Innovation to support science....

ZYMO RESEARCH

*The Beauty of Science is to Make Things Simple*

# INSTRUCTION MANUAL

# *Quick*-DNA™ Fecal/Soil Microbe 96 Kit

Catalog No. **D6011**

## Highlights

- Rapid, high-throughput (96-well) method for the isolation of inhibitor-free, PCR-quality DNA from microbes including Gram positive and Gram negative bacteria, fungi, algae, protozoa, etc. in fecal and soil samples in as little as 50 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.

## Contents






**ZYMO RESEARCH CORP.**

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## Product Contents

Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product, please call 1-888-882-9682.

| ***Quick*-DNA™ Fecal/Soil Microbe 96 Kit** (Kit Size) | **D6011** (2x96 Preps.) | **Storage Temperature** |
|---|---|---|
| **ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm)** | 2 | Room Temp. |
| **BashingBead™ Buffer** | (2) 40 ml | Room Temp. |
| **Genomic Lysis Buffer**[1] | 150 ml | Room Temp. |
| **DNA Pre-Wash Buffer**[2] | 50 ml | Room Temp. |
| **g-DNA Wash Buffer** | 100 ml | Room Temp. |
| **DNA Elution Buffer** | (2) 10 ml | Room Temp. |
| **Prep Solution** | 30 ml | Room Temp. |
| **Deep-Well Block** | 2 | Room Temp. |
| **Silicon-A™ Plate** | 2 | Room Temp. |
| **Silicon-A™-HRC Plate** | 2 | Room Temp. |
| **Collection Plate** | 2 | Room Temp. |
| **Elution Plate** | 6 | Room Temp. |
| **Cover Foil** | 4 | 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.

[1] For optimal performance, add beta-mercaptoethanol to 0.5% (v/v) i.e., 750 µl per 150 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 ºC for 30 minutes and mix by inversion. DO NOT MICROWAVE.

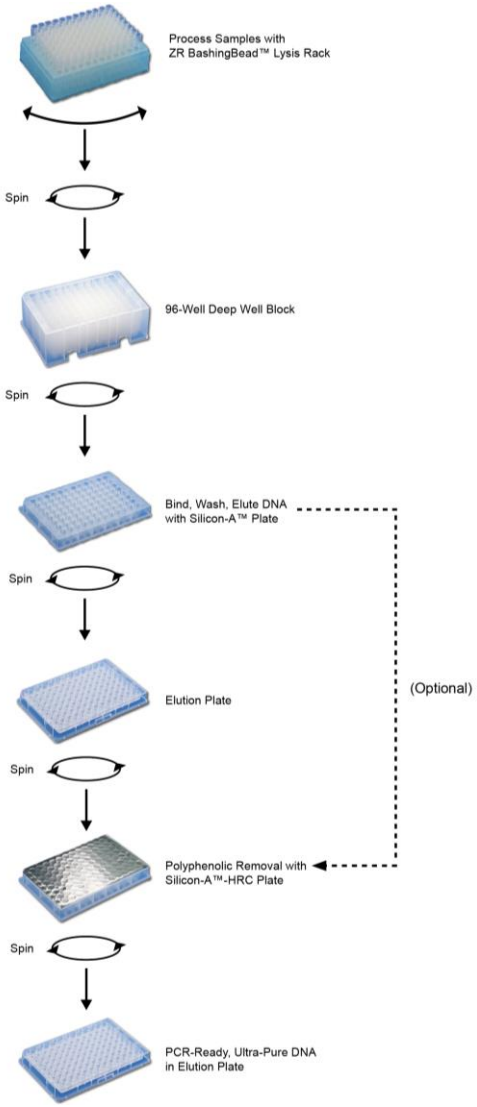
## Specifications

- **Format** – Bead Beating, 96-Well Plate Purification.
- **Sample Sources** – Host, bacterial, fungal, algal, protozoan, viral DNA can be isolated from up to 80 mg sample of mammalian feces or up to 135 mg 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, 10 – 20 mg (wet weight) bacterial/fungal cells[3] 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 sized fragments 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 100 µl (50 µl minimum) **DNA Elution Buffer** per sample.
- **Equipment** – Centrifuge w/ microplate carrier, 96-well plate/block disruptor or pulverizer.

[3] This equates to approximately $2x10^8$ bacterial cells and $2x10^7$ yeast cells.

## Product Description

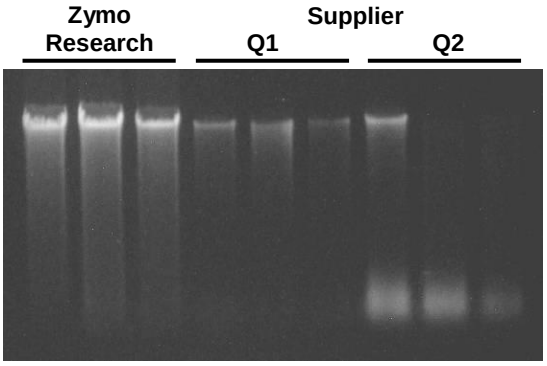
The ***Quick*-DNA™ Fecal/Soil Microbe 96 Kit** is designed for the simple, rapid, and high-throughput (96-well) isolation of inhibitor-free, PCR-quality DNA from a variety of fecal (including humans, birds, rats, mice, cattle, etc.) and soil (including clay, sandy, silty, peaty, chalky, and loamy soils) samples. The kit can be used to successfully isolate DNA from tough-to-lyse Gram positive and Gram negative bacteria, fungi, algae, protozoa, etc. that inhabit fecal and soil samples. The procedure is easy and can be completed in as little as 50 minutes: fecal samples (≤ 80 mg each) or soil samples (≤ 135 mg) are added directly to a **ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm)** and rapidly and efficiently lysed by bead beating (e.g., 2010 GenoGrinder®, Page 6) without the use of organic denaturants or proteinases. The DNA is then isolated and purified using our Zymo-Spin™ Technology, which is subsequently filtered to remove PCR inhibitors. The DNA is ideal for downstream molecular-based applications including PCR, arrays, genotyping, etc. A schematic of the ***Quick*-DNA™ Fecal/Soil Microbe 96 Kit** procedure is shown below.



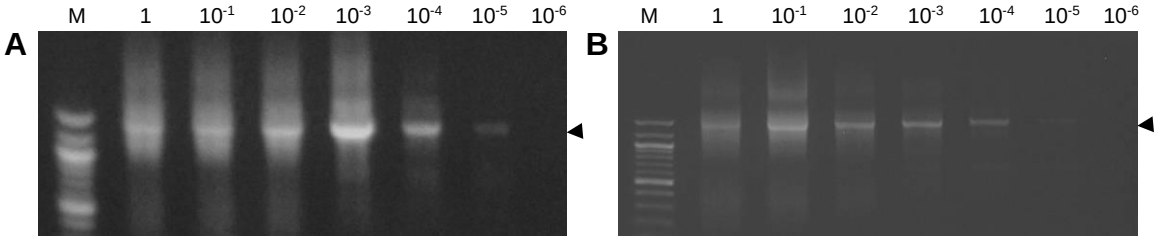

**DNA/RNA Shield™** (R1100-50, R1100-250) can be used to stabilize nucleic acids and inactivate infectious agents in a variety of samples, without the need for reagent removal.

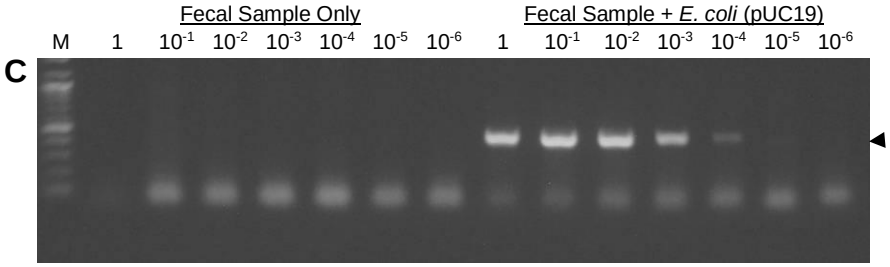
## Fecal DNA Isolation

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. Panels 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 µl) of eluted DNA (100 µl) 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

**A**

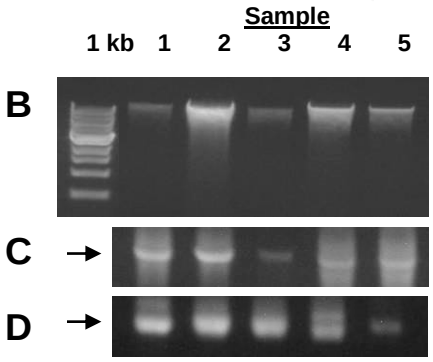
**Sample 1**
Class: Sand
<5% Organic
<10% Silt/Clay

**Sample 2**
Class: Sandy Clay Loam
<5% Organic
<50% Silt/Clay

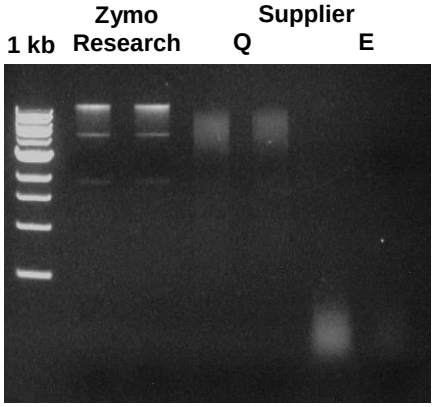
**Sample 3**
Class: Sandy Loam
<5% Organic
<40% Silt/Clay
Slightly Hydrophobic

**Sample 4**
Class: Sandy Loam
7-8% Organic
<25-30% Silt/Clay
Coarse Sand

**Sample 5**
Class: Sandy Loam
5-6% Organic
<25% Silt/Clay
Fine Gravel




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.

## 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., 750 µl per 150 ml.

1. Add ≤ 80 mg of fecal sample or ≤135 mg of soil to the tubes of a **ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm)**. Add 400 µl **BashingBead™ Buffer** to each tube and cap tightly.

   ***Note:*** *Alternatively, add 10-20 mg (wet weight) fungal and/or bacterial cells[1] that have been resuspended in up to 50 µl of water or isotonic buffer (e.g., PBS) to the tubes of a ZR BashingBead™ Lysis Rack.*

2. Secure in a 96-well block/plate bead beater (e.g., 2010 GenoGrinder®) and process samples. Optimization of processing time/speed will be necessary for complete sample lysis.

   ***Note:*** *Processing times may be as little as one minute when using high-speed cell disrupters (e.g., 2000 GenoGrinder®, page 6). See manufacturer's literature for operating information.*

3. Centrifuge the ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm) at ≥ 3,000 x *g* (5,000 x *g* max.) for 5 minutes.

4. Transfer up to 250 µl supernatant to each well of a **96-Well Block**.

5. Binding Preparation:

| Feces and All Non-Soil Samples | OR | Soil Samples |
|---|---|---|
| Add 750 µl of **Genomic Lysis Buffer** to the filtrate in the 96-Well block from Step 4. | | Add 500 µl of **Genomic Lysis Buffer** and 250 µl of 95% ethanol to the filtrate in the 96-Well Block from Step 4. |

   Cover completely with **Cover Foil** and mix thoroughly by vortexing for 2 minutes. Centrifuge the 96-Well Block at ≥ 3,000 x *g* (5,000 x *g* max.) for 5 minutes.

6. Remove or pierce foil and transfer 500 µl from the wells of Step 5 to the wells[2] of a **Silicon-A™ Plate,** mounted on a **Collection Plate**. Centrifuge the assembly at ≥ 3,000 x *g* (5,000 x *g* max.) for 5 minutes.

7. Discard the flow-through from the Collection Plate and repeat Step 6.

8. Add 200 µl **DNA Pre-Wash Buffer** to the wells of the Silicon-A™ Plate, mounted on the emptied Collection Plate, and centrifuge the assembly at ≥ 3,000 x *g* for 5 minutes.

9. Add 500 µl **g-DNA Wash Buffer** to the wells of the Silicon-A™ Plate on the Collection Plate and centrifuge the assembly at ≥ 3,000 x *g* for 5 minutes.

10. Prepare the **Silicon-A™-HRC Plate[3]** by mounting it on an **Elution Plate**. Add 150 µl **Prep Solution** to the wells by piercing through the cover foil. Incubate at room temperature for 5 minutes and centrifuge the assembly at exactly 3,500 x *g* for 5 minutes.

11. Place the Silicon-A™ Plate directly onto a prepared Silicon-A™-HRC Plate, and then mount the assembly on a new Elution Plate (this new assembly is a 3 plate stack).

12. Add 100 µl (50 µl minimum) **DNA Elution Buffer** directly to the matrices to the Silicon-A™ Plate on top. Centrifuge the assembly at exactly 3,500 x *g* for 3 minutes.

    Eluted, ultra-pure DNA is now ready for use in your experiments, or the Elution Plate can be covered with Cover Foil for storage of the DNA.

[1] This equates to approximately $2x10^8$ bacterial cells and $2x10^7$ yeast cells.

[2] Be careful to avoid pipetting debris that can clog the wells of the Silicon-A™ Plate.

[3] Make sure the matrices are located at the bottom of the wells of the Silicon-A™-HRC Plate by firmly tapping the plate against a flat surface.

GenoGrinder® is a registered trademark of Spex SamplePrep®, LLC

## Ordering Information

| Product Description | Format | Catalog No. | Kit Size |
|---|---|---|---|
| ***Quick*-DNA™ Fecal/Soil Microbe Miniprep Kit** | Spin Column | D6010 | 50 preps. |
| ***Quick*-DNA™ Fecal/Soil Microbe 96 Kit** | 96-Well | D6011 | 2x96 preps. |

| For Individual Sale | Catalog No. | Amount |
|---|---|---|
| **Genomic Lysis Buffer** | D3004-1-150 | 150 ml |
| **BashingBead™ Buffer** | D6001-3-40 | 40 ml |
| **DNA Pre-Wash Buffer** | D3004-5-50 | 50 ml |
| **g-DNA Wash Buffer** | D3004-2-100 | 100 ml |
| **DNA Elution Buffer** | D3004-4-10 | 10 ml |
| **Prep Solution** | D6035-1-30 | 30 ml |
| **ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm)** | S6002-96-3 | 1 rack |
| **96-Well Block** | P1001-2 | 2 blocks |
| **Silicon-A™ Plate** | C2001 | 2 plates |
| **Silicon-A™-HRC Plate** | C2009 | 2 plates |
| **Collection Plate** | C2002 | 2 plates |
| **Elution Plate** | C2003 | 2 plates |

### *The Ultimate Combination For High-Throughput Sample Lysis!*

***High-Throughput BashingBead™ Kits From Zymo Research & The 2010 GenoGrinder® Instrument From Spex SamplePrep.***

***High-Throughput Lysis of Tough or Frozen Samples in Minutes!***

| Description | Cat. No. | Amount |
|---|---|---|
| **2010 GenoGrinder®** w/ 2 x 96-well block head adapter | S6006 | 1 unit |
| **Aluminum CryoBlock** w/ 48 x 2.0 ml Tube Adapter | S6006-1 | 1 pair |

GenoGrinder and accessories for sale in USA only. Visit www.spexcsp.com for a distributor near you.