



INSTRUCTION MANUAL

Quick-DNA™ Fecal/Soil Microbe Miniprep Kit Catalog No. D6010

Highlights

- Rapid method for the isolation of inhibitor-free, PCR-quality DNA (up to 25 µg/prep) from microbes including Gram-positive and Gram-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 Miniprep Kit (Kit Size)	D6010 (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™ III-HRC Filters	50	Room Temp.
Zymo-Spin™ IICR 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 150 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 50 – 100 mg (wet weight) fungal/bacterial cells⁴ can be isolated.
- **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 25 µg total DNA is eluted into 100 µl (50 µl minimum) **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.

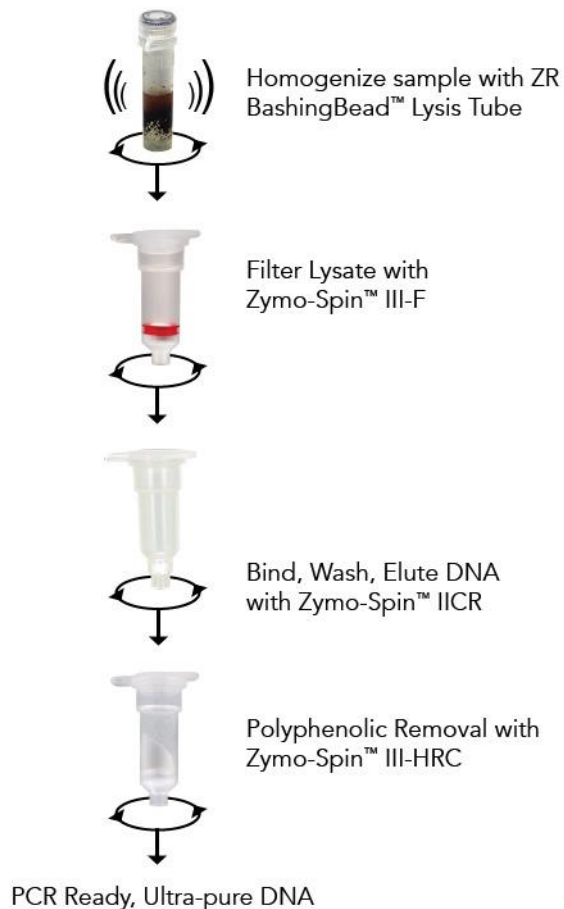
⁴ This equates to approximately 10⁹ bacterial cells and 10⁸ yeast cells.

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 Miniprep 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 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 15 minutes: fecal samples (≤ 150 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. Zymo-Spin™ Technology is then used to isolate the DNA, 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 Miniprep Kit** procedure is shown below.

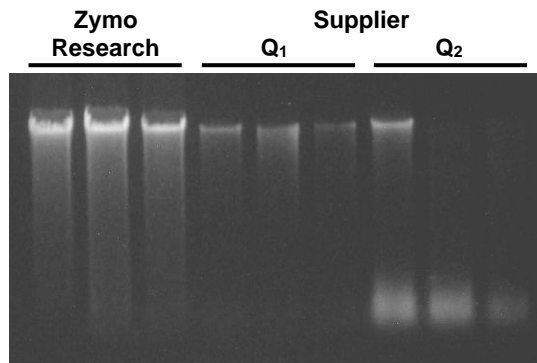


DNA/RNA Shield™ (R1100-50, R1100-250) can be used to stabilize nucleic acids and inactivate infectious agents in a variety of samples, without the need for reagent removal.

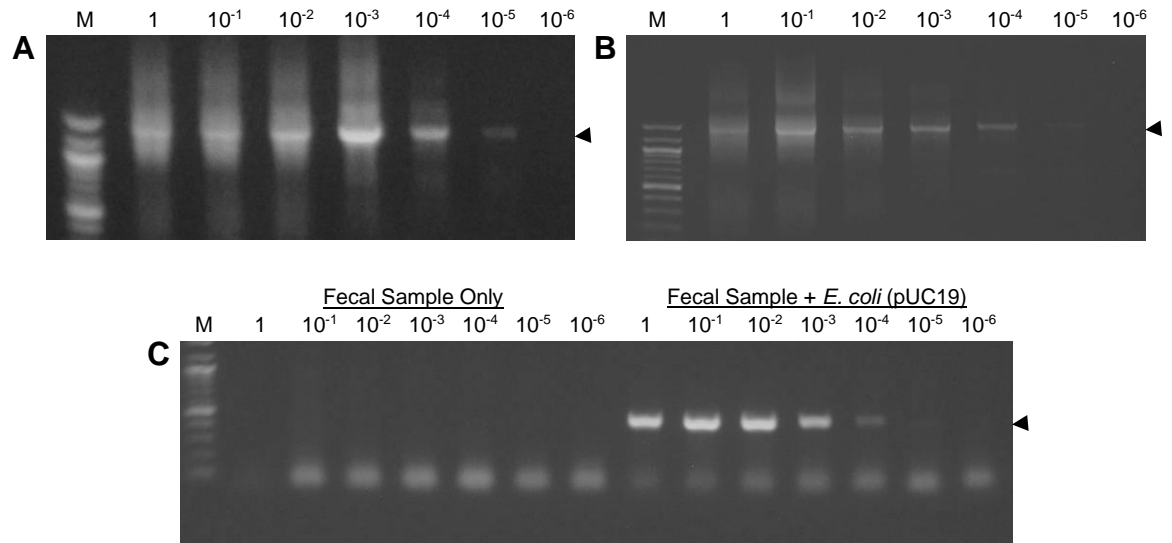
For rapid, robust, and simple purification of high quality, inhibitor-free DNA from any sample including feces, soil, water, biofilms, swabs, saliva, body fluids, etc. use the **ZymoBIOMICS™ DNA Miniprep Kit (D4300)**.

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.

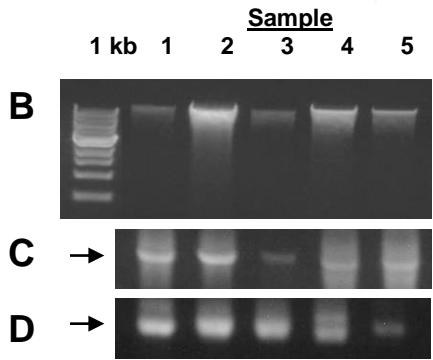
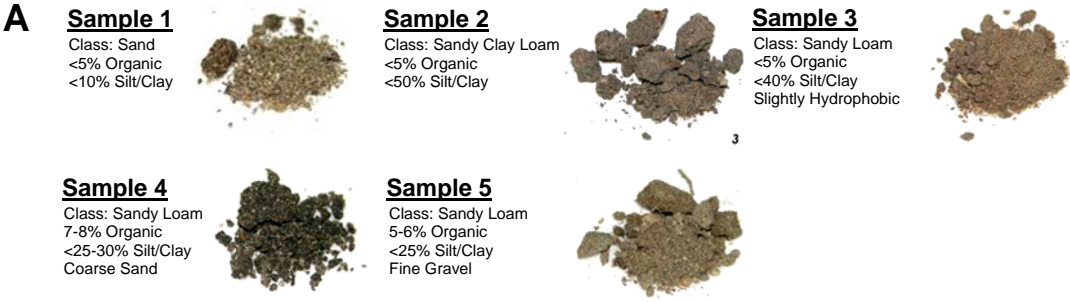


Comparison of DNA yields from rat feces using the **Quick-DNA™ Fecal/Soil Microbe Kit** and kits from suppliers Q₁ and Q₂. Equivalent amounts of feces were processed using each kit and then equal volumes of eluted DNA were analyzed in a 0.8% (w/v) agarose/ethidium bromide gel. Samples were processed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

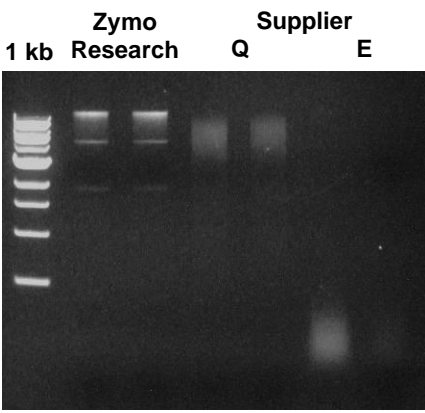


PCR of DNAs from rat and human fecal samples isolated with the **Quick-DNA™ Fecal/Soil Microbe Kit**. **Panel A** and **B** show the results of PCR with DNA isolated from rat and human fecal samples, respectively, using primers specific for prokaryotic 16S rRNA. **Panel C** shows the results of PCR of 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 ≤ 150 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 tube¹.

Note: Alternatively, add water sample² or 50-100 mg (wet weight) fungal/bacterial cells³ 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: Required processing time will vary depending on the device and application and therefore should be evaluated on a case by case basis.

For example, processing times may be as little as 3 minutes when using high-speed cell disrupters (e.g., the portable TerraLyzer™ Sample Processor, FastPrep® -24, or similar) or as long as 20 minutes when using lower speeds (e.g., Disruptor Genie™, or standard benchtop vortexes). See manufacturer's literature for operating information.

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.
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™ IICR Column**⁴ 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™ IICR 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™ IICR Column and centrifuge at 10,000 x g for 1 minute.
10. Transfer the Zymo-Spin™ IICR Column to a clean 1.5 ml microcentrifuge tube and add 100 µl (50 µl minimum) **DNA Elution Buffer** directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA^{5, 6}.
11. 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.
12. 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 Cap tube tightly to prevent leakage.

² For water samples, filter using desired non-silica based filter (not provided). Cut the filter into small pieces before adding to the lysis tube.

³ This equates to approximately 10⁹ bacterial cells and 10⁸ yeast cells.

⁴ The Zymo-Spin™ IICR Column has a maximum capacity of 800 µl.

⁵ 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.

⁶ If fungi or bacterial cultures were sampled, the DNA is now suitable for PCR as well as other downstream applications.

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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 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	D3004-2-50	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™ IICR Columns	C1078-50	50
OneStep™ PCR Inhibitor Removal Kit	D6030	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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