



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR Soil Microbe DNA MidiPrep™

Catalog No. **D6101**

Highlights

- Simple, efficient isolation of humic-free, PCR-quality DNA from microbes in soil, sludge, sediment, and sand in as little as 25 minutes including tough-to-lyse bacteria, fungi, algae, and protozoa.
- State-of-the-art, ultra-high density **BashingBeads™** are fracture resistant and chemically inert.
- Can be used with any bead mill, disrupter, or vortex that can accommodate standard 50 ml conical centrifuge tubes.
- Omits the use of organic denaturants as well as proteinases.

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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

ZR Soil Microbe DNA MidiPrep™ (Kit Size)	D6101 (25 preps.)	Storage Temperature
ZR BashingBead™ Lysis/Filtration Tubes	25	Room Temp.
Lysis Solution	150 ml	Room Temp.
Soil DNA Binding Buffer	500 ml	Room Temp.
DNA Pre-Wash Buffer*	15 ml	Room Temp.
Soil DNA Wash Buffer	50 ml	Room Temp.
DNA Elution Buffer	16 ml	Room Temp.
Zymo-Spin™ V-E Columns w/ Zymo-Midi Filters™	25	Room Temp.
Zymo-Spin™ IV-HRC Spin Filters (Green Tops)	50	Room Temp.
Collection Tubes	100	Room Temp.
Instruction Manual	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.

* A precipitate may have formed in the DNA Pre-Wash Buffer during shipping. To completely resuspend the buffer, incubate the bottle at 30 – 37 °C for 30 minutes and mix by inversion. DO NOT MICROWAVE.

Specifications

- **Format** – Bead Beating, Spin/Vacuum Filtration, and Spin Column Purification
- **Sample Sources** – DNA is isolated from bacteria, fungi, protozoa, and algae in up to 5 g of soil. However, 2.5 g is recommended for most applications. The amount of sample will vary depending on the composition of the sample: process more material for wet muddy samples and less for dry sandy samples. DNA can also be isolated directly from isolated (pelleted) fungus and bacteria (see protocol).
- **DNA Purity** – High quality, humic/fulvic-free DNA is eluted with **DNA Elution Buffer** making it perfect for PCR. $A_{260}/A_{280} \geq 1.8$, $A_{260}/A_{230} \geq 2.0$
- **DNA Size Limits** – Capable of recovering genomic DNA sized fragments from 100 bp to ≥ 40 kb. Typical fragment sizes range from 25 kb-35 kb. If present, parasitic and viral DNA will also be recovered.
- **DNA Recovery** – Typically, up to ~125 µg total DNA is eluted into ≥ 150 µl **DNA Elution Buffer** per sample.
- **Equipment** – Centrifuge, Vacuum Source and Manifold, Microcentrifuge, Cell Disrupter/Pulverizer w/ 50 ml Tube Adapter

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

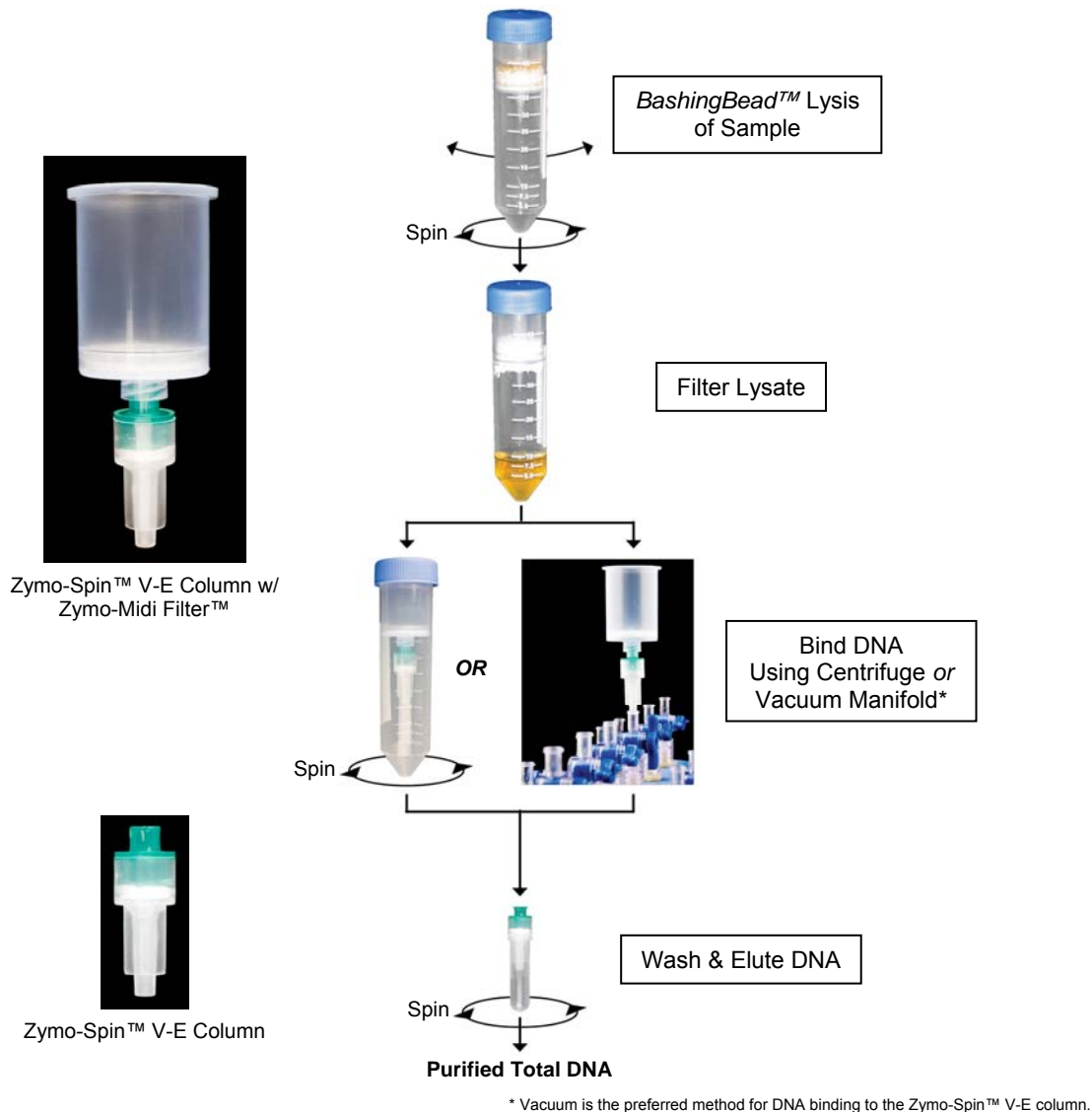
For purification of up to 25 µg DNA/prep use the **ZR Soil Microbe DNA Kit™ (D6001)**. For high throughput purification (96-well) use the **ZR-96 Soil Microbe DNA Kit™ (D6002)**.

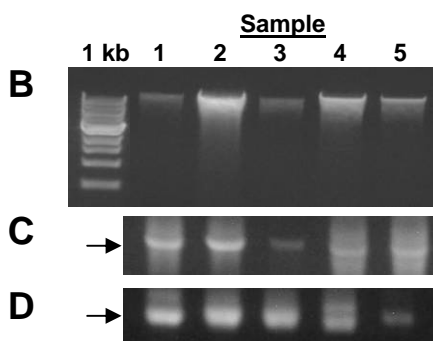
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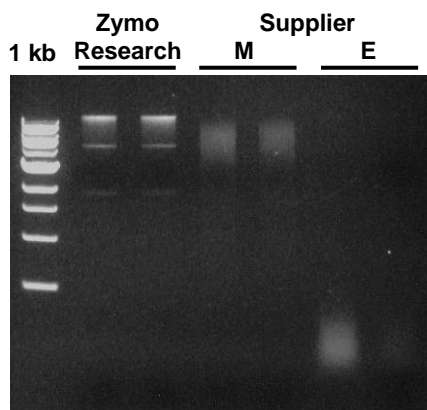
Product Description

The **ZR Soil Microbe DNA MidiPrep™** is designed for the simple, rapid isolation of humic-free, PCR-quality DNA from microbes in soil. The kit can be used to successfully isolate *metagenomic* DNA from tough-to-lyse bacteria, fungi, protozoa, and algae that inhabit a range of samples including clay, sandy, silty, peaty, chalky, and loamy soils. Soil samples (≤ 5 g) are added to a **ZR BashingBead™ Lysis/Filtration Tube** where microbes are rapidly and efficiently lysed by bead beating in a uniquely designed lysis buffer. Our *Fast-Spin* column technology is then used to isolate the DNA which is subsequently filtered to remove humic acids/polyphenols that inhibit PCR. The entire procedure can be performed in as little as 25 minutes, and there is no need for organic denaturants or proteinases. The **ZR Soil Microbe DNA MidiPrep™** can also be used to successfully isolate DNA from cultured bacteria, fungi, and yeast. A schematic of the procedure is shown below.





The **ZR Soil Microbe DNA Kit™** can be used to isolate high quality DNA from a variety of soil types which yields robust products following PCR. **Panel A:** Physical characteristics of sampled soils (1-5) (Ref. 1). **Panel B:** Microbial DNA was isolated from soil samples (1-5) using the **ZR Soil Microbe DNA Kit™**. Approximately 10% of the eluted DNA was then separated in a 0.8% (w/v) agarose/ethidium bromide gel. **Panels C and D** show the results of PCR of microbial DNA isolated from the samples with primers specific for prokaryotic 16S rRNA (**C**) or eukaryotic rRNA (**D**). In the figures, the 1kb size marker (NEB) is as indicated and the arrows show the prokaryotic 16S rRNA and eukaryotic rRNA PCR products.



DNA isolated from *Saccharomyces cerevisiae* (strain TMY18) using the **ZR Soil Microbe DNA Kit™** is high-quality and structurally intact. Equivalent amounts of yeast were processed using the **ZR Soil Microbe DNA Kit™** or the kits from suppliers M and E. Equal volumes of eluted DNA were then analyzed in a 0.8% (w/v) agarose/ethidium bromide gel. The size marker is a 1 kb ladder (NEB).

Reference:

1. Soil and Plant Laboratory, Inc. P.O. Box 11744, Santa Ana, California 92711

For **Technical Assistance**, please contact those at **Zymo Research's Technical Department** at 1-888-882-9682 or E-mail to tech@zymoresearch.com.

Protocol

Before Starting: (soil samples only) **Zymo-Spin™ IV-HRC Spin Filters** (green tops) need to be prepared prior to use by: 1) snapping off the base, 2), inserting into a **Collection Tube**, and 3), spinning in a microcentrifuge at exactly 8,000 x g for 3 minutes.

1. Add 2.5 grams (5 g max.)¹ of soil sample to the bead/filter chamber of a **ZR BashingBead™ Lysis/Filtration Tube**. Add 6 ml **Lysis Solution** to the sample, cap tube², and process.

*(To prevent the **Lysis Solution** from leaking into the bottom of the 50 ml tube, place the **ZR BashingBead™ Lysis/Filtration Tube** on its side prior to processing).*

Alternatively, add 250-500 mg (wet weight) fungal and/or bacterial cells that have been resuspended in 6 ml of Lysis Solution to a **ZR BashingBead™ Lysis/Filtration Tube**.

2. Secure in a bead beater fitted with a 50 ml tube holder assembly (see page 6) to process samples. Optimization of processing time/speed will be necessary for complete sample lysis.

Processing times may be as little as 40 seconds when using high-speed cell disrupters (e.g., FastPrep®-24, Geno/Grinder®, page 6). See manufacturer's literature for operating information.

3. Centrifuge the **ZR BashingBead™ Lysis/Filtration Tube** in a centrifuge at $\geq 3,000 \times g$ (5,000 x g max.) for 5 minutes.
4. Remove bead/filter chamber from the top of the **ZR BashingBead™ Lysis/Filtration Tube** and transfer supernatant³ from the bottom of the tube to a clean 50 ml tube (not provided). Add 18 ml **Soil DNA Binding Buffer** to the supernatant (~3:1) and vortex to mix.
5. Filter the entire mixture from Step 4 using a **Zymo-Spin™ V-E Column/Zymo-Midi Filter™** assembly mounted on a vacuum manifold⁴ (see diagram on page 2) with a vacuum source set at ≥ 600 mm Hg.
6. Disconnect the **Zymo-Spin™ V-E Column/Zymo-Midi Filter™** assembly and transfer the **Zymo-Spin™ V-E Column** to a **Collection Tube**. Spin the column at 10,000 x g for 1 minute in a microcentrifuge⁵, then add 300 μ l **DNA Pre-Wash Buffer** to the column and spin at 10,000 x g for 1 minute. Discard the flow through.
7. Add 400 μ l **Soil DNA Wash Buffer** to the column and centrifuge at 10,000 x g for 1 minute. Discard flow through and repeat wash step.
8. Transfer the **Zymo-Spin™ V-E Column** to a 1.5 ml microcentrifuge tube and add 150 μ l **DNA Elution Buffer** directly to the column matrix⁶. Wait for 1 minute and then centrifuge at 10,000 x g for 1 minute to elute the DNA⁷.

If fungal or bacterial cultures were sampled, the DNA is now suitable for PCR as well as other downstream applications.

9. Transfer the eluted DNA from Step 10 to a prepared **Zymo-Spin™ IV-HRC Spin Filter** (green top) (see above) in a clean 1.5 ml microcentrifuge tube and centrifuge at exactly 8,000 x g for 1 minute.

The filtered DNA is suitable for PCR, other downstream applications, or storage.

Notes:

¹ Although 2.5 g is recommended for most applications, the amount of sample will vary depending on its composition: process more material for wet muddy samples and less for dry sandy samples.

² Cap tube tightly to prevent leakage.

³ Be careful to avoid the pelleted material at the bottom of the tube when transferring the supernatant.

⁴ Alternatively, the **Zymo-Spin™ V-E Column/Zymo-Midi Filter™** assembly can be placed in a 50 ml tube and centrifuged at 2,000 x g max. for 5 minutes. Filtration of the entire mixture will require several spins. Empty the flow through from the tube after each spin. **CAUTION: Make sure the connection between the column and filter is secure (finger tight) prior to centrifugation!**

⁵ Leave the rotor cover off the microcentrifuge if clearance with the column tops is a problem.

⁶ DNA yields can be increased by performing a second elution and pooling the eluates.

⁷ In some cases a brown-colored pellet may form at the bottom of the tube after centrifugation. Avoid this pellet when collecting the eluted DNA.

Ordering Information

Product Description	Catalog No.	Kit Size
ZR Soil Microbe DNA Kit™	D6001	50 preps.
ZR-96 Soil Microbe DNA Kit™	D6002	2x96 preps.
ZR Soil Microbe DNA MidiPrep™	D6101	25 preps.

For Individual Sale	Catalog No.	Amount
ZR BashingBead™ Lysis/Filtration Tubes (50 ml) w/ 0.5 mm Beads	S6010	25 tubes
Lysis Solution	D6001-3-150	150 ml
Soil DNA Binding Buffer	D6001-1-500	500 ml
DNA Pre-Wash Buffer	D3004-5-15	15 ml
Soil DNA Wash Buffer	D6001-2-50	50 ml
DNA Elution Buffer	D3004-4-16	16 ml
Zymo-Spin™ V-E Columns w/ Zymo-Midi Filters™	C1021-25	25 columns/filters
Zymo-Spin™ IV-HRC Spin Filters (Green Tops)	C1010-50	50 filters
Collection Tubes	C1001-50	50 tubes
	C1001-500	500 tubes
	C1001-1000	1,000 tubes

Compatible Lysis Instruments...

Bullet Blender™



NEXT >>> ADVANCE

Homogenize tissue or disrupt/lyse cells in minutes. **The Bullet Blender™**, is a vortexer (at a low setting), a cell disruptor and a tissue homogenizer (at a high setting) all in one unit. No parts contact the samples, eliminating any possibility of cross contamination.

Description	Cat No.
DX50B Bullet Blender™ Blue 50 Accommodates 9 x 50 ml tubes. Features fan cooling.	S6007-1

FastPrep®



The **FastPrep®-24 Instrument** is a unique, high-speed benchtop homogenizer that employs a powerful, proprietary technology for the rapid lysis of almost any sample in 40 seconds or less. The FastPrep® Instrument makes it possible to isolate DNA, RNA and protein from sources that are virtually impossible to lyse without the use of its rapid reciprocating motion.

Description	Cat No.
FastPrep® Instrument	S6005
BigPrep™ Attachment Accommodates 2 x 50 ml tubes.	S6005-4



2000 Geno/Grinder®



The **2000 Geno/Grinder® Instrument** is a unique instrument that provides vigorous up-and-down grinding/pulverizing action. The Geno/Grinder® instrument makes it possible to prepare plant materials such as seeds, stems, roots, leaves, and certain animal tissue. Can accommodate (2) 96-well plates/blocks for high-throughput sample processing.

Description	Cat No.
2000 Geno/Grinder® Instrument	S6006
50 ml Tube Holder/Cryo Block Assembly Accommodates 12 x 50 ml tubes/block.	S6006-3



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Environmental DNA & RNA Purification Products From Zymo Research

Product	Description	Kit Size	Cat No.
<i>Tough-to-Lyse and Environmental Sample DNA Isolation</i>			
ZR Soil Microbe DNA Kit™ ZR-96 Soil Microbe DNA Kit™ ZR Soil Microbe DNA MidiPrep™	Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 2x96 preps. 25 preps.	D6001 D6002 D6101
ZR Fungal/Bacterial DNA Kit™ ZR-96 Fungal/Bacterial DNA Kit™ ZR Fungal/Bacterial DNA MidiPrep™	Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 2x96 preps. 25 preps.	D6005 D6006 D6105
ZR Fecal DNA Kit™ ZR-96 Fecal DNA Kit™ ZR Fecal DNA MidiPrep™	Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 2x96 preps. 25 preps.	D6010 D6011 D6110
ZR Tissue & Insect DNA Kit-5™ ZR Tissue & Insect DNA Kit-25™ ZR-96 Tissue & Insect DNA Kit™ ZR Tissue & Insect DNA MidiPrep™	Spin Column Format (up to 5 µg/prep.) Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 50 preps. 2x96 preps. 25 preps.	D6015 D6016 D6017 D6115
ZR Plant/Seed DNA Kit™ ZR-96 Plant/Seed DNA Kit™ ZR Plant/Seed DNA MidiPrep™	Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 2x96 preps. 25 preps.	D6020 D6021 D6120
<i>Tough-to-Lyse and Environmental Sample RNA Isolation</i>			
ZR Fungal/Bacterial RNA MicroPrep™ ZR Fungal/Bacterial RNA MiniPrep™	Spin Column Format (up to 5 µg/prep.) Spin Column Format (up to 25 µg/prep.)	50 preps. 50 preps.	R2010 R2014
ZR Plant RNA MiniPrep™	Spin Column Format (up to 25 µg/prep.)	50 preps.	R2024
ZR Tissue & Insect RNA MicroPrep™	Spin Column Format (up to 5 µg/prep.)	50 preps.	R2030