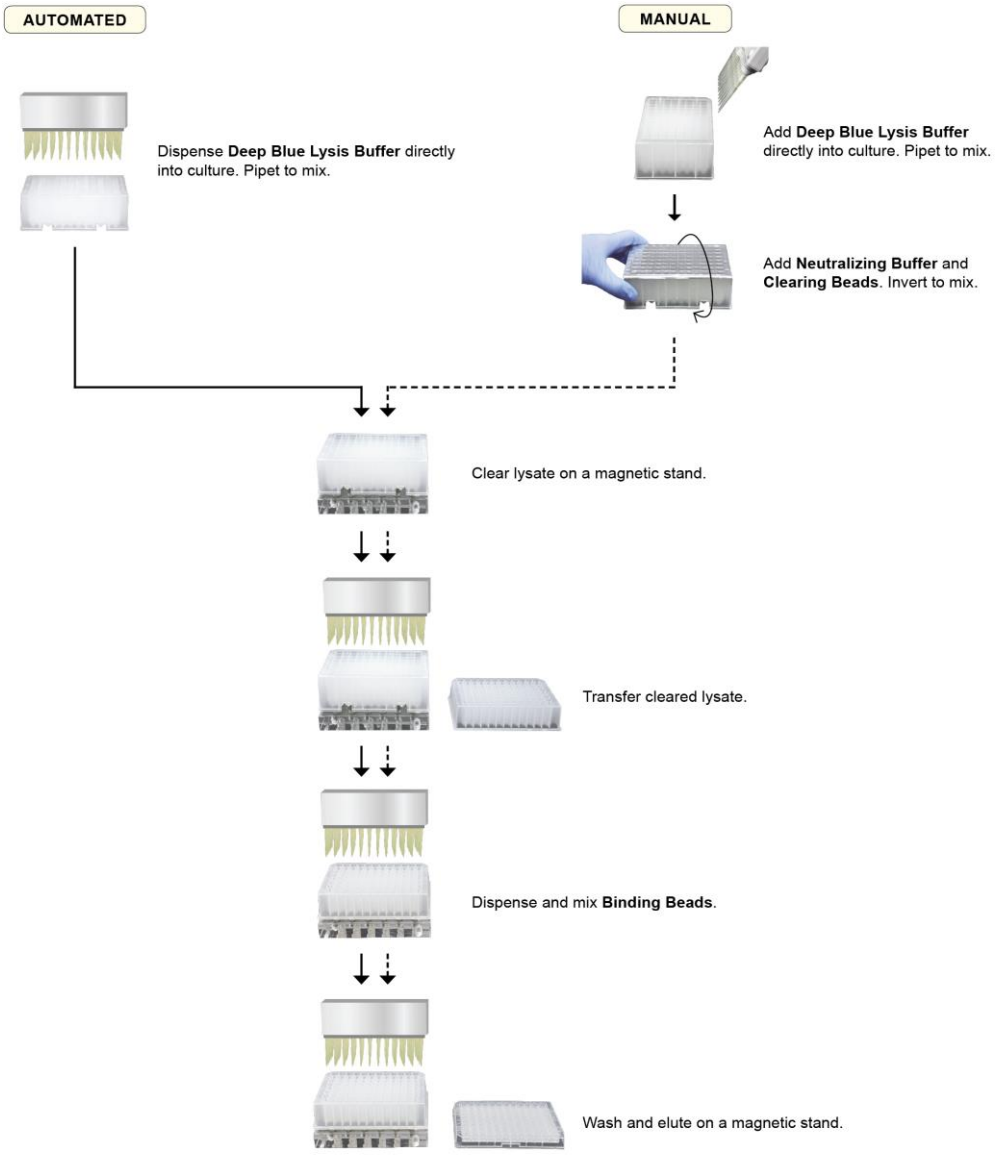
Specifications

- **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 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).

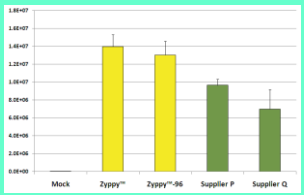
Product Description:

The **Zyppy™-96 Plasmid MagBead Miniprep** is the fastest high-throughput (96-well), *Pellet-Free* method available for efficient isolation of plasmid DNA from *E. coli*. The kit features a modified 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 bacterial cultures in a 96-well block. Buffer neutralization and lysate separation steps are expedited using a specially designed **Neutralization Buffer**. Plasmid DNA is then purified using the featured magnetic bead based technology. This straightforward procedure is compatible for either automated *or* manual processing.

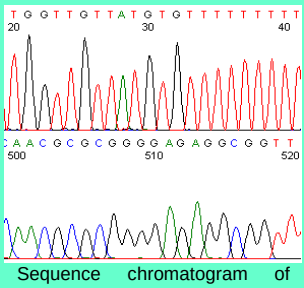
Eluted plasmid DNA is of the *highest quality*, endotoxin-free, and is well suited for use in restriction endonuclease digestion, DNA ligation, PCR, transcription, sequencing, and other sensitive downstream applications including transfection. An overview of the purification procedure is shown below.



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



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.

Buffer Preparation:

1. Add 208 ml of 95% ethanol to the 48 ml **Zyppy™ Wash Buffer** concentrate 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.

Considerations for Automated Applications:

Reagent Volumes: The total volumes (rounded to the nearest ml) of each reagent needed to process all the samples in a 96-well block is given below…

12 ml	**Deep Blue Lysis Buffer**
50 ml	**Neutralization Buffer**
5 ml	**Clearing Beads**
4 ml	**Binding Beads**
30 ml	**Endo-Wash Buffer**
90 ml	**Zyppy™ Wash Buffer**
6 ml	**Zyppy™ Elution Buffer**

Fill reagent reservoirs and place **96-Well Block**s according to the electronic setup (protocol) of your platform.

Protocol: Automated Procedure

Growing 96-Well Bacterial Cultures

1. Remove the seal from the **96-Well Block** and dispense 750 µl of LB medium, containing the appropriate antibiotic, into each well.
   *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 an **Air-Permeable Sealing Cover**. Incubate cultures in an incubator/shaker for 24 hours at 37°C with constant shaking at 250-300 rpm.

Purification of Plasmid DNA

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

1. Remove the **96-Well Block** from the incubator and discard the **Air-Permeable Sealing Cover** and transfer to a robotic (automated) platform

2. Dispense 100 µl of Deep Blue Lysis Buffer to each well containing culture and vortex for 10 seconds. Let sit for 5 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. Dispense 450 µl of **Neutralization Buffer** (yellow) and vortex for 45 seconds.
   Note: The sample will turn a dark yellow color when buffer neutralization is complete.

4. Dispense 50 µl **MagClearing Beads** and vortex for 10 seconds.

5. Transfer **96-Well Block** to a magnetic stand (sold separately)[2] and allow to sit for 5 minutes while beads separate from the lysate. From a depth of about half the length of the well, aspirate and transfer the cleared lysate (~750 µl) to a Collection Plate. Remove 96-Well Block from magnetic stand and discard.
   Note: Small amounts of bead carry-over should have no adverse effects on the procedure.

6. Dispense 30 µl of **MagBinding Beads**[1] to each well of the **Collection Plate** and vortex to mix. Incubate at room temperature for 10 minutes, each 30 seconds vortex for 5 seconds to re-suspend MagBinding Beads. Transfer the Collection Plate onto the magnetic stand for 5 minutes until beads pellet. With the Collection Plate still on the magnetic stand, aspirate and discard cleared lysates.

7. Transfer Collection Plate off magnetic stand and dispense 200 µl of **Endo-Wash Buffer** to each well of the plate. Vortex for 30 seconds to re-suspend beads and then transfer Collection Plate back onto magnetic stand for 2 minutes. With the Collection Plate still on the magnetic stand, aspirate and discard Endo-Wash Buffer.

(continued on next page)

Notes:

[1] The Clearing Beads and Binding Beads may have settled to the bottom while shipping. Ensure complete re-suspension by vortexing before every use.

[2] A strong-field magnetic stand is recommended (e.g., ZR-96 MagStand, Cat. No. P1005)

Protocol (continued):

8. Transfer Collection Plate off magnetic stand and dispense 400 µl **Zyppy™ Wash Buffer** to each well. Vortex for 30 seconds to re-suspend beads and then transfer Collection Plate back onto magnetic stand for 2 minutes. With the Collection Plate still on the magnetic stand, aspirate and discard Zyppy™ Wash Buffer.

9. Repeat *Step 8.*

10. For the removal of residual ethanol, transfer the **Collection Plate** onto a heating element (65°C) for 30 minutes.
    Note: Once dried, the bead pellets will no longer appear glossy

11. Transfer **Collection Plate** off the heating element. Dispense 40 µl of **Zyppy™ Elution Buffer**[1] to each well of the Collection Plate and vortex for 10 seconds to re-suspend beads. Place plate back onto heating element and incubate for 5 minutes, each minute vortexing for 5 seconds to re-suspend beads.

12. Transfer the plate back onto the magnetic stand for 1 minute. With the **Collection Plate** still on the magnetic stand, transfer the eluates (~30µl) to a provided **Elution Plate**. The eluted plasmid DNA is ready for immediate use, or the **Elution Plate** can be sealed with a provided 96-Well Plate Cover Foil and stored at -20°C.

Notes:

[1]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.

Protocol: Manual Procedure

This procedure can be adapted to be preformed manually by using a multi-channel pipet.

Growing 96-Well Bacterial Cultures

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 an **Air-Permeable Sealing Cover**. Incubate cultures in an incubator/shaker for 24 hours at 37°C with constant shaking at 250-300 rpm.

Purification of Plasmid DNA

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

1. Pre-heat a heat block or similar to 65°C.
   Note: This step is optional and intended to facilitate air-drying and removal of ethanol from the samples. Alternatively, samples can be air dried at room temperature.

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

3. Add 100 µl of **Deep Blue Lysis Buffer** directly to each well containing the culture. 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 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.*

4. Pierce foil to add 450 µl of cold **Neutralization Buffer** and then 50 µl **Clearing Beads**[2] 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 gently 4-6 times until lysate is completely neutralized[1].
   Note: The sample will turn a yellow/brown color when neutralization is complete.

5. Place the **96-Well Block** on a magnetic stand (sold separately)[3] and allow to sit for 3-5 minutes until beads have separated from the lysate. Pierce foil, and from a depth of about half the length of the well, aspirate and transfer the cleared lysates (~750 µl) to the wells of a **Collection Plate**.
   Note: Small amounts of bead carry-over should have no adverse effects on the procedure.

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Notes:

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

[2] The Clearing beads and DNA Binding beads may have settled to the bottom while shipping. Ensure complete re-suspension by vortexing before every use.

[3] A strong-field magnetic stand is recommended (e.g., ZR-96 MagStand, Cat. No. P1005)

Protocol (continued):

6. Add 30 µl of **Binding Beads**[2] to each well and mix by pipetting up and down 2-3 times. Incubate at room temperature for 10 minutes. Place the **Collection Plate** onto the magnetic stand for 2-3 minutes until the beads pellet. With the **Collection Plate** on the magnetic stand, aspirate and discard the cleared lysates.

7. Remove the **Collection Plate** from the magnetic stand and then add 200 µl of **Endo-Wash Buffer** to each well of the plate. Completely re-suspend beads by pipetting and place **Collection Plate** back onto the magnetic stand for 2-3 minutes until the beads pellet. With the plate on the magnetic stand, aspirate and discard the **Endo-Wash Buffer**.

8. Remove the **Collection Plate** from the magnetic stand and add 400 µl of **Zyppy™ Wash Buffer** to each well. Completely re-suspend beads by pipetting and then place the plate back onto the magnetic stand for 2-3 minutes until beads pellet. With the plate on the magnetic stand, aspirate and discard as much **Zyppy™ Wash Buffer** as possible without disrupting the bead pellet.

9. Repeat *Step 8.*

10. To remove residual ethanol, place the **Collection Plate** onto a heating block or similar (65°C) for 10-15 minutes. Alternatively, the plate can be air dried for 30 minutes at room temperature.
    Note: Once dry, the pellet will no longer appear glossy.

11. Add 40 µl of **Zyppy™ Elution Buffer**[1] to each well of the **Collection Plate** and completely re-suspend beads by pipetting. Place plate back onto heating block and incubate for 10 minutes. Place the plate on the magnetic stand for 2-3 minutes until beads pellet.

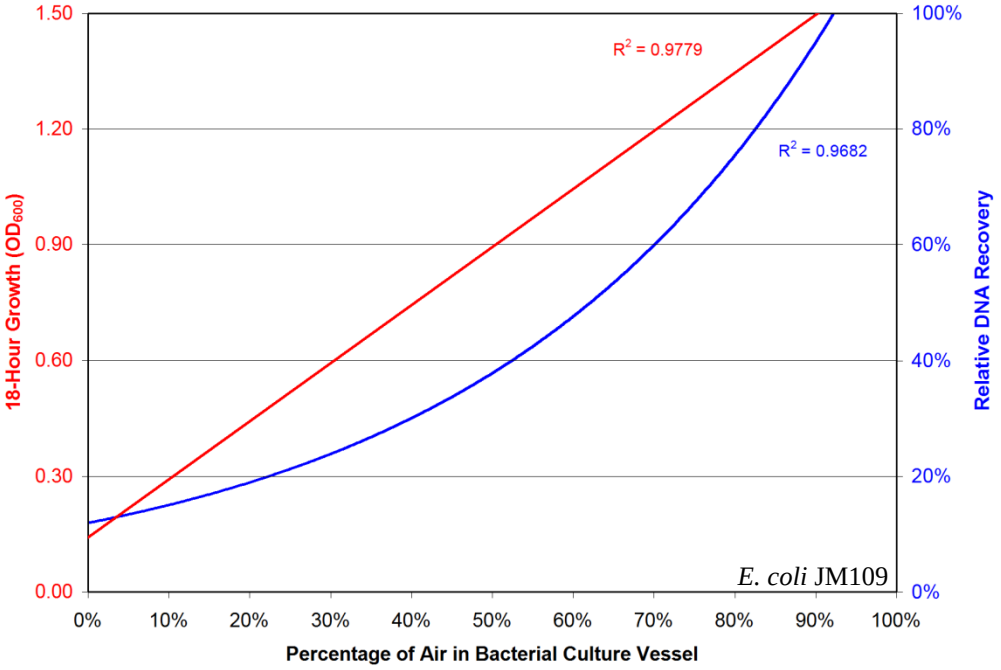
12. With the **Collection Plate** on the magnetic stand, transfer the eluates to a provided **Elution Plate** without disturbing the bead pellets. The eluted plasmid DNA is ready for immediate use, or the **Elution Plate** can be sealed with the provided **96-Well Plate Cover Foil** and stored at -20°C.

Notes:

[1]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 (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 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 an additional 5-10 minutes on a heating element and re-suspend beads every 5 minutes. Also, 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 Buffer**. • Insufficient washes: be sure to remove collection plate from magnet and fully re-suspend beads during wash steps. • The bead pellet contained residual ethanol from wash buffer. Be sure to completely dry beads before final elution step.
RNA in eluate	• Ensure **Neutralization Buffer** is stored between 4-8 ºC. Allow lysate to sit an additional 2-3 minutes before adding **Binding Beads** .
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.	D4100 D4101 D4102

For Individual Sale	Amount	Catalog No.
Deep Blue Lysis Buffer	30 ml 48 ml	D4041-1-30 D4041-1-48
Neutralization Buffer (yellow)	100 ml 200 ml	D4036-2-100 D4036-2-200
Clearing Beads	10 ml 20 ml 40 ml	D4100-1-10 D4100-1-20 D4100-1-40
Binding Beads	8 ml 16 ml 24 ml	D4100-2-8 D4100-2-16 D4100-2-24
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
ZR-96 MagStand	1	P1005

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)