



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR Soil Microbe DNA Kit™

Catalog No. **D6001**

Highlights

- Simple, efficient isolation of humic-free DNA from microbes in soil, sludge, sediment, and sand in as little as 15 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 2 ml 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 Kit™ (Kit Size) | D6001 (50 preps.) | Storage Temperature |
|---------------------------------------------|----------------------|------------------------|
| ZR BashingBead™ Lysis Tubes | 50 tubes | Room Temp. |
| Lysis Solution | 40 ml | Room Temp. |
| Soil DNA Binding Buffer | 100 ml | Room Temp. |
| DNA Pre-Wash Buffer* | 15 ml | Room Temp. |
| Soil DNA Wash Buffer | 50 ml | Room Temp. |
| DNA Elution Buffer | 10 ml | Room Temp. |
| Zymo-Spin™ IV Spin Filters (Orange Tops) | 50 filters | Room Temp. |
| Zymo-Spin™ IV-HRC Spin Filters (Green Tops) | 50 filters | Room Temp. |
| Zymo-Spin™ IIC Columns | 50 columns | Room Temp. |
| Collection Tubes | 200 tubes | 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 Column.
- **Sample Sources** – DNA is isolated from bacteria, fungi, protozoa, and algae in up to 0.25 g of soil or from fungal/bacterial cells directly (see protocol).
- **DNA Purity** – High quality, humic-free DNA is eluted with **DNA Elution Buffer** making it perfect for PCR. $A_{260}/A_{280} > 1.8$
- **DNA Size Limits** – > 1 kb
- **DNA Recovery** – Typically, up to 25 µg total DNA is eluted into 100 µl (25 µl minimum) **DNA Elution Buffer** per sample. For DNA 75 bp to 10 kb, the recovery is 70-90%. For DNA 11 kb to 23 kb the recovery is 50-70%.
- **Equipment** – Microcentrifuge, vortex, cell disrupter/pulverizer (optional)

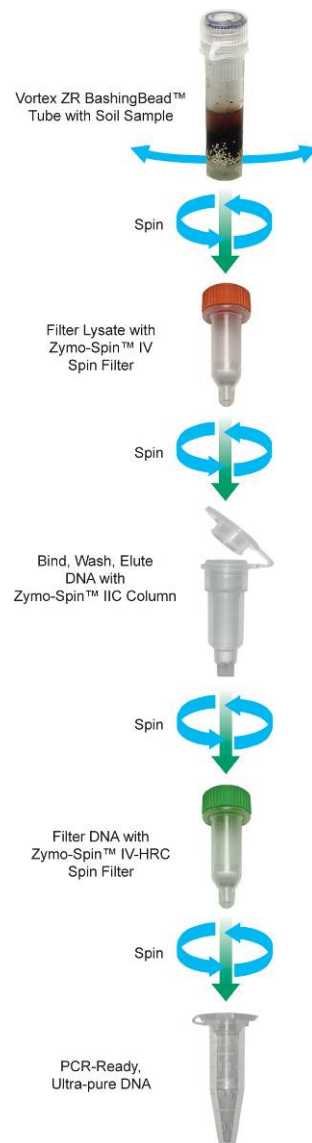
Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution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

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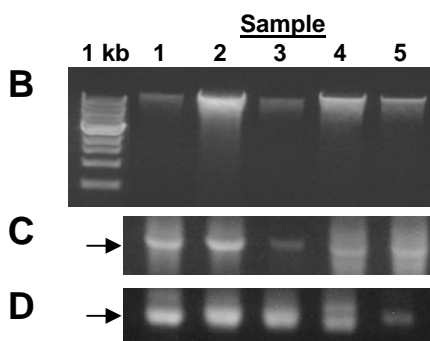
Product Description

The **ZR Soil Microbe DNA Kit™** 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 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 are added to a **ZR BashingBead™ Lysis Tube** where microbes are rapidly and efficiently lysed by bead beating (e.g. FastPrep®-24 Instrument, page 6) 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 15 minutes, and there is no need for organic denaturants or proteinases. The **ZR Soil Microbe DNA Kit™** can also be used to successfully isolate DNA from cultured bacteria, fungi, and yeast. A schematic of the procedure is shown below.

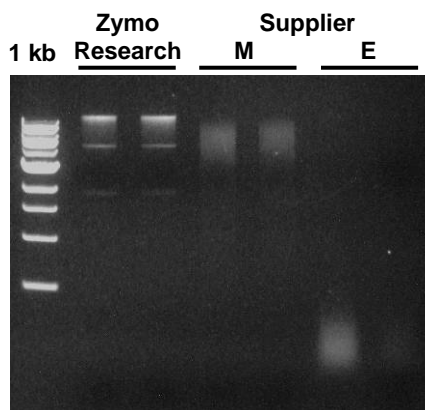


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The **ZR Soil Microbe DNA Kit™** can be used to isolate high quality DNA from a variety of soil types which yields robust products following PCR. **Panel A:** Physical characteristics of sampled soils (1-5) (Ref. 1). **Panel B:** Microbial DNA was 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).

References:

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 up to 0.25 grams of soil sample to a **ZR BashingBead™ Lysis Tube**. Add 750 µl **Lysis Solution** to the tube.

Alternatively, add 50-100 mg (wet weight) fungal and/or bacterial cells that have been resuspended in up to 200 µl of water or isotonic buffer (e.g., PBS) to a **ZR BashingBead™ Lysis Tube**.

2. Secure in a bead beater fitted with a 2 ml tube holder assembly (e.g., Disruptor Genie™) and process at maximum speed for 5 minutes.

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

3. Centrifuge the **ZR BashingBead™ Lysis Tube** in a microcentrifuge at $\geq 10,000 \times g$ for 1 minute.

4. Transfer up to 400 µl supernatant to a **Zymo-Spin™ IV Spin Filter** (orange top) in a **Collection Tube** and centrifuge at 7,000 rpm ($\sim 7,000 \times g$) for 1 minute.

5. Add 1,200 µl of **Soil DNA Binding Buffer** to the filtrate in the **Collection Tube** from Step 4.

6. Transfer 800 µl of the mixture from Step 5 to a **Zymo-Spin™ IIC Column** in a **Collection Tube** and centrifuge at 10,000 x *g* for 1 minute.

7. Discard the flow through from the **Collection Tube** and repeat Step 6.

8. Add 200 µl **DNA Pre-Wash Buffer** to the **Zymo-Spin™ IIC Column** in a new **Collection Tube** and centrifuge at 10,000 x *g* for 1 minute.

9. Add 500 µl **Soil DNA Wash Buffer** to the **Zymo-Spin™ IIC Column** and centrifuge at 10,000 x *g* for 1 minute.

10. Transfer the **Zymo-Spin™ IIC Column** to a clean 1.5 ml microcentrifuge tube and add 100 µl **DNA Elution Buffer** directly to the column matrix. Centrifuge at 10,000 x *g* for 30 seconds to elute the DNA.

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

11. 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 now suitable for PCR and other downstream applications.

If the HRC matrix is dry, add 400-600 µl water prior to prepping the filter.

Cap tube tightly to prevent leakage.

Alternatively, a standard bench top vortex can be used although the overall yield of DNA may be lower.

Snap off the base of the **Zymo-Spin™ IV Spin Filter** prior to use.

The **Zymo-Spin™ IIC Column** has a maximum capacity of 800 µl.

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.

Disruptor Genie™ is a trademark of Scientific Industries, Inc.
FastPrep® is a registered trademark of Qbiogene, Inc.

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Ordering Information

| Product Description | Catalog No. | Kit Size |
|--------------------------|-------------|-----------|
| ZR Soil Microbe DNA Kit™ | D6001 | 50 preps. |

| For Individual Sale | Catalog No. | Amount |
|---------------------------------------------|-------------|-------------|
| ZR BashingBead™ Lysis Tubes | S6002-50 | 50 tubes |
| Lysis Solution | D6001-3-40 | 40 ml |
| Soil DNA Binding Buffer | D6001-1-100 | 100 ml |
| DNA Pre-Wash Buffer | D3004-5-15 | 15 ml |
| Soil DNA Wash Buffer | D6001-2-50 | 50 ml |
| DNA Elution Buffer | D3004-4-10 | 10 ml |
| Zymo-Spin™ IV Spin Filters (Orange Tops) | C1007-50 | 50 filters |
| Zymo-Spin™ IV-HRC Spin Filters (Green Tops) | C1010-50 | 50 filters |
| Zymo-Spin™ IIC Columns | C1011-50 | 50 columns |
| Collection Tubes | C1001-50 | 50 tubes |
| | C1001-500 | 500 tubes |
| | C1001-1000 | 1,000 tubes |

Lysis Instruments



| Description | Cat. No. | Amount |
|-------------------------------------------------------------------------------------------------------------------------------------|-------------|--------|
| Disruptor Genie™, 120V w/ 2 ml tube holder assembly. | S6001-2-120 | 1 unit |
| Disruptor Genie™, 240V w/ 2 ml tube holder assembly. | S6001-2-240 | 1 unit |
| TurboMix Attachment, 2 ml Permanently mounts to most existing Vortex Genie™ mixers converting them to a Disruptor Genie™. | S6004-2 | 1 unit |

The **Disruptor Genie™** with 2 ml tube holder assembly from Scientific Industries, Inc. (Cat. No. S6001-2 from Zymo Research Corp.)

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The Ultimate Combination For Efficient Sample Lysis!

BashingBead™ Kits From Zymo Research & The FastPrep®-24 Instrument From MP Biomedicals.



**Lyse Any Tough or Frozen Sample
in 40 Seconds or Less!**

| Description | Cat. No. | Amount |
|------------------------------------------------------------------------|----------|--------|
| FastPrep®-24 Instrument (Supplied w/ 24 x 2 ml head adapter) | S6005 | 1 unit |

FastPrep®-24 with 24 x 2 ml head assembly (Cat. No. S6005 from Zymo Research)

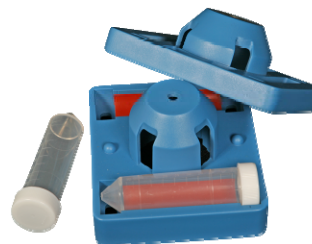
FastPrep® Accessories



For -80°C Lysis

| Description | Cat. No. | Amount |
|-------------------------------------------------------------|----------|--------|
| HiPrep™ Attachment (Accommodates 48 x 2 ml tubes) | S6005-1 | 1 unit |

| Description | Cat. No. | Amount |
|---------------------------------------------------------------|----------|--------|
| CryoPrep™ Attachment (Accommodates 24 x 2 ml tubes) | S6005-2 | 1 unit |



| Description | Cat. No. | Amount |
|----------------------------------------------------------------|----------|--------|
| TeenPrep™ Attachment (Accommodates 12 x 15 ml tubes) | S6005-3 | 1 unit |

| Description | Cat. No. | Amount |
|--------------------------------------------------------------|----------|--------|
| BigPrep™ Attachment (Accommodates 2 x 50 ml tubes) | S6005-4 | 1 unit |

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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 |