ZYMO RESEARCH

DNA Purification Made Simple

Quick-DNA™ Fecal/Soil Microbe 96 Kit

DNA from fecal, soil, and microbial samples.

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.

Catalog Numbers:
D6011

Scan with your smart-phone camera to view the online protocol/video.

Table of Contents

Product Contents

Quick-DNA™ Fecal/Soil Microbe 96 Kit	D6011 (2 x 96 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	-

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 30–37ºC for 30 minutes and mix by inversion. DO NOT MICROWAVE.

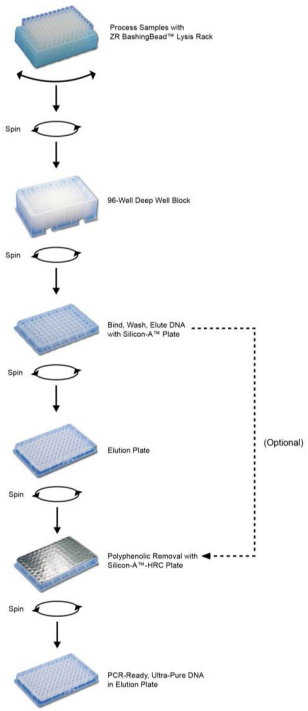
Specifications

- **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[1] 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 100 µl (50 µl minimum) **DNA Elution Buffer** per sample.
- **Equipment –** Centrifuge w/ microplate carrier, 96-well plate/block disruptor or pulverizer.

1 This equates to approximately 2 x 10^8 bacterial cells and 2 x 10^7 yeast cells.

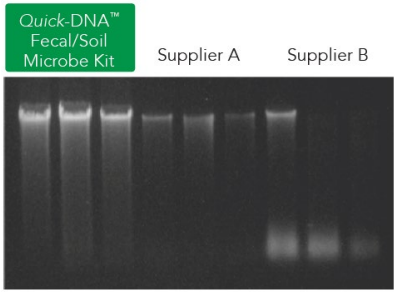
Product Description

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 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.

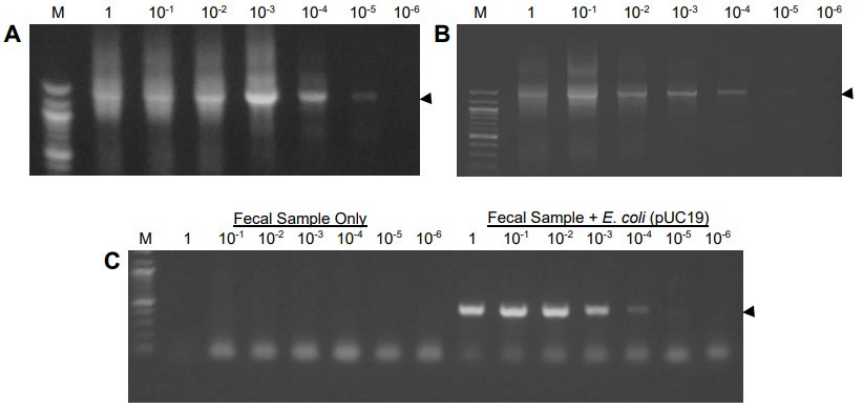


DNA/RNA Shield™ (R1100-50, R1100-250) can be used to stabilize nucleic acids and inactivate infectious agents in a variety of samples, without the need for reagent removal.

Fecal DNA Isolation

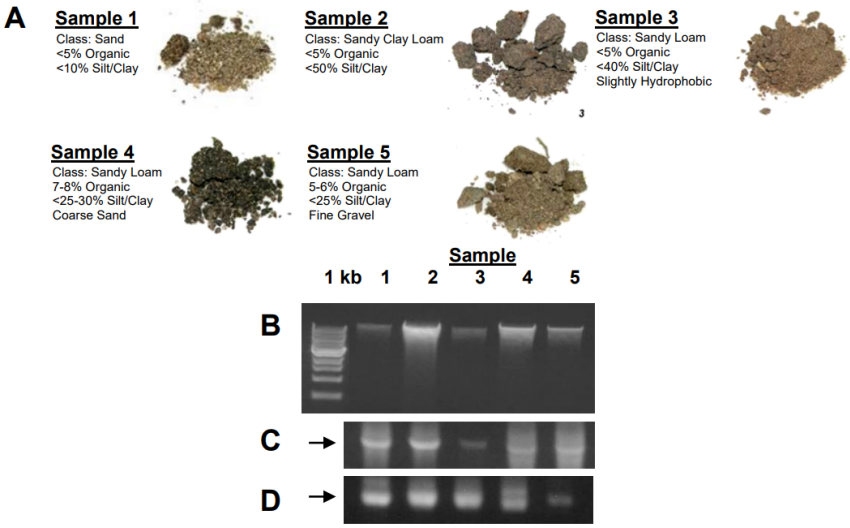


Comparison of DNA yields from rat feces using the ***Quick*-DNA™ Fecal/Soil Microbe Kit** and kits from suppliers Q1 and Q2. Equivalent amounts of feces were processed using each kit and then equal volumes of eluted DNA were analyzed in a 0.8% (w/v) agarose/ethidium bromide gel. Samples were processed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

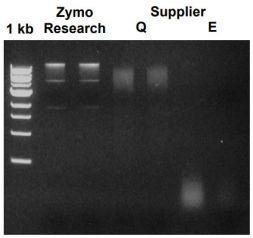


PCR of DNAs from rat and human fecal samples isolated with the ***Quick*-DNA™ Fecal/Soil Microbe Kit**. Panels A and B show the results of PCR with DNA isolated from rat and human fecal samples, respectively, using primers specific for prokaryotic 16S rRNA. Panel C shows the results of PCR of DNA isolated 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



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, CA 92711

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 bead beater fitted with the appropriate holder assembly for your bead beating module and process using optimized beat beating conditions (speed and time) for your device (see Appendix).

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. Add 750 µl of **Genomic Lysis Buffer** 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.

---

1 This equates to approximately 2 x 10^8 bacterial cells and 2 x 10^7 yeast cells.
2 Be careful to avoid pipetting debris that can clog the wells of the Silicon-A™ Plate.

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.

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.

Appendix

Optimized Lysis Protocols for Bead-Beating

The following conditions with different mechanical lysis machines were validated with minimum bias using the **ZymoBIOMICS™ Microbial Community Standard**.

1 Vortex Genie® with 2 ml BashingBead™ Tubes

Recommended for ease of use and accessibility

Use Microtube Adaptor (Scientific Industries, Inc. Cat. No. S5001-7)

1. 40 minutes of continuous bead beating (max of 18 tubes per adaptor)

2 Bertin Precellys® Evolution with 2 ml BashingBead™ Tubes

Recommended for ease of use and ultra-high speed

1. 1 minute on at 9,000 rpm
2. 2 minutes rest
3. Repeat cycle 4 times for a total of 4 minutes of bead beating

3 MP Fastprep®-24 with 2 ml BashingBead™ Tubes

Maximum of 20 tubes. The weight of >20 tubes may cause a system error

1. 1 minute on at max speed
2. 5 minutes rest
3. Repeat cycle 5 times for a total of 5 minutes of bead beating

4 Omni Bead Ruptor® Elite with 2 ml BashingBead™ Tubes

1. 1 minute on at 6 m/s
2. 5 minutes rest
3. Repeat cycle 3 times for a total of 3 minutes of bead beating

5 Biospec Mini-BeadBeater-16 with 2 ml BashingBead™ Tubes

1. 1 minute at maximum speed
2. 5 minutes rest
3. Repeat cycle 5 times for a total of 5 minutes of bead beating

6 Biospec Mini-BeadBeater-96 with 2 ml BashingBead™ Tubes

1. 5 minutes on at Max RPM
2. 5 minutes rest
3. Repeat cycle 4 times for a total of 20 minutes of bead beating

7 Biospec Mini-BeadBeater-96 with 96 well BashingBead™ Lysis Rack

1. 5 minutes on at Max RPM
2. 5 minutes rest
3. Repeat cycle 8 times for a total of 40 minutes of bead beating

X TissueLyser II

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

X TissueLyser LT

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

X Retsch Mixer Mill MM 400

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

Ordering Information

Product Description	Catalog No.	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 96 Kit**	D6011	2 x 96 Preps.

Individual Kit Components	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

100% satisfaction guarantee on all Zymo Research products, or your money back.

Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

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 the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not 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.

ZYMO RESEARCH

The **BEAUTY** *of* **SCIENCE** *is to Make Things* **SIMPLE**®

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682