ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Zyppy™ Plasmid Midiprep Kit

Catalog Nos. **D4025 & D4026** (Patent Pending)

Highlights

- The fastest, simplest method for purifying the highest quality endonuclease-free plasmid DNA.
- *Pellet-Free* procedure omits conventional cell-pelleting and re-suspension steps.
- Innovative *colored buffers* permit error-free visual identification of complete bacterial cell lysis.
- A unique Midiprep allowing for more yield from less culture.

Contents

ZYMO RESEARCH CORP.

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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 Midiprep Kit (Kit Size)	**D4025** (25 Preps.)	**D4026** (50 Preps.)	**Storage Temperature**
7X Lysis Buffer[1] (Blue)	30 ml	60 ml	Room Temp.
Neutralization Buffer[2] (Yellow)	100 ml	200 ml	4-8 ºC
Endo-Wash Buffer	30 ml	60 ml	Room Temp.
Zyppy™ Wash Buffer (concentrate)[3]	12 ml	24 ml	Room Temp.
Zyppy™ Elution Buffer	5 ml	10 ml	Room Temp.
Zymo-Spin™ V-E Columns[4]	25	50	Room Temp.
Zymo-Midi Filter™[4] (Blue)	25	50	Room Temp.
Collection Tubes	50	50	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] 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 200µg/ml. Store at 4-8° C

[3] Add 52 ml of 95% ethanol to the 12 ml Zyppy™ Wash Buffer concentrate (D4025), or 104 ml of 95% ethanol to the 24 ml Zyppy™ Wash Buffer concentrate (D4026) before use.

[4] The Zymo-Spin™ V-E and Zymo-Midi Filter™ are pre-assembled as a single unit.

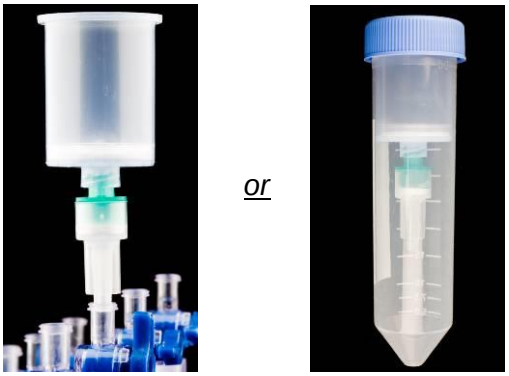
* Caution: 7X Lysis Buffer contains NaOH and Neutralization Buffer contains chaotropic reagents. Please use proper safety precautions with these reagents.

Product Description:

The **Zyppy™ Plasmid Midiprep 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, and then purify using our *Fast-Spin* column technology. Additionally, the innovative colored buffers included in the kit permits error-free visualization identification of complete bacterial cell lysis and neutralization.

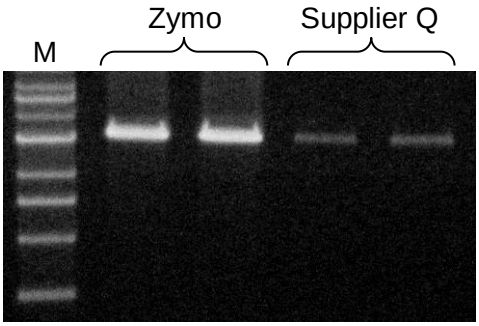
The **Zyppy™ Plasmid Midiprep Kit** is the *fastest* and *simplest* method available to efficiently separate plasmid DNA from *E. coli*. The plasmid DNA is of the *highest quality*, is endotoxin-free, and is well suited for use in bacterial transformation, restriction endonuclease digestion, DNA ligation, PCR, transcription, sequencing, and other sensitive downstream applications including transfection.

Lysate pre-clearing using…



Vacuum *or* Centrifugation

The Zymo-Midi Filter™ attaches to the Zymo-Spin™ V-E spin column for lysate pre-clearing using a vacuum manifold or centrifuge (see pictures above). The Zymo-Midi Filter™ is removed from the spin column for washing and elution steps.



EcoRI digestion of plasmid DNA (pGEM®) isolated from a 6 ml *E. coli* culture using the Zyppy™ Plasmid Midiprep Kit or a kit from Supplier Q. Performed in duplicate. M, ZR 1 kb DNA Marker (Zymo Research).

pGEM® is a registered trademark of Promega Corporation.

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

Specifications:

- **DNA Purity:** Plasmid DNA is well suited for ligation, sequencing, restriction endonuclease digestion, *in vitro* transcription, transfection and other sensitive applications requiring pure DNA. Typical $Abs_{260/280}$ index is ≥1.8.
- **Plasmid DNA Yield:** up to 50 µg per 6 ml of culture, depending on the plasmid copy number, culture growth conditions, and strain of *E. coli* utilized.
- **Plasmid DNA Size:** Up to 25 kb.
- **Recovery Volume:** ≥150 µl.
- **Procedure:** Performed at room temperature, between 15-30°C, using a centrifuge and/or a vacuum manifold and a microcentrifuge.

Buffer Preparation:

1. **Neutralization Buffer** contains RNase A at a concentration of 200 µg/ml. Store Buffer at 4-8°C.
2. Add 52 ml of 95% ethanol to the 12 ml **Zyppy™ Wash Buffer** concentrate (D4025), or 104 ml of 95% ethanol to the 24 ml **Zyppy™ Wash Buffer** (D4026) concentrate before use.
3. 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.

Vacuum Pump and Manifold:

- The vacuum pump should be a single- or double-staged unit capable of producing approximately 650 mm Hg pressure at the vacuum manifold.
- This product is compatible with any conventional vacuum-based manifold.

Vacuum Protocol (Recommended):

The following procedure is performed at room temperature. Make sure that *E. coli* culture was grown with enough aeration, see page 6.

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

1. Add 6 ml of bacterial culture in LB medium to a 15 or 50 ml conical tube.

   Alternatively, centrifuge up to 12ml, (or for low-copy number plasmids, 24ml) of bacterial culture in a 50 ml conical tube for 10 minutes at ≥3,400 x g. Discard the supernatant. Add 6 ml of TE or water to the bacterial cell pellet and completely re-suspend by vortexing or pipetting.

   ***IMPORTANT:** If culture is grown in a medium other than LB or contains a high-copy number plasmid, the culture volume processed must not exceed 12 ml. Processing an excess of bacteria will reduce lysis efficiency resulting in lower yields of plasmid DNA.*

2. Add 1 ml of **7X Lysis Buffer (Blue)** to the sample and mix by gently inverting the tube 2-4 times. Let sit at room temperature for 5 minutes.[1]

3. Add 3.5 ml of cold **Neutralization Buffer (Yellow)** and invert 4-6 times to 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 if needed** to ensure complete neutralization.[2]

4. Place the **Zymo-Midi Filter™/Zymo-Spin™ V-E** column assembly onto a vacuum manifold.

5. Add the entire mixture into the blue **Zymo-Midi Filter™** column, let the cell debris float to the surface and turn on the vacuum until al of the liquid has passed completely through both columns.

6. Remove and discard the blue **Zymo-Midi Filter™** column from the top of the **Zymo-Spin™ V-E** column.

7. Transfer the **Zymo-Spin™ V-E** column to a collection tube and using a microcentrifuge, spin at ≥11,000 *x g* (or top speed) for 30 seconds to remove any retained lysate.

8. Add 400 µl of **Endo-Wash Buffer** to the **Zymo-Spin™ V-E** column and centrifuge at ≥11,000 x *g* (or top speed) for 30 seconds.

9. Add 400 µl of **Zyppy™ Wash Buffer** and centrifuge at ≥11,000 x *g* (or top speed) for 30 seconds. Discard the flow through. Repeat this step and centrifuge at top speed for additional minute to eliminate any residue **Wash Buffer** from the column.

10. Transfer the **Zymo-Spin™ V-E** column into a clean 1.5 ml microcentrifuge tube then add 150 µl of **Zyppy Elution Buffer**[3] to the center of the column. Incubate at room temperature for one minute, then centrifuge at ≥11,000 x *g* (or top speed) for 1 minute 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 five or less at a time. Continue with the next set of five samples after the first set has been neutralized and mixed thoroughly.

[2] Incomplete neutralization may result in poor lysate filtration and lower DNA yield and quality.

[3] The Zyppy™ Elution Buffer contains 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.

Centrifugation Protocol:

The following procedure is performed at room temperature. Make sure that *E. coli* culture was grown with enough aeration, see page 6.

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

1. Add 6 ml of bacterial culture in LB medium to a 15 or 50 ml conical tube.

   Alternatively, centrifuge up to 12ml, (or for low-copy number plasmids, 24ml) of bacterial culture in a 50 ml conical tube for 10 minutes at ≥3,400 x g. Discard the supernatant. Add 6 ml of TE or water to the bacterial cell pellet and completely re-suspend by vortexing or pipetting.

   IMPORTANT: *If culture is grown in a medium other than LB or contains a high-copy number plasmid, the culture volume processed must not exceed 12 ml. Processing an excess of bacteria will reduce lysis efficiency resulting in lower yields of plasmid DNA.*

2. Add 1 ml of **7X Lysis Buffer (Blue)** to the sample and mix by gently inverting the tube 4-6 times. Let sit at room temperature for 5 minutes.[1]

3. Add 3.5 ml of cold **Neutralization Buffer (Yellow)** and invert 4-6 times to 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.[2]

4. Place the **Zymo-Midi Filter™/Zymo-Spin™ V-E** column assembly into a clean 50 ml conical tube.

5. Add the entire mixture into the blue **Zymo-Midi Filter™** column, place the cap on the conical tube, and centrifuge at 500 x *g* for 6 minutes.

6. Remove and discard the blue **Zymo-Midi Filter™** column from the top of the **Zymo-Spin™ V-E** column.

7. Transfer the **Zymo-Spin™ V-E** column to a collection tube and using a microcentrifuge, spin at ≥11,000 *x g* (or top speed) for 30 seconds to remove any retained lysate.

8. Add 400 µl of **Endo-Wash Buffer** to the **Zymo-Spin™ V-E** column and centrifuge at ≥11,000 x *g* (or top speed) for 30 seconds.

9. Add 400 µl of **Zyppy™ Wash Buffer** and centrifuge at ≥11,000 x *g* (or top speed) for 1 minute. Discard the flow through. Repeat this step and centrifuge at top speed for additional minute to eliminate any residue **Wash Buffer** from the column.

10. Transfer the **Zymo-Spin™ V-E** column into a clean 1.5 ml microcentrifuge tube and then add 150 µl of **Zyppy Elution Buffer**[3] to the center of the column. Incubate at room temperature for one minute, then centrifuge at ≥11,000 x *g* (or top speed) for 1 minute to elute the plasmid DNA.

Notes:

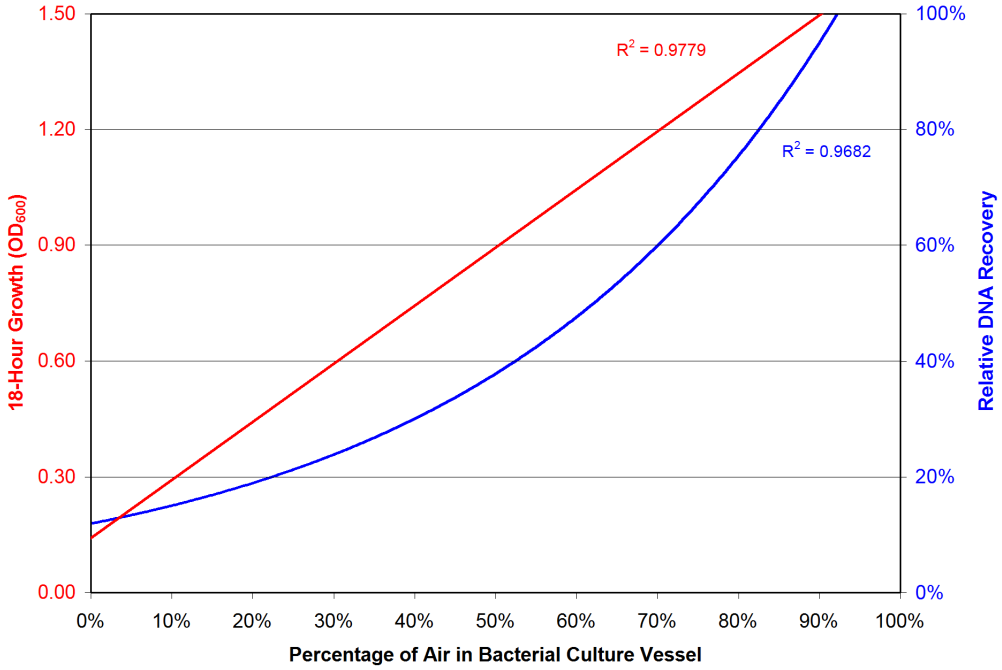
[1] Excessive lysis can result in denatured plasmid DNA. If processing a large number of samples, we recommend working with groups of five or less at a time. Continue with the next set of five samples after the first set has been neutralized and mixed thoroughly.

[2] Incomplete neutralization may result in poor lysate filtration and lower DNA yield and quality.

[3] The Zyppy™ Elution Buffer contains 0.1 mM EDTA. If required, pure water 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. This is the most common reason for low plasmid DNA yield. The optimal culture volume to air volume ratio is 1:4 or less. 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 $O.D._{600}$ ≥2.0.
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. • Insufficient centrifugation: make sure that all centrifugation steps are performed at the indicated speed. If a lower centrifuge speed is used, then extend the centrifugation time to compensate.
Over loading column	• *If culture is grown in a medium other than LB or contains a high-copy number plasmid, the culture volume processed must not exceed 12 ml. Processing an excess of bacteria will reduce lysis efficiency resulting in lower yields of plasmid DNA*.

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 eluate on 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. • Ethanol contamination in eluate. Centrifuge the spin column matrix to dryness as indicated in the protocol prior to adding the Zyppy™ Elution Buffer.

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.
RNA in eluate	• Neutralization 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 Neutralization buffer has been 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. • Spin down cellular debris prior to lysate filtration on column. • Overgrown culture. Older cultures may contain more genomic DNA contamination than fresh cultures.

Ordering Information

Product Description	Kit Size	Catalog No.
Zyppy™ Plasmid Midiprep Kit	25 preps. 50 preps.	D4025 D4026

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™ V-E Columns w/ Zymo-Midi Filters™	25	C1021-25
Collection Tubes	50 500 1000	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