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The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-DNA™ Fecal/Soil Microbe Microprep Kit Catalog No. **D6012**

Highlights

- Rapid method for the isolation of inhibitor-free, PCR-quality DNA (up to 5 µg/prep) from microbes including Gram positive and negative bacteria, fungi, algae, protozoa, etc. in fecal and soil samples in as little as 20 minutes.
- State-of-the-art, ultra-high density **BashingBeads™** are fracture resistant and chemically inert.
- 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

| Quick-DNA™ Fecal/Soil Microbe Microprep Kit (Kit Size) | D6012 (50 Preps.) | Storage Temperature |
|---|--------------------------|----------------------------|
| ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm) | 50 | Room Temp. |
| BashingBead™ Buffer | 40 ml | Room Temp. |
| Genomic Lysis Buffer¹ | 100 ml | Room Temp. |
| DNA Pre-Wash Buffer² | 15 ml | Room Temp. |
| g-DNA Wash Buffer | 50 ml | Room Temp. |
| DNA Elution Buffer | 10 ml | Room Temp. |
| Prep Solution | 30 ml | Room Temp. |
| Zymo-Spin™ III-F Filters | 50 | Room Temp. |
| Zymo-Spin™ II-µHRC Filters | 50 | Room Temp. |
| Zymo-Spin™ IC Columns | 50 | Room Temp. |
| Collection Tubes | 200 | Room Temp. |
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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 For optimal performance, add beta-mercaptoethanol to 0.5% (v/v) i.e., 500 µl per 100 ml.

² 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** – Host, bacterial, fungal, algal, protozoan, viral DNA can be isolated from up to 50 mg of feces or up to 250 mg of soil. The amount of soil sample processed will vary depending on the composition of the sample: process more soil material for wet muddy samples and less for dry sandy samples. Additionally, water³ or 10 – 20 mg (wet weight) fungal/bacterial cells⁴ can be processed.
- **DNA Purity** – High quality, inhibitor-free DNA is eluted with **DNA Elution Buffer** suitable for the amplification of bacterial, protist, and/or mammalian templates ($A_{260}/A_{280} > 1.8$).
- **DNA Size Limits** – Capable of recovering genomic DNA up to and above 40 kb. In most instances, mitochondrial DNA and viral DNA (if present) will also be recovered.
- **DNA Recovery** – Typically, up to 5 µg total DNA is eluted into 20 µl **DNA Elution Buffer** per sample.
- **Equipment** – Microcentrifuge, vortex, cell disrupter/disrupter (recommended).

³ For water samples, use desired filter to collect sample (not provided) and cut the filter into small pieces before adding to the lysis tube.

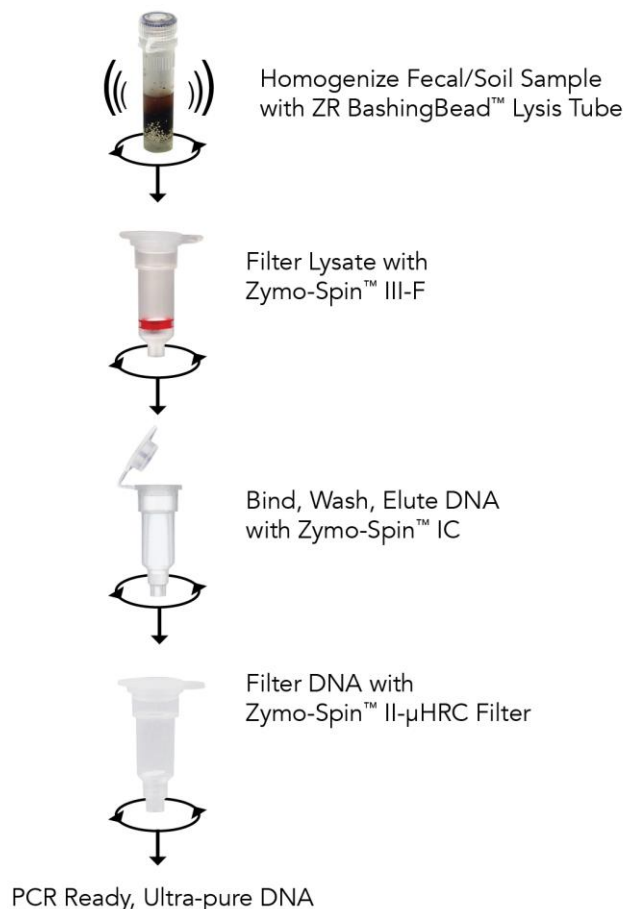
⁴ This equates to approximately 2×10^8 bacterial cells and 2×10^7 yeast cells.

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended for use in diagnostic procedures. 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. Disruptor Genie™ is a trademark of Scientific Industries, Inc. and FastPrep® is a registered trademark of QbioGene, Inc.

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Product Description

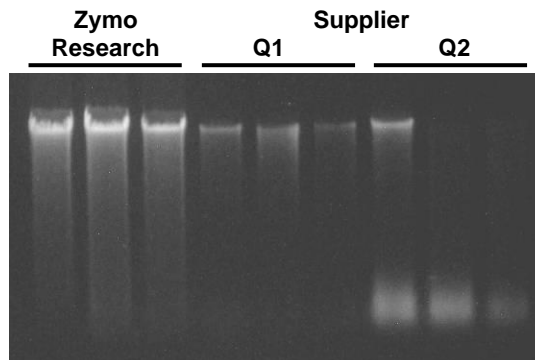
The **Quick-DNA™ Fecal/Soil Microbe Microprep Kit** is designed for the simple, rapid isolation of inhibitor-free, PCR-quality DNA from a variety of fecal (including humans, birds, rats, mice, cattle, etc.) and soil (including clay, sandy, silty, peaty, chalky, and loamy soils) samples. The kit can be used to successfully isolate DNA from tough-to-lyse Gram positive and negative bacteria, fungi, algae, protozoa, etc. that inhabit fecal and soil samples. The procedure is easy and can be completed in as little as 15 minutes: fecal samples (≤ 50 mg each) or soil samples (≤ 250 mg each) are added directly to a **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)** and rapidly and efficiently lysed by bead beating without the use of organic denaturants or proteinases. The DNA is then isolated using our Zymo-Spin™ Technology, which is subsequently filtered to remove humic acids/polyphenols that inhibit PCR. The DNA is ideal for downstream molecular-based applications including PCR, arrays, genotyping, etc. A schematic of the **Quick-DNA™ Fecal/Soil Microbe Microprep Kit** procedure is shown below.



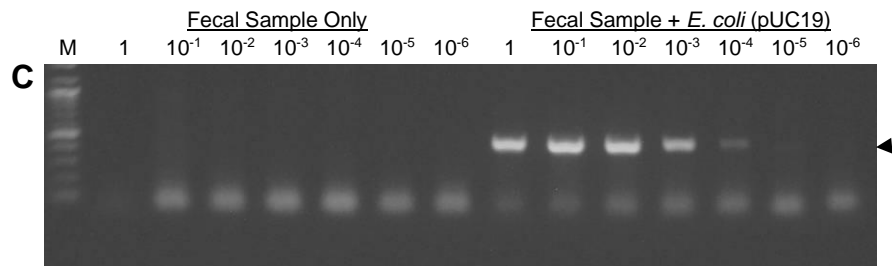
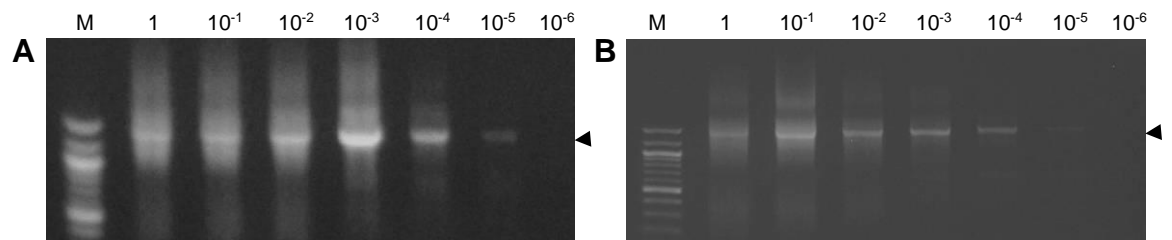
DNA/RNA Shield™ (R1100-50, R1100-250) can be used to stabilize nucleic acids and inactivate infectious agents in a variety of samples, without the need for reagent removal.

Fecal DNA Isolation

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

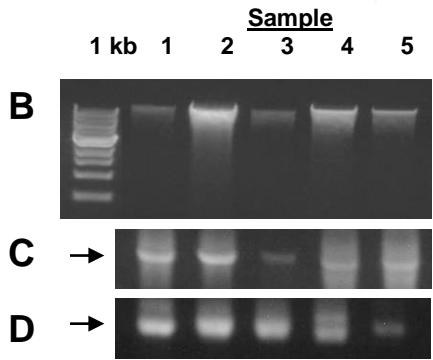
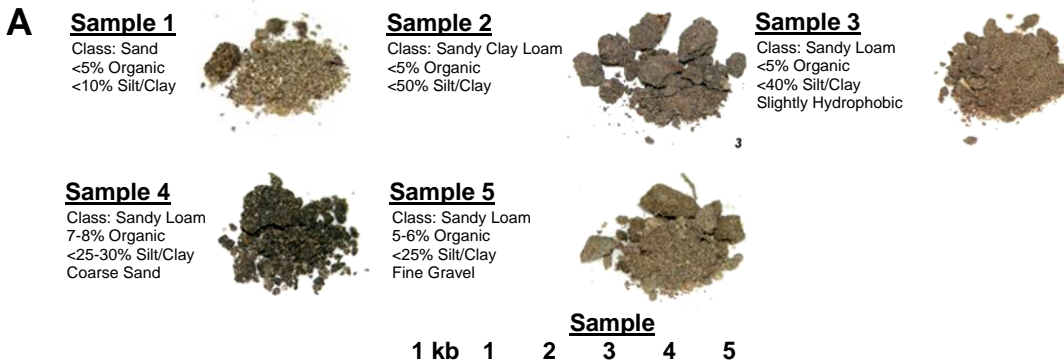


Comparison of DNA yields from rat feces using the **Quick-DNA™ Fecal/Soil Microbe Kit** and kits from suppliers Q1 and Q2. Equivalent amounts of feces were processed using each kit and then equal volumes of eluted DNA were analyzed in a 0.8% (w/v) agarose/ethidium bromide gel. Samples were processed in triplicate.

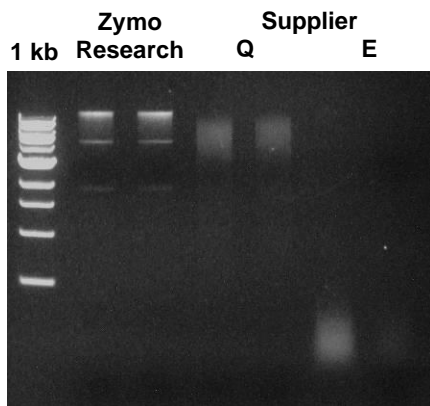


PCR of DNAs from rat and human fecal samples isolated with the **Quick-DNA™ Fecal/Soil Microbe Kit**. **Panel A** and **B** show the results of PCR with DNA isolated from rat and human fecal samples, respectively, using primers specific for prokaryotic 16S rRNA. **Panel C** shows the results of PCR of DNA isolated from human feces with and without the addition of *E. coli* containing pUC19 plasmid DNA (indicated at the top of the image) using primers specific for the pUC19 sequence. In each case, amplicons were analyzed in a 1.5% (w/v) agarose / ethidium bromide gel using a UV imager. Numbers above each lane of the gel images are the volumetric equivalent (in μ l) of eluted DNA (100 μ l) used for PCR. Arrows mark the relative migration of amplicons in the gels, and M is a 100 bp DNA ladder (NEB).

Soil Microbe DNA Isolation



The **Quick-DNA™ Fecal/Soil Microbe 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 **Quick-DNA™ Fecal/Soil Microbe 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 1 kb 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 **Quick-DNA™ Fecal/Soil Microbe Kit** is high-quality and structurally intact. Equivalent amounts of yeast were processed using the **Quick-DNA™ Fecal/Soil Microbe Kit** or the kits from suppliers Q 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.

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Protocol

For optimal performance, add beta-mercaptoethanol (user supplied) to the **Genomic Lysis Buffer** to a final dilution of 0.5% (v/v) i.e., 500 µl per 100 ml.

1. Add ≤50 mg of fecal sample or ≤250 mg of soil sample to a **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)**. Add 750 µl **BashingBead™ Buffer** to the tube and cap tightly.

Note: Alternatively, add water sample¹ or 10 – 20 mg (wet weight) bacterial/bacterial cells² 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 and process at maximum speed for ≥ 5 minutes.

Note: Processing time will vary based on sample input and bead beater. Times may be as little as 5 minutes when using high-speed cell disrupters (FastPrep® -24) or as long as 20 minutes when using lower speeds (e.g., Disruptor Genie®).

3. Centrifuge the ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm) in a microcentrifuge at ≥10,000 x g for 1 minute.

4. Transfer up to 400 µl supernatant to a **Zymo-Spin™ III-F Filter** in a **Collection Tube** and centrifuge at 8,000 x g for 1 minute. Discard the Zymo-Spin™ III-F Filter.

5. Binding preparation:

| Feces and All Non-Soil Samples | OR | Soil Samples |
|---|----|---|
| Add 1,200 µl of Genomic Lysis Buffer to the filtrate in the Collection Tube from Step 4. Mix well. | | Add 800 µl of Genomic Lysis Buffer and 400 µl of 95% ethanol to the filtrate in the Collection Tube from Step 4. Mix well. |

6. Transfer 800 µl of the mixture from Step 5 to a **Zymo-Spin™ IC Column³** 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™ IC Column in a new Collection Tube and centrifuge at 10,000 x g for 1 minute.

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

10. Transfer the Zymo-Spin™ IC Column to a clean 1.5 ml microcentrifuge tube and add ≥ 20 µl **DNA Elution Buffer** directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA^{4, 5}.

11. Place a **Zymo-Spin™ II-µHRC Filter** in a clean Collection Tube and add 600 µl **Prep Solution**. Centrifuge at 8,000 x g for 3 minutes.

12. Transfer the eluted DNA to a prepared Zymo-Spin™ II-µHRC Filter in a clean 1.5 ml microcentrifuge tube and centrifuge at exactly 16,000 x g for 3 minutes.

The filtered DNA is now suitable for PCR and other downstream applications.

¹ For water samples, filter using desired non-silica based filter (not provided). Cut the filter into small pieces before adding to the lysis tube.

² This equates to approximately 2x10⁸ bacterial cells and 2x10⁷ yeast cells.

³ The Zymo-Spin™ IC Column has a maximum capacity of 800 µl.

⁴ 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.

⁵ If fungi or bacterial cultures were sampled, the DNA is now suitable for PCR as well as other downstream applications.

Ordering Information

| Product Description | Catalog No. | Kit Size |
|--|-------------|-------------|
| Quick-DNA™ Fecal/Soil Microbe Microprep Kit | D6012 | 50 preps. |
| Quick-DNA™ Fecal/Soil Microbe Miniprep Kit | D6010 | 50 preps. |
| Quick-DNA™ Fecal/Soil Microbe Midiprep Kit | D6110 | 25 preps. |
| Quick-DNA™ Fecal/Soil Microbe 96 Kit | D6011 | 2x96 preps. |

| For Individual Sale | Catalog No. | Amount |
|---|-------------|--------|
| ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm) | S6012-50 | 50 |
| BashingBead™ Buffer | D6001-3-40 | 40 ml |
| Genomic Lysis Buffer | D3004-1-100 | 100 ml |
| DNA Pre-Wash Buffer | D3004-5-15 | 15 ml |
| g-DNA Wash Buffer | D6010-2-50 | 50 ml |
| DNA Elution Buffer | D3004-4-10 | 10 ml |
| Prep Solution | D6035-1-30 | 30 ml |
| Zymo-Spin™ III-F Filters | C1057-50 | 50 |
| Zymo-Spin™ IC Columns | C1004-50 | 50 |
| Collection Tubes | C1001-50 | 50 |
| | C1001-500 | 500 |
| | C1001-1000 | 1,000 |

Lysis Instruments



| Description | Cat. No. | Amount |
|---|-------------|--------|
| Disruptor Genie™, 120V w/ 2 ml tube holder assembly. | S6001-2-120 | 1 unit |
| Disruptor Genie™, 230V w/ 2 ml tube holder assembly. | S6001-2-230 | 1 unit |
| TurboMix Attachment, 2 ml Permanently mounts to most existing Vortex Genie™ mixers converting them to a Disruptor Genie™. | S6004 | 1 unit |

The **Disruptor Genie™** with 2 ml tube holder assembly from Scientific Industries, Inc. (Cat. No. S6001-2-120 from Zymo Research Corp.)

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