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Produktinformation

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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-DNA™ Fecal/Soil Microbe 96 Magbead Kit

Catalog No. **D6010-FM, D6011-FM, D6012-FM**

Highlights

- Rapid method for the high throughput isolation of inhibitor-free, PCR-quality DNA (up to 25 µg/prep) from Gram positive and negative bacteria, fungi, algae, protozoa, etc. in fecal and soil samples in as little as 2 hours.
- 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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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 96 Magbead Kit** (Kit Size)	**D6010-FM** (2x 96 preps.)	**D6011-FM** (2x 96 preps.)	**D6012-FM** (2x 96 preps.)	**Storage Temperature**
Lysis Solution	150 ml	-	150 ml	Room Temp.
***Quick*-DNA MagBinding Buffer**	150 ml	150 ml	150 ml	Room Temp.
DNA Pre-Wash Buffer[1]	250 ml	250 ml	250 ml	Room Temp.
g-DNA Wash Buffer	200 ml x 2	200 ml x 2	200 ml x 2	Room Temp.
DNA Elution Buffer	50 ml	50 ml	50 ml	Room Temp.
MagBinding Beads	12 ml	12 ml	12 ml	Room Temp.
ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm)	2	-	-	Room Temp.
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	-	-	200	Room Temp.
Instruction Manual	1	1	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] 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

- **Sample Sources** – Host, bacterial, fungal, algal, protozoan, viral DNA can be effectively isolated from a ≤ 100 mg sample of mammalian feces or ≤ 200 mg soil. Additionally, water[1] or 5 – 20 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 50 µl (37.5 µl minimum) **DNA Elution Buffer** per sample.
- **Equipment** – Centrifuge fitted with a 96 well microplate carrier, 96 Well Magnetic Stand, Liquid handler or other robotic sample processor, 96 well plate heat block, 2 mL 96 well plates and reagent carriers[3] (user supplied).

[1] For water samples, filter using desired filter (not provided). Cut the filter into small pieces and place into BashingBead Module. Alternatively up to 250 µl water can be processed directly.

[2] This equates to approximately 2 x 10^8 bacterial cells, 2 x 10^7 yeast cells and 2 x 10^6 mammalian cells.

[3] 96-Well Blocks (P1001-2) can be purchased separately at www.zymoresearch.com

Product Description

The ***Quick*-DNA™ Fecal/Soil Microbe 96 Magbead Kit** is designed for the simple and 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 90 minutes per 96 samples. Fecal (≤100 mg) and soil (≤200 mg) samples 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 MagBinding Bead Technology, which features our Inhibitor Removal Technology built into the buffer system. Simply bind, wash, and elute with the ***Quick*-DNA™ Fecal/Soil Microbe 96 Kit** to recover DNA that is ideal for downstream molecular-based applications including PCR, arrays, genotyping, etc.

Innovation Pure and Simple™

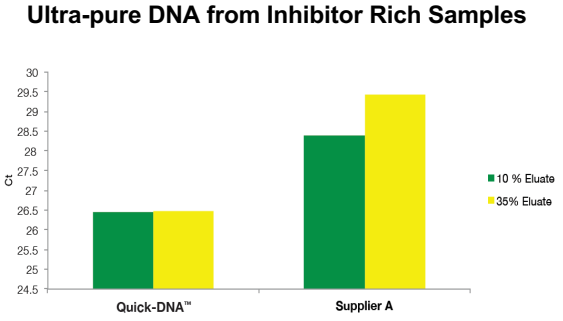


Figure 1. The ***Quick*** **DNA Fecal/Soil Microbe 96 Magbead Kit** provides inhibitor-free DNA even when challenged with extremely inhibitor rich samples. Real-time PCR was used to evaluate eluates recovered using the ***Quick*** **DNA Fecal/Soil Microbe 96 Magbead Kit** or Supplier A. Reaction volumes consisted of either 10% or 35% of the eluate from each kit to detect the presence of PCR inhibitors. Each reaction contained 10 ng of *Brettanomyces* DNA. Delayed and/or no amplification indicates PCR inhibition from inefficient inhibitor removal. N=8.

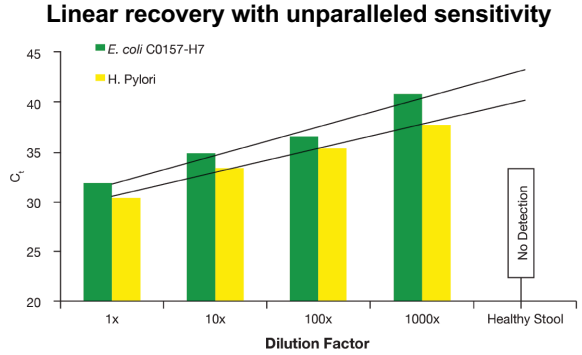


Figure 2. The ***Quick*-DNA™ Fecal/Soil Microbe 96 Magbead kit** produces linear recovery of DNA for sensitive applications, detecting pathogenic organisms such as E. *coli* and H. *pylori* in assays with up to a 1000x dilution factor. A dilution series of stool spiked with $1x10^6$ E. *coli* cells and H. *Pylori* infected stool was extracted using the ***Quick*-DNA™ Fecal/Soil Microbe 96 Magbead Kit,** showing effective purification and qPCR amplification, even at 1000:1 dilution. Healthy stool sample controls did not amplify pathogenic organisms compared to the spiked stools. N=8.

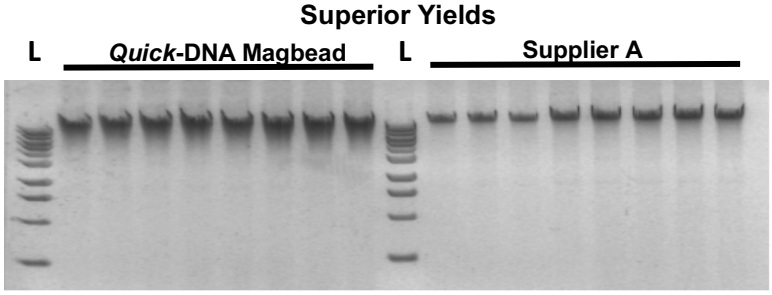
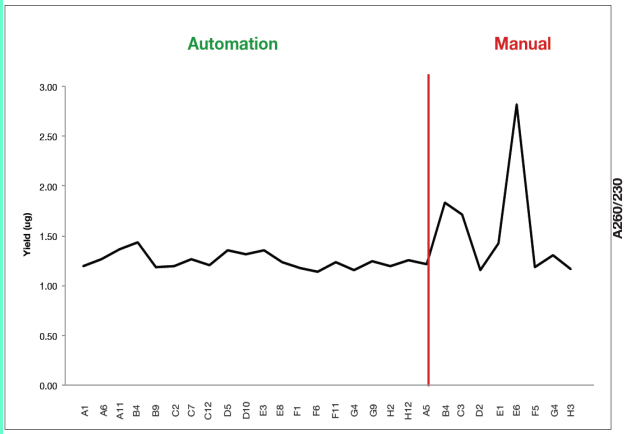


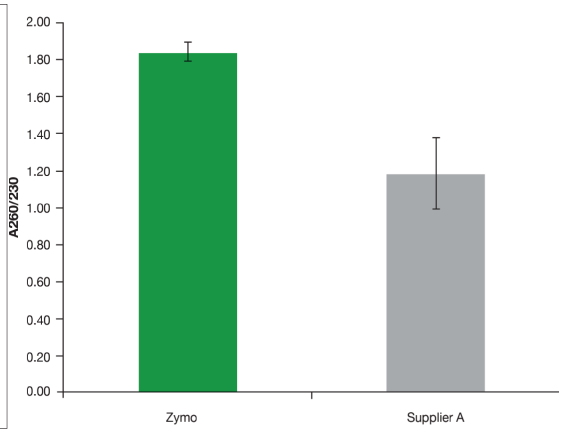
Figure 3. The ***Quick*-DNA™ Fecal/Soil Microbe 96 Magbead Kit** provides superior yields when compared to Supplier A. 80 mg of feces was processed using each kit according to the manufactures' recommended protocol. DNA was eluted using 100 µl **DNA Elution Buffer**. 6 µl of each sample was analyzed in a 1.0% (w/v) agarose/ethidium bromide gel. Samples were processed in triplicate. L is a 1Kb ladder.

Reliability and Consistency

A) Consistent Yields Across Multiple Samples



B) Reliable, Clean Samples



C) No Cross Contamination Between Sample Wells

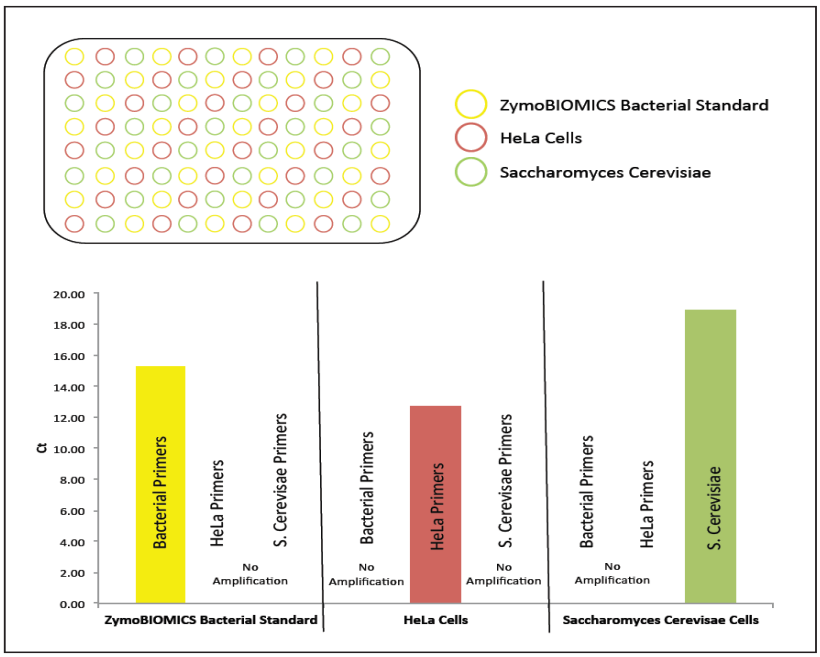


Figure 4A). The ***Quick*-DNA™ Fecal/Soil Microbe 96 Magbead Kit** provides consistent yields when DNA is purified from 20 mg of human fecal sample using an automated liquid handling system. Consistent and replicable yields were observed when DNA isolation was performed on an automated liquid handler. Samples were evaluated using spectrophotometric analysis in technical duplicates.
B). The ***Quick*-DNA™ Fecal/Soil Microbe 96 Magbead** reliably purifies clean DNA from 20 mg of fecal sample, providing DNA with A260/230 values ≥1.80 and more consistent values compared to Supplier A. DNA was quantified using spectrophotometric analysis in technical duplicates. N=8.
C). Coupling the ***Quick*-DNA™ Fecal/Soil Microbe 96 Magbead** with a liquid handler, isolated DNA is free from cross contamination when purified across a standard 96-well plate. Plate was setup with alternating rows of ZymoBIOMICS™ Microbial Community Standards, HeLa cells, and *S. Cerevisiae* cells, and DNA was purified simultaneously from these samples on a liquid handling platform. Samples were evaluated using quantitative PCR with primer sets targeted at the bacterial 16S gene, the human LINE gene, and the fungal ITS gene. PCR was performed in technical duplicates.

Protocol

Sample Lysis

For all mixing steps, either pipette mix or shake at 1200 rpm

For optimal performance, add beta-mercaptoethanol (user supplied) to the Quick-DNA MagBinding Buffer to a final dilution of 0.5% (v/v) *i.e.*, 500 µl per 100 ml.

1. Add sample to the **ZR BashingBead™ Module** using the table below.
    a. If using **ZR BashingBead™ Lysis Rack**, add 650 µl **Lysis Solution.**
    b. If using **ZR BashingBead™ Lysis Tubes**, add 750 µl **Lysis Solution.**

Sample Type	Maximum Input
Feces	100 mg
Soil	200 mg
Liquid Samples[1] and Swab Collections[2]	250 µl
Cells (Suspended in DNA/RNA Shield™ or isotonic buffer, *e.g.* PBS)	5-20 mg (wet weight) (2×10^8 bacterial, 2×10^7 yeast cells, 2×10^6 mammalian cells)
Samples in DNA/RNA Shield™ (10% v/v Sample)	200 µl

2. Secure in a bead beater fitted with the appropriate holder assembly for your bead beating module and process at maximum speed for 5 minutes.

    Note: *Processing time will vary based on sample input and bead beater. Times may be as little as 5 minutes when using high-speed cell disrupters (FastPrep® -24) or as long as 20 minutes when using lower speeds (e.g., Disruptor Genie®).*

3. Centrifuge the ZR BashingBead™ Lysis Module:

    a. If using **ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm),** centrifuge at ≥4,000 x g for 5 minutes.
    b. If using **ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm),** centrifuge at ≥10,000 x g for 1 minute.

For automated scripts and **Technical Assistance** regarding generation of scripts for automated platforms, contact **Zymo Research's Technical Department** at 1-888-882-9682 or E-mail to tech@zymoresearch.com.

For all buffer additions, mix well by pipetting up and down several times and/or (if available) by vortexing at ~1,200 rpm.

[1]For water samples, filter using desired filter (not provided). Cut the filter into small pieces and place into ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm).

[2]Swabs can also be cut or broken and placed directly in bead beating tube.

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[3]This alternative step may result in a slight (≤5%) loss of genomic DNA and complete loss of nucleic acids less than 100 bp in size. For ordering information on ZymoBIOMICS™ DNase/RNase Free Water, see page 16.

[4] The DNA is now suitable for all downstream applications.

[5] For optimal spectrophotometric quantification, eluate may be centrifuged at 4,000 x g for 5 minutes and transfer eluate to a new plate while avoiding the pellet.
For all buffer additions, mix well by pipetting up and down several times and/or (if available) by vortexing at ~1,200 rpm.

Sample Purification

4. Transfer 200 µl supernatant to the deep-well block (not provided). Add 600 µl **Quick-DNA MagBinding Buffer**.

   For samples with excessive amounts of solid particulate, centrifuge at 4,000 x g for 5 minutes to reduce clogging.

5. Dispense 25 µl of **MagBinding Beads** to each well. Mix well by pipette or shaker plate for 10 minutes.
6. Transfer the plate to a magnetic stand until beads pellet, then aspirate and discard the supernatant. Remove the 96-Well Block from the magnetic stand.
7. Dispense 900 µl of **DNA Pre-Wash Buffer** and mix well by pipette or shaker plate for 5 minutes.
8. Transfer the plate to a magnetic stand until beads pellet, then aspirate and discard the supernatant. Remove the 96-Well Block from the magnetic stand.
9. Dispense 900 µl **gDNA Wash Buffer** and mix well by pipette or shaker plate for 5 minutes.
10. Transfer the plate to a magnetic stand until beads pellet, then aspirate and discard the supernatant. Remove the 96-Well Block from the magnetic stand.
11. Repeat the wash (Steps 9-10).
12. Transfer the 96-well block onto a heating element (65°C) until beads dry (approximately 10 minutes). If no heating element is available, air dry for approximately 20-30 minutes.

    Note: *Alternatively, to avoid heating step, keep the pelleted beads on the magnetic stand, add 900 µl of* ***DNA Elution Buffer*** *to the wells,* ***DO NOT MIX****, and incubate for 1 minute. Remove the supernatant and proceed with Step 12.*[3]

13. Dispense 50 µl of **DNA Elution Buffer** to each well and re-suspend beads. Mix the beads well for 10 minutes and then transfer the plate onto the magnetic stand for 2-3 minutes until the beads pellet.
14. Transfer the supernatant (containing the eluted DNA) to a clean **Elution Plate**[4,5].

Automation Setup Guide

For optimal performance, add beta-mercaptoethanol (user supplied) to the Quick-DNA MagBinding Buffer to a final dilution of 0.5% (v/v) *i.e.*, 500 µl per 100 ml.

1. Add 75 ml of **Quick-DNA MagBinding Buffer** to a 96 well reagent trough.
2. Add 100 ml of **DNA-Pre Wash Buffer** to a 96 well reagent trough.
3. Add 200 ml (one bottle) of **gDNA Wash Buffer** to a 96 well reagent trough.
4. Add 25 ml of **DNA Elution Buffer** to a 96 well reagent trough.
5. Vortex the **Magbinding Beads** vigorously for 30 seconds, then add 6 ml to a 96 well low dead volume reagent trough.

Automation Protocol

1. Place the following components on the deck prior to initialization of the protocol:
    a. Place five 96-well racks of 300 µl standard volume tips on the deck.
    b. Place five 96-well racks of 1000 µl high volume tips on the deck.
    c. Place three 96-well racks of 50 µl low volume tips on the deck.
    d. Place a magnetic stand on the deck.
    e. Place a 96-well 96-well block on the deck.
    f. Place the pre-homogenized bead beating module on the deck in the appropriate carrier and remove the lids.
    g. Place two empty 96-well reagent troughs for waste disposal.
2. If a Heater/Shaker module is installed, begin preheating to 65° C before proceeding to the next step.
3. Aspirate 200 µl lysate from the bead beating module 20 mm from the container bottom.
4. Dispense 200 µl lysate into the empty 96-well block and discard the pipette tips.
5. Aspirate 600 µl **Quick-DNA MagBinding Buffer** from the appropriate reagent trough.
6. Dispense 600 µl **Quick-DNA MagBinding Buffer** into the 96-well block at a height of 2mm from the container bottom. After dispensing, pipette mix (400 µl for 15 cycles) 2 mm from the container bottom.
7. Premix the **MagBinding Beads** (50 µl for 10 cycles). Aspirate 25 µl **MagBinding Beads.**
8. Dispense 25 µl **MagBinding Beads** to the 96-well block 2 mm from the container bottom.
9. Using 1000 µl high volume tips, mix the lysate (600 µl for 25 cycles).
10. Transfer the 96-well block to a shaking device and shake at 1200 rpm for 10 minutes[1].
11. Transfer the 96-well block to a 96-well magnetic stand; allow it to stand for 2 minutes.
12. Using a slow aspirate mode (≤ 50 µl/s flow rate) remove 830 µl supernatant and discard.
13. Transfer the 96-well block from the magnetic stand to a normal plate carrier.
14. Aspirate 900 µl **DNA Pre-Wash Buffer**.
15. Dispense 900 µl **DNA Pre-Wash Buffer** into the 96-well block 2 mm from the container bottom. After dispensing, pipette mix (400 µl for 25 cycles).
16. Transfer the 96-well block to a shaking device and shake at 1200 rpm for 2 minutes.
17. Transfer the 96-well block to a 96-well magnetic stand; allow it to stand for 2 minutes.
18. Using a slow aspirate mode (≤ 50 µl/s flow rate) remove 900 µl supernatant and discard.
19. Transfer the 96-well block from the magnetic stand to a normal plate carrier.

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[1]If a shaker module is not installed, pipette mix for the duration of the shaking step

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[3]This alternative step may result in a slight (≤5%) loss of genomic DNA and complete loss of nucleic acids less than 100 bp in size.

[4] For optimal spectrophotometric quantification, eluate may be centrifuged at 4,000 x g for 5 minutes and transfer eluate to a new plate while avoiding the pellet.

20. Aspirate 900 µl **gDNA Wash Buffer**.
21. Dispense 900 µl **gDNA Wash Buffer** into the 96-well block at a height of 2 mm from the container bottom. After dispensing, pipette mix (400 µl for 25 cycles).
22. Transfer the 96-well block to a shaking device and shake at 1200 rpm for 2 minutes.
23. Transfer the 96-well block to a 96-well magnetic stand; allow it to stand for 2 minutes.
24. Using a slow aspirate mode (≤ 50 µl/s flow rate) remove 900 µl supernatant and discard.
25. Transfer the 96-well block from the magnetic stand to a normal plate carrier.
26. Aspirate 900 µl **gDNA Wash Buffer**.
27. Dispense 900 µl **gDNA Wash Buffer** into the 96-well block at a height of 2 mm from the container bottom. After dispensing, pipette mix (400 µl for 25 cycles).
28. Transfer the 96-well block to a shaking device and shake at 1200 rpm for 2 minutes.
29. Transfer the 96-well block to a 96-well magnetic stand; allow it to stand for 2 minutes.
30. Using a slow aspirate mode (≤ 50 µl/s flow rate) remove 900 µl supernatant and discard.
31. Proceed with one of the following methods depending on accessibility to a heating device:

    Note: *Alternatively, to avoid heating step, keep the pelleted beads on the magnetic stand, add 900 µl of* ***DNA Elution Buffer*** *to the wells,* ***DO NOT MIX,*** *and incubate for 1 minute.[3] Remove the supernatant and proceed with Step 32.*

32. Aspirate 50 µl **DNA Elution Buffer.**
33. Dispense 50 µl **DNA Elution Buffer** into the 96-well block at a height of 2mm from the container bottom. After dispensing, pipette mix (40 µl for 25 cycles).
34. Transfer the 96-well block to a shaking device and shake at 800 rpm for 3 minutes.
35. Transfer the 96-well block to a magnetic stand; allow it to stand for 2 minutes.
36. Aspirate 40 µl **DNA Elution Buffer** from the 96-well block.
37. Dispense 40 µl **DNA Elution Buffer** containing the eluted DNA to the elution plate. The DNA is now ready for downstream applications.[4]

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 Microbe 96 Kit**	D6011	2x96 preps.
***Quick*-DNA™ Fecal/Soil Microbe 96 Magbead Kit** (includes ZR BashingBead™ Lysis Rack)	D6010-FM	2x96 preps.
***Quick*-DNA™ Fecal/Soil Microbe 96 Magbead Kit** (includes ZR BashingBead™ Lysis Tubes)	D6012-FM	2x96 preps.
***Quick*-DNA™ Fecal/Soil Microbe 96 Magbead Kit** (Lysis Matrix Not Included)	D6011-FM	2x96 preps.

For Individual Sale	Catalog No.	Amount
***Quick*-DNA MagBinding Buffer**	D4077-1-150	150 ml
DNA Pre-Wash Buffer	D3004-5-250	250 ml
gDNA Wash Buffer	D3004-2-200	100 ml
DNA Elution Buffer	D3004-4-50	50 ml
MagBinding Beads	D4100-4-12	12 ml
Lysis Solution	D6001-3-150	150 ml
ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm)	S6002-96-3	2 Racks
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	S6012-50	50 Tubes