ZYMO RESEARCH

DNA Purification Made Simple™

Quick-DNA™ Fecal/Soil Microbe Midiprep Kit

DNA from fecal, soil, and microbial samples.

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.

Catalog Numbers:
D6110

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

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Product Contents

Quick-DNA™ Fecal/Soil Microbe Midiprep Kit	D6110 (25 Preps.)	Storage Temperature
ZR BashingBead™ Lysis/Filtration Tubes	25	Room Temp.
BashingBead™ Buffer	150 ml	Room Temp.
Genomic Lysis Buffer[1]	250 ml (2x)	Room Temp.
DNA Pre-Wash Buffer[2]	15 ml	Room Temp.
g-DNA Wash Buffer	50 ml	Room Temp.
DNA Elution Buffer[3]	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[4]	50	Room Temp.
Collection Tubes	100	Room Temp.
Instruction Manual	1	-

[1] For optimal performance, add beta-mercaptoethanol to 0.5% (v/v) *i.e.,* 1.25 ml per 250 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.
[3] The **DNA Elution Buffer** contains 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.
[4] Matrix in the HRC filter may appear dehydrated or powdery. This is normal.
[5] HRC filter chemistry will skew the A260/A230 purity ratio value. This will not affect any downstream processes. For accurate purity ratios, quantify sample prior to passing through the HRC filter.

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).[1]

- **DNA Size Limits –** On average, post bead beating, genomic DNA is between 15-20 kb depending on initial quality of the sample. In most instances, mitochondrial DNA and viral DNA (if present) will also be recovered.

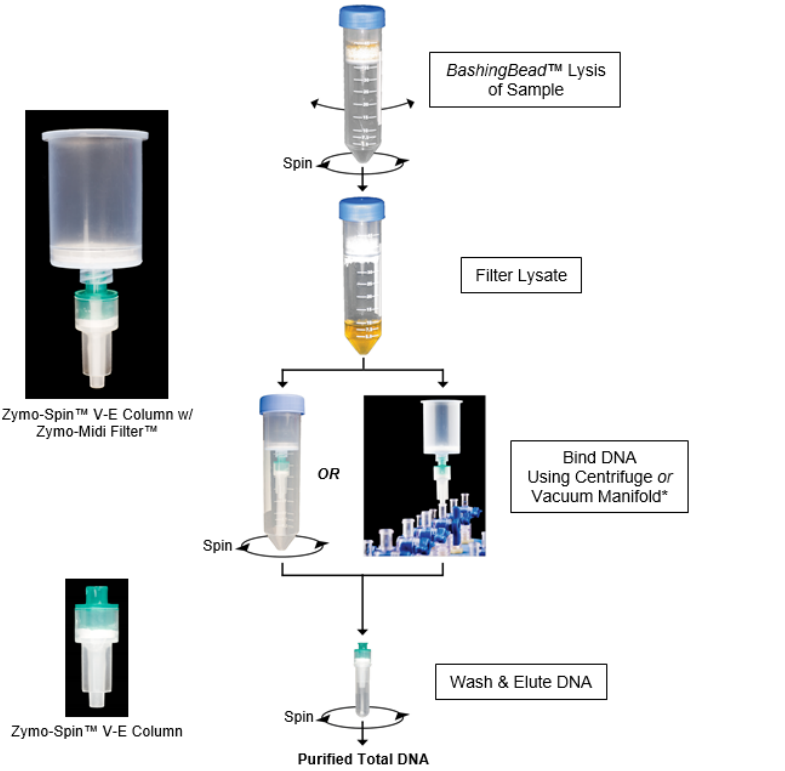
- **DNA Recovery –** Typically, up to ~125 µg total DNA is eluted into ≥150 µl **DNA Elution Buffer** per sample.

- **Equipment –** Centrifuge, vacuum source and manifold, microcentrifuge, cell disrupter or pulverizer w/ 50 ml tube adapter

[1] For microbiome or metagenomic applications, we recommend using the **ZymoBIOMICS® DNA** product line.

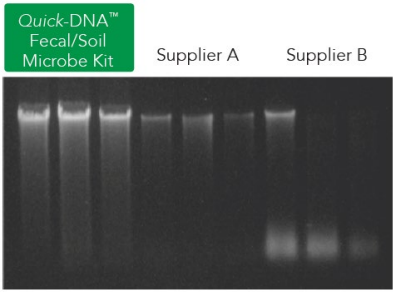
Product Description

The ***Quick*-DNA™ Fecal/Soil Microbe Midiprep Kit**[1] 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**[2], 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.

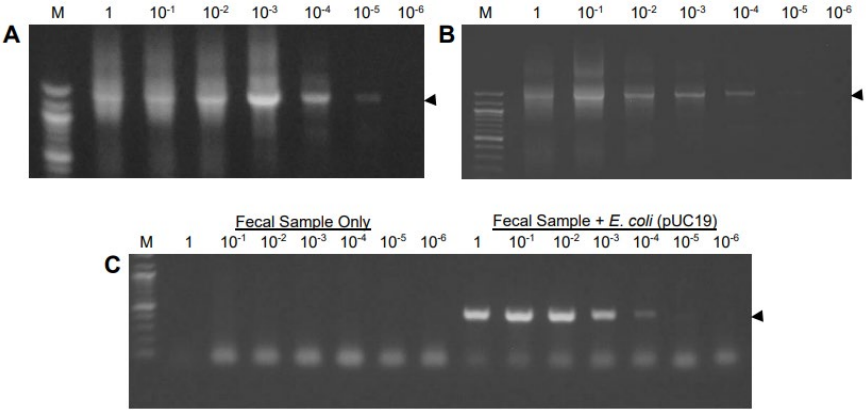


[1] For microbiome or metagenomic applications, we recommend using the **ZymoBIOMICS® DNA** product line.
[2] **DNA/RNA Shield™ (R1100-50, R1100-250)** can be used to stabilize nucleic acids and inactivate infectious agents in a variety of samples, without the need for reagent removal prior to extraction.

Fecal DNA Isolation

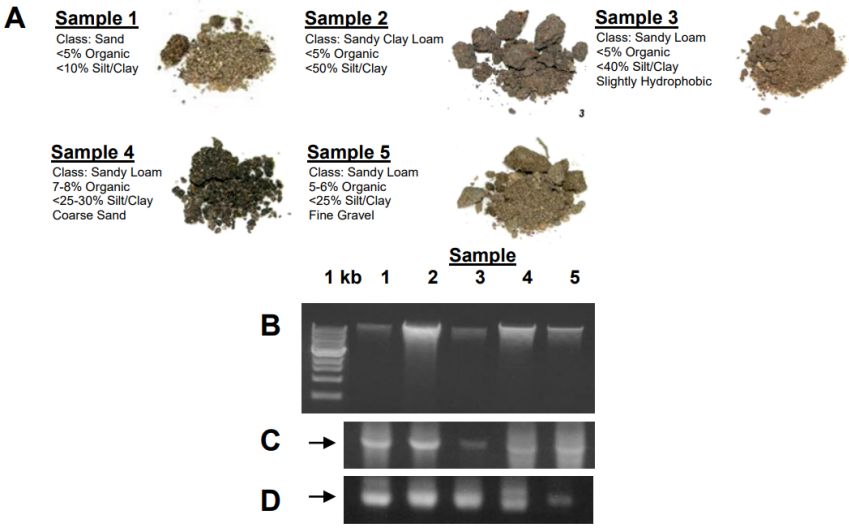


Comparison of DNA yields from rat feces using the ***Quick*-DNA™ Fecal/Soil Microbe Kit** and kits from suppliers A and B. Equivalent amounts of feces were processed using each kit and then equal volumes of eluted DNA were analyzed in a 0.8% (w/v) agarose/ethidium bromide gel. Samples were processed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

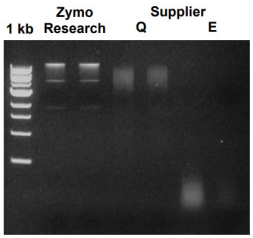


PCR of DNAs from rat and human fecal samples isolated with the ***Quick*-DNA™ Fecal/Soil Microbe Kit**. Panels A and B show the results of PCR with DNA isolated from rat and human fecal samples, respectively, using primers specific for prokaryotic 16S rRNA. Panel C shows the results of PCR of DNA isolated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

This protocol consists of: (I) Buffer Preparation and (II) DNA Purification

(I) Buffer Preparation

- ✓ For optimal performance, add ~100% beta-mercaptoethanol (user supplied) to the **Genomic Lysis Buffer** to a final dilution of 0.5% (v/v) *i.e.*, 1.25 ml per 250 ml.

(II) DNA Purification

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.

   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.

   *For samples stored in **DNA/RNA Shield™**, see **Samples in DNA/RNA Shield™** (**pg. 8**).*

2. Secure in a bead beater fitted with a 50 ml tube holder assembly to process samples. Optimization of processing time/speed will be necessary for complete sample lysis.

   ***Recommended:** Bead beat using the Vortex Genie® 2 (S5001) with the Horizontal 50 ml Tube Holder (S5001-5). See manufacturer's literature for specific operating information. See page 12 for additional recommendations.*

   ***Optional Stopping Point:** Samples can be stored after Step 2 at -80°C.*

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).

5. Add 18 ml of **Genomic Lysis Buffer** to the supernatant. Mix well.

[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.

6. Filter the entire mixture from Step 5 using a **Zymo-Spin™ V-E Column/Zymo-Midi Filter™** assembly mounted on a vacuum manifold[3] (see diagram on page 3) with a vacuum source set at ≥ 600 mm Hg.

7. 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.

8. 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.

9. 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.*

10. Place a **Zymo-Spin™ III-HRC Filter**[7,8] in a clean Collection Tube and add 600 µl **Prep Solution.** Centrifuge at 8,000 x *g* for 3 minutes.

11. Transfer the eluted DNA to a prepared the 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.

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[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.

[7] Matrix in the HRC filter may appear dehydrated or powdery. This is normal.

[8] HRC filter chemistry will skew the A260/A230 purity ratio value. This will not affect any downstream processes. For accurate purity ratios, quantify sample prior to passing through the HRC filter.

Appendix

Samples in DNA/RNA Shield™

DNA/RNA Shield™ *ensures nucleic acid stability during sample storage and transport at ambient temperatures. There is no need for refrigeration or specialized equipment. DNA/RNA Shield™ effectively lyses cells and inactivates nucleases and infectious agents (virus), and it is compatible with various collection and storage devices (vacutainers, swabs, nasal, buccal, fecal, etc.).*

DNA/RNA Shield™ *purchased separately (R1100 or R1200).*

1. For samples collected in DNA/RNA Shield™, transfer up to 6 ml of sample into the **ZR BashingBead™ Lysis/Filtration Tube**.

   Note: If using < 6 ml sample, increase the volume to 6 ml using **BashingBead™ Buffer** or **DNA/RNA Shield™** before continuing.

2. Continue from Step 2 of the main protocol (**pg. 6**).

Troubleshooting

For **Technical Assistance**, please contact 1-888-882-9682 or E-mail tech@zymoresearch.com

Problem	Possible Causes and Suggested Solutions
Background Contamination	**Contaminated workspace or equipment**. Clean workspace, centrifuge, and pipettes with 10% bleach routinely to avoid contamination. Make sure bags of columns and buffer bottles are properly sealed for storage. Use of these outside a clean room or hood can result in contamination.
DNA Degradation	**DNase contamination:** Check pipettes, pipette tips, microcentrifuge tubes, etc. for DNase contamination and exercise the appropriate precautions during the DNA purification procedure. If water is used to elute the DNA, ensure that DNase-free water is used.
Low DNA Yield	**Incomplete sample lysis:** Bead beating devices that oscillate in a single dimension (only vertically or only horizontally) have been observed to inefficiently lyse very recalcitrant species. Devices that oscillate three-dimensionally or in a figure-8 motion often lyse efficiently. Bead beating parameters will require optimization for complete sample lysis. Processing times will vary based on bead beater and sample type. See manufacturer's literature for specific operating information. **Incomplete debris removal:** For high density samples, ensure lysate is centrifuged properly to pellet insoluble debris following bead beating. Ensure that none of the debris is transferred to the **Zymo-Spin™ V-E Column/Zymo-Midi Filter™**. **Too much input material used.** If the lysate does not pass through the column or is extremely viscous, use less input material. Too much sample input can cause cellular debris to overload the column and insufficient flow. Consult the Sample Sources under Specifications for information on your input limit based on sample.

Problem	Possible Causes and Suggested Solutions
Low DNA Yield (cont.)	**Incomplete elution:** Ensure the DNA Elution Buffer hydrates the matrix for 5 minutes at room temperature before centrifugation. To increase yields, heat the DNA Elution Buffer to 60ºC before use. You can also load the eluate a second time, incubate at room temperature for 3 minutes, and centrifuge again.
Low DNA Purity	**Improper handling:** The column tip can be contaminated with wash buffer flow through. Ensure the tip does not touch the flow through. Empty the collection tube or use a new collection tube when instructed. **Insufficient centrifugation:** Ensure the indicated centrifugation times and speeds are used. Increase the centrifugation time of the final wash step by one minute to ensure complete wash buffer removal. When applicable, centrifuge at the maximum given speed. **III-HRC Filter:** HRC filter chemistry will skew the A260/A230 purity ratio value. This will not affect any downstream processes. For accurate purity ratios, quantify sample prior to passing through the HRC filter.

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/Filtration Tubes (50 ml) w/ 0.5 mm Beads	S6010	25 Pack
BashingBead™ Buffer	D6001-3-40 D6001-3-150	40 ml 150 ml
Genomic Lysis Buffer	D3004-1-50 D3004-1-100 D3004-1-150 D3004-1-200 D3004-1-250	50 ml 100 ml 150 ml 200 ml 250 ml
DNA Pre-Wash Buffer	D3004-5-15 D3004-5-30 D3004-5-50 D3004-5-250	15 ml 30 ml 50 ml 250 ml
g-DNA Wash Buffer	D3004-2-50 D3004-2-100 D3004-2-200 D3004-2-250 D3004-2-400	50 ml 100 ml 200 ml 250 ml 400 ml
DNA Elution Buffer	D3004-4-1 D3004-4-4 D3004-4-10 D3004-4-16 D3004-4-50	1 ml 4 ml 10 ml 16 ml 50 ml
Zymo-Spin™ V-E Columns w/ Zymo-Midi Filters™	C1021-25	25 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
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
Horizontal 50 ml Tuber Holder for Vortex-Genie® 2	S5001-5	1 Unit
2010 Geno/Grinder®	S6006	1 Unit
50 ml Tube Holder/Cryo Block Assembly for Geno/Grinder®	S6006-3	2 Blocks
FastPrep™ 24, 120V	S6005	1 Unit
BigPrep™ Adapter (2 x 50 ml Tubes) for FastPrep-24	S6005-4	1 Unit

Lysis conditions must be optimized for complete sample disruption. Processing time and speed can vary depending on the instrument used. Refer to the instrument manufacturer's guidelines for optimal settings.

Complete Your Workflow

✓ **Storage and Preservation of Nucleic Acids at Ambient Temperature**

DNA/RNA Shield™ and Collection Devices	Size	Catalog No.
DNA/RNA Shield™ Reagent	50 ml 250 ml	R1100-50 R1100-250
DNA/RNA Shield™ Reagent (2x Concentrate)	25 ml 125 ml	R1200-25 R1200-125
DNA/RNA Shield™ Fecal Collection Tube	10 pack	R1101
DNA/RNA Shield™ Lysis Tubes (Microbe)	50 pack	R1103

✓ **Accurate Quantification and Robust Amplification of Microbial DNA**

ZymoTaq DNA Polymerase	Size	Catalog No.
ZymoTaq™ PreMix	50 Rxns. 200 Rxns.	E2003 E2004
ZymoTaq™ DNA Polymerase	50 Rxns. 200 Rxns.	E2001 E2002
ZymoTaq™ qPCR PreMix	50 Rxns. 200 Rxns.	E2054 E2055
Femto Bacterial DNA Quantification Kit	100 Rxns.	E2006
Femto Fungal DNA Quantification Kit	100 Rxns.	E2007

✓ **Innovative Next-Gen Sequencing Solutions for Microbial DNA Detection**

NGS Library Prep Kits	Size	Catalog No.
***Quick*-16S Plus NGS Library Prep Kits** Available Target Regions: V4, V1-V2, V1-V3, and V3-V4, UDI	96 Rxns.	D6430 (V4) D6434 (V1-V2) D6440 (V1-V3) D6421 (V3-V4, UDI)
***Quick*-ITS Plus NGS Library Prep Kits**	96 Rxns.	D6425
***Quick*-16S Full-Length Library Prep Kits**	96 Rxns.	D6450

Notes

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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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