ZYMO RESEARCH

DNA Purification Made Simple

Quick-DNA™ Fecal/Soil Microbe Miniprep Kit

DNA from fecal, soil, and microbial samples.

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.

Catalog Numbers:
D6010

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

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Product Contents

Quick-DNA™ Fecal/Soil Microbe Miniprep Kit	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 Buffer[1]	100 ml	Room Temp.
DNA Pre-Wash Buffer[2]	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.
Instruction Manual	1	-

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

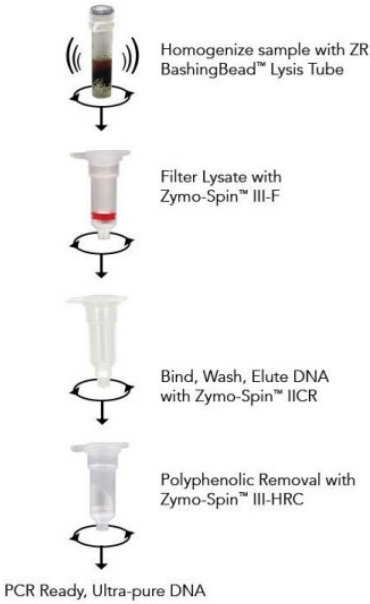
Specifications

- **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, water[1] or 50 – 100 mg (wet weight) fungal/bacterial cells[2] 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).

1 For water samples, use desired filter to collect sample (not provided) and cut the filter into small pieces before adding to the lysis tube.
2 This equates to approximately 10^9 bacterial cells and 10^8 yeast cells.

Product Description

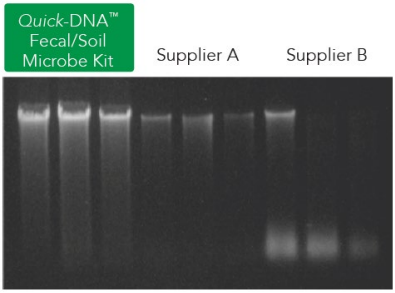
The ***Quick*-DNA™ Fecal/Soil Microbe Miniprep Kit** is designed for the simple, rapid isolation of inhibitor-free, PCR-quality DNA from a variety of fecal (including humans, birds, rats, mice, cattle, etc.) and soil (including clay, sandy, silty, peaty, chalky, and loamy soils) samples. The kit can 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.



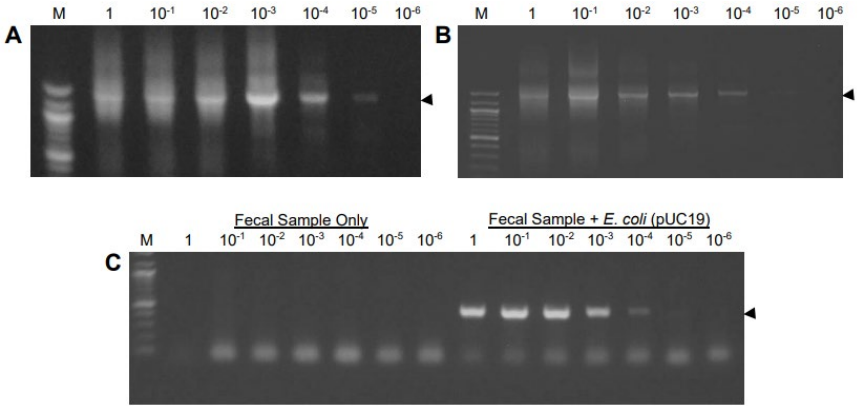
DNA/RNA Shield™ (R1100-50, R1100-250) can be used to stabilize nucleic acids and inactivate infectious agents in a variety of samples, without the need for reagent removal.

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

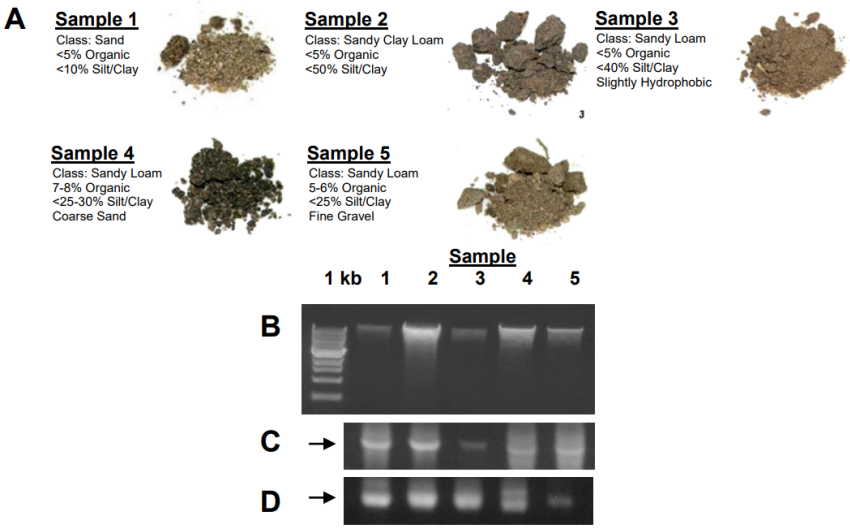


Comparison of DNA yields from rat feces using the ***Quick*-DNA™ Fecal/Soil Microbe Kit** and kits from suppliers A and B. Equivalent amounts of feces were processed using each kit and then equal volumes of eluted DNA were analyzed in a 0.8% (w/v) agarose/ethidium bromide gel. Samples were processed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

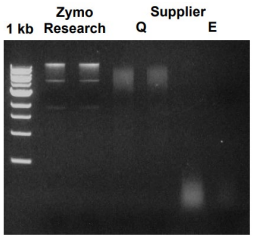


PCR of DNAs from rat and human fecal samples isolated with the ***Quick*-DNA™ Fecal/Soil Microbe Kit**. Panels A and B show the results of PCR with DNA isolated from rat and human fecal samples, respectively, using primers specific for prokaryotic 16S rRNA. Panel C shows the results of PCR of DNA isolated 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, California 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.*, 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 tube[1].

   ***Note**: Alternatively, add water sample[2] or 50-100 mg (wet weight) fungal/bacterial cells[3] that have been resuspended in up to 200 µl of water or isotonic buffer (e.g., PBS) to a **ZR BashingBead™ Lysis Tube**.*

   ***Note**: For samples stored in DNA/RNA Shield™, add up to 1 ml to a ZR BashingBead™ Lysis Tube. Do not add BashingBead™ Buffer and proceed to Step 2.*

2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process using optimized beat beating conditions (speed and time) for your device (see Appendix).

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. Add 1,200 µl of **Genomic Lysis Buffer** 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.

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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 10^9 bacterial cells and 10^8 yeast cells.
4 The **Zymo-Spin™ IICR Column** has a maximum capacity of 800 µl.

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.

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.

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
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	S6012-50	50 Tubes
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 Pack
Zymo-Spin™ IICR Columns	C1078-50	50 Pack
***OneStep*™ PCR Inhibitor Removal Kit**	D6030	50 Preps.
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 Pack 500 Pack 1,000 Pack

Lysis Instruments	Catalog No.	Amount
Horizontal Microtube Holder	S5001-7	1 Unit
Vortex-Genie® 2, 120V	S5001	1 Unit
Vortex-Genie® 2, 230V	S5002	1 Unit
Digital Vortex-Genie® 2, 120 V	S5003	1 Unit
Digital Vortex-Genie® 2, 230 V	S5004	1 Unit

The Vortex-Genie® 2 paired with the Horizontal Microtube Holder has been validated for efficient microbial lysis with the ZR BashingBead Lysis Tubes (0.1 & 0.5 mm). See the Appendix for more details.

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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.

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