INSTRUCTION MANUAL

Quick-DNA™ Fecal/Soil Microbe Midiprep Kit

Catalog No. **D6110**

Highlights

- Simple, efficient isolation of humic-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 25 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.

Phone: (949) 679-1190 ▪ Toll Free: (888) 882-9682 ▪ Fax: (949) 266-9452 ▪ info@zymoresearch.com ▪ www.zymoresearch.com

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 Midiprep Kit** (Kit Size)	**D6110** (25 Preps.)	**Storage Temperature**
ZR BashingBead™ Lysis/Filtration Tubes	25	Room Temp.
BashingBead™ Buffer	150 ml	Room Temp.
Genomic Lysis Buffer[1]	2 x 250 ml	Room Temp.
DNA Pre-Wash Buffer[2]	15 ml	Room Temp.
g-DNA Wash Buffer	50 ml	Room Temp.
DNA Elution Buffer	16 ml	Room Temp.
Prep Solution	30 ml	Room Temp.
Zymo-Spin™ V-E Columns w/ Zymo-Midi Filters™	25	Room Temp.
Zymo-Spin™ III-HRC Filters	50	Room Temp.
Collection Tubes	100	Room Temp.
Instruction Manual	1	Room Temp.

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

Specifications

- **Format** – Bead Beating, Spin/Vacuum Filtration, and Spin Column Purification
- **Sample Sources** – Host, bacterial, fungal, algal, protozoan, viral DNA can be isolated from up to 375 mg of feces or up to 5 g of soil (2.5 g recommended). 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, DNA can be isolated directly from pelleted fungi and bacteria.
- **DNA Purity** – High quality, humic/fulvic-free DNA is eluted with **DNA Elution Buffer** making it perfect for PCR. **(**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 ~125 µg total DNA is eluted into ≥150 µl **DNA Elution Buffer** per sample.
- **Equipment** – Centrifuge, vacuum source and manifold, microcentrifuge, cell disrupter or pulverizer w/ 50 ml tube adapter.

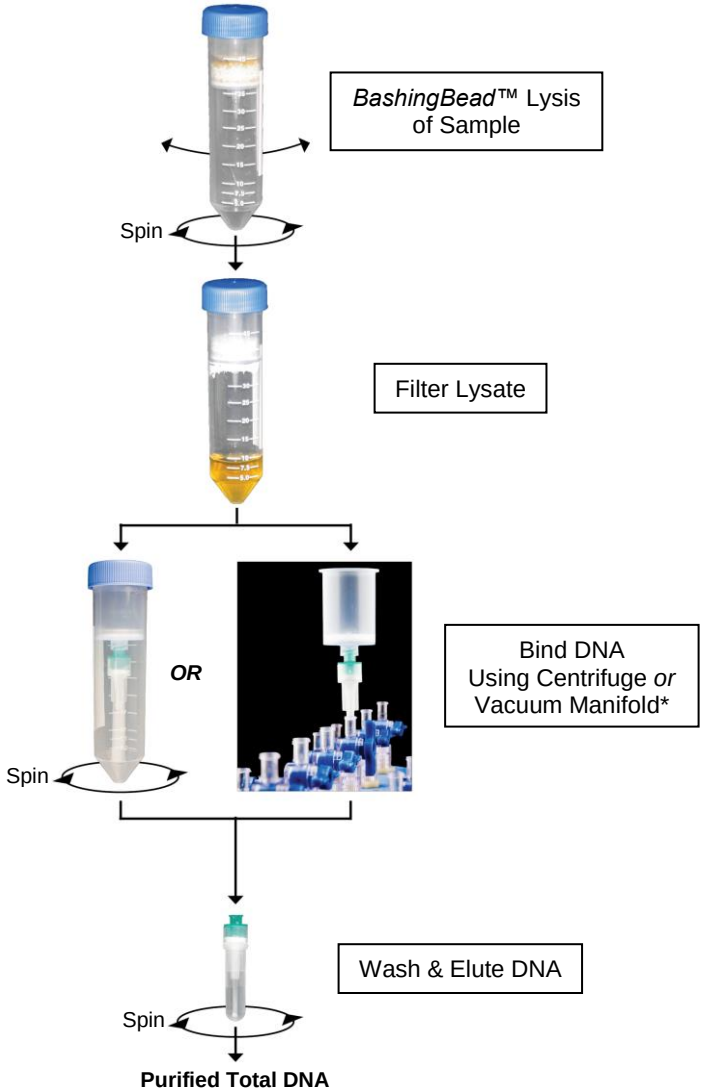
For rapid, robust, and simple purification of high quality, inhibitor-free DNA from any sample including feces, soil, water, biofilms, swabs, saliva, body fluids, etc. use the **ZymoBIOMICS™ DNA Miniprep Kit (D4300).**

Product Description

The ***Quick*-DNA™ Fecal/Soil Microbe Midiprep 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 procedure is easy and can be completed in as little as 25 minutes: fecal samples (≤375 mg each) or soil samples (≤ 5 g) are added directly to a **ZR BashingBead™ Lysis/Filtration Tube**, where microbes are 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 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. A schematic of the ***Quick*-DNA™ Fecal/Soil Microbe Midiprep Kit** procedure is shown below.

Zymo-Spin™ V-E Column w/
Zymo-Midi Filter™

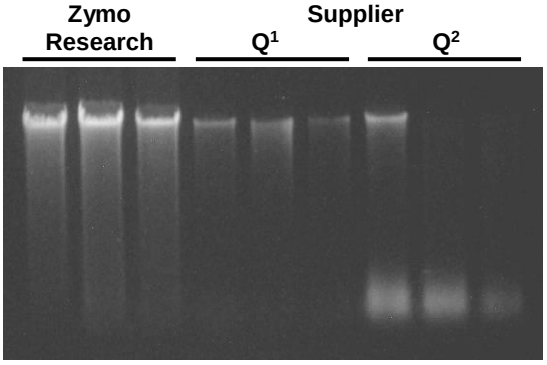
Zymo-Spin™ V-E Column



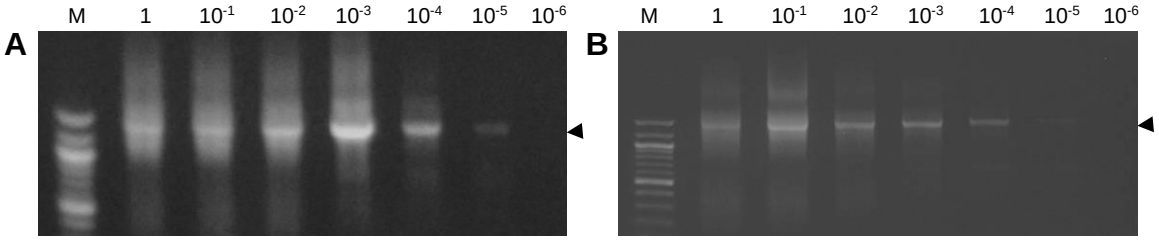
* Vacuum is the preferred method for DNA binding to the Zymo-Spin™ V-E column.

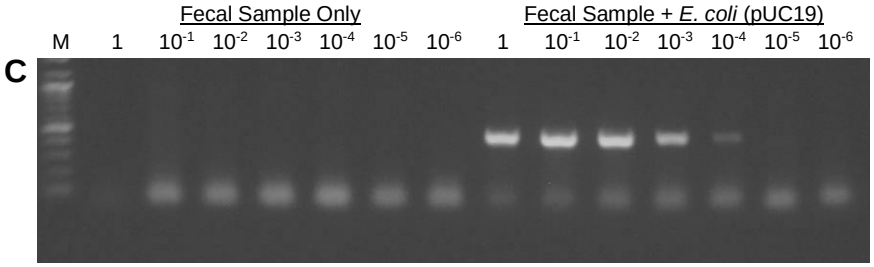
Fecal DNA Isolation

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 Q^1 and Q^2. 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

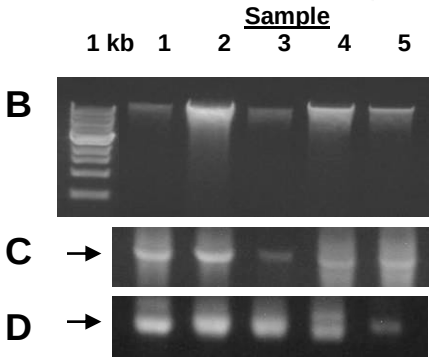
Sample 1
Class: Sand
<5% Organic
<10% Silt/Clay

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

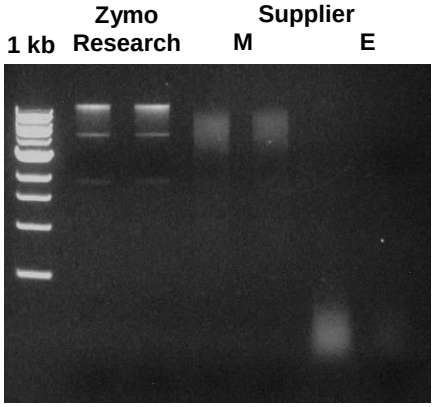
Sample 3
Class: Sandy Loam
<5% Organic
<40% Silt/Clay
Slightly Hydrophobic

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 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

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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., 2.5 ml per 500 ml.

1. Add 2.5 grams (5 g max.)[1] of soil sample or up to 375 mg of fecal samples to the bead/filter chamber of a **ZR BashingBead™ Lysis/Filtration Tube**. Add 6 ml **BashingBead™ Buffer** to the sample, cap tube tightly, and process.

   Note: *To prevent the **BashingBead™ Buffer** 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 **BashingBead™ Buffer** to a **ZR BashingBead™ Lysis/Filtration Tube**.

2. Secure in a bead beater fitted with a 50 ml tube holder assembly (see page 7) to 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 bead beaters (e.g., 2000 GenoGrinder®, page 4). See manufacturer's literature for specific operating information.*

3. Centrifuge the ZR BashingBead™ Lysis/Filtration Tube in a centrifuge at ≥3,000 x *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 supernatant[2] from the bottom of the tube to a clean 50 ml tube (not provided).

Feces and All Non-Soil Samples	OR	Soil Samples
Add 18 ml of **Genomic Lysis Buffer** to the supernatant. Mix well.		Add 12 ml of **Genomic Lysis Buffer** and 4 ml of 95% ethanol to the supernatant. Mix well.

5. Filter the entire mixture from Step 4 using a **Zymo-Spin™ V-E Column/Zymo-Midi Filter™** assembly mounted on a vacuum manifold[3] (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 microcentrifuge[4], then add 300 µl **DNA Pre-Wash Buffer** to the column and spin at 10,000 x *g* for 1 minute. Discard the flow through.

7. Add 400 µl **g-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 µl **DNA Elution Buffer** directly to the column matrix[5]. Wait for 1 minute and then centrifuge at 10,000 x *g* for 1 minute to elute the DNA[6].

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

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

10. Transfer the eluted DNA to a prepared Zymo-Spin™ III-HRC 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] 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] Be careful to avoid the pelleted material at the bottom of the tube when transferring the supernatant.

[3] 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!*

[4] Leave the rotor cover off the microcentrifuge if clearance with the column tops is a problem.

[5] DNA yields can be increased by performing a second elution and pooling the eluates.

[6] 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
***Quick*-DNA™ Fecal/Soil Microbe Miniprep Kit**	D6010	50 preps.
***Quick*-DNA™ Fecal/Soil Microbe 96 Kit**	D6011	2x96 preps.
***Quick*-DNA™ Fecal/Soil Microbe Midiprep Kit**	D6110	25 preps.

For Individual Sale	Catalog No.	Amount
ZR BashingBead™ Lysis/Filtration Tubes (50 ml) w/ 0.5 mm Beads	S6010	25
Genomic Lysis Buffer	D3004-1-100	100 ml
BashingBead™ Buffer	D6001-3-150	150 ml
DNA Pre-Wash Buffer	D3004-5-15	15 ml
g-DNA Wash Buffer	D3004-2-50	50 ml
DNA Elution Buffer	D3004-4-16	16 ml
Zymo-Spin™ V-E Columns w/ Zymo-Midi Filters™	C1021-25	25
OneStep™ PCR Inhibitor Removal Kit	D6030	50
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1,000

Compatible Lysis Instruments…

Bullet Blender™

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 sam les, 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

2010 Geno/Grinder®

The **2010 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.
2010 Geno/Grinder Instrument	S6006
50 ml Tube Holder/Cryo Block Assembly Accommodates 12 x 50 ml tubes/block.	S6006-3

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