October 2024

EZ2® PowerFecal® Pro DNA/RNA Kit Instructions for Use

48

For automated purification of total Nucleic Acid, DNA, or RNA from stool material using EZ2 Connect instruments

Sample to Insight

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Kit Contents

EZ2 PowerFecal Pro DNA/RNA Kit **Catalog no.** **No. of reactions**	**(48)** **954634** **48**
PowerBead Pro Tubes	2 mL (50)
Solution CD1†	40 mL
Solution CD2	15 mL
EZ2 PowerFecal Pro Cartridges†	48
Disposable Tip Holders	50
Disposable Filter-Tips	50
Elution Tubes 1.5 ml	50
Sample Tubes 2 ml	50
Q-Card‡	1
Quick-Start Protocol	1

† Contains chaotropic salt. Not compatible with disinfecting agents containing bleach; see page 6 for Safety Information.

‡ The information encoded in the bar code on the Q-Card is needed for reagent data tracking using the EZ2 Connect instruments.

Intended Use

The EZ2® PowerFecal Pro DNA/RNA Kit® is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Shipping and Storage

The EZ2 PowerFecal Pro DNA/RNA Kit is shipped at ambient temperature. Upon receipt, store Solution CD2 at 2–8°C. Store all other kit components dry at room temperature (15–25°C).

When stored properly, buffers and reagent cartridges are stable until the expiration date on the Q-Card and the kit label.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION

DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Solution CD1 and buffers in the EZ2 PowerFecal Pro DNA/RNA Kit cartridge contain chaotropic salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

If liquid containing potentially infectious agents is spilt on the EZ2 Connect instrument, please refer to the instrument user manual for decontamination instructions.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of EZ2 PowerFecal Pro DNA/RNA Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The EZ2 PowerFecal Pro DNA/RNA Kit comprises a new proprietary one-step lysis and inhibitor removal technology for a more streamlined automatic handling of stool and gut samples. The procedure allows a fully automated processing of samples after lysis to reduce manual steps and hands-on time significantly for more convenience.

Principle and procedure

The EZ2 PowerFecal Pro DNA/RNA Kit efficiently lyses microbial cells and removes inhibitory substances commonly found in stool, such as polysaccharides, heme compounds and bile salt using a novel combined one-step lysis and inhibitor removal procedure. The lysis buffer, Solution CD1, IRT buffer, Solution CD2, and an organic phase separation agent such as phenol chloroform isoamyl alcohol ensure both effective mechanical and chemical lysis using bead beating tubes and efficient inhibitor depletion during centrifugation after lysis and disruption. The supernatant is placed on the EZ2 Connect instrument and either total NA or DNA only or RNA only can be extracted in a fully automated workflow. The workflow is optimized for microbial nucleic acid extraction, but is not selective; all nucleic acid, including the generally smaller proportion deriving from the host, will be purified. Nucleic acids are eluted in 100 µL RNase-free water. For the extraction of DNA only or RNA only, the corresponding enzyme: RNaseA or RNase-free DNase I Set need to be purchased separately.

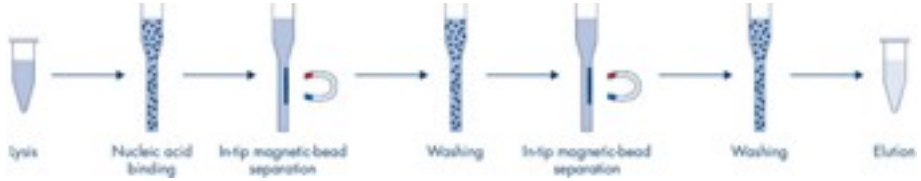
Please note that the use of phenol chloroform isoamyl alcohol is crucial for the depletion of the downstream inhibitors. At the same time, it also deactivates RNases which can be released during disruption, allowing the extraction of high-quality RNA. For detailed information about alternatives to phenol chloroform isoamyl alcohol, see "Appendix: Phenol-free Lysis and IRT" on page 23.

The recommended amount of starting material is 50 mg of stool. For typical samples, this amount provides yields between 10–25 µg DNA and 20–90 µg RNA. The maximum amount that can be processed in the EZ2 PowerFecal Pro DNA/RNA Kit is 100 mg. Attempting to process much larger amounts could lead to negative effects such as tip clogging or inefficient washing, which will decrease the final yield and quality of nucleic acid. These recommendations are based on the total amount of stool material, not total volume; for example, 50 mg of stool in 200 µL of transport buffer can still be processed.

Automation

The EZ2 Connect instruments can perform all steps following lysis and disruption of the sample. This automation is based on magnetic particle technology and includes nucleic acid binding, optional enzymatic digestion, washing, and elution. Up to 24 samples can be processed in a single run.

Magnetic-particle technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles. Total nucleic acid, DNA only, or RNA only is isolated from lysates in one step through its binding to the silica surface on the particles in the presence of a chaotropic salt. The particles are subsequently separated from the lysates using a magnet. An additional washing step removes any residual contaminants. Finally, nucleic acids are efficiently eluted.



Equipment and Reagents Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

The following materials are not included in the kit, and are to be supplied by user:

- For RNA-only protocol: RNase-free DNase Set (50) (cat.no. 79254),
- For DNA-only protocol: RNase A (17,500 U) (cat.no. 19101)
- For all protocols: Phenol–chloroform–isoamyl alcohol (25:24:1) pH 6.5-8
- Microcentrifuge (with rotor for 2 mL tubes)
- Equipment for sample disruption and homogenization, one of the following:
    - Vortex Genie 2 and Vortex Adapter for 24 (1.5–2 mL) tubes (cat. no. 13000-V1-24)
    - TissueLyser III (cat. no. 9003240) with adapter sets for use with the PowerBead Pro Tubes (TissueLyser Adapter Set 2 x 24 [cat. no. 69982] or 2 mL Tube Holder [cat. no. 11993], in conjunction with Plate Adapter Set, cat. no. 11990)

Important Notes

Handling and storage of starting material

The yield and integrity of nucleic acids isolated from microbes in stool is greatly influenced by the state of the digestive system, diet of the individual, and the length of time between collection of the sample and preservation. The main components of stool are water (between 65–85%), bacterial cells, undigested food and fiber, bile, and bilirubin (which is derived from dead red blood cells). To a lesser degree, cellular components that have been shed from the walls of the gastrointestinal tract can also be found in stool. Host DNA can also be found in these samples, but is normally a minor component compared to microbial DNA. Nucleic acids isolated from stool typically appear to have some level of degradation using standard analysis methods because of the relatively high content of dead and decaying cells.

To optimize the quality of nucleic acids from stool, process the sample as quickly as possible after collection. The PowerProtect DNA/RNA reagent enables stabilization of stool samples at room temperature. See the relevant handbook for processing recommendations. Freezing the samples at –65 to –90°C will also preserve the quality of nucleic acids. If freezing at ultralow temperatures is not possible, freezing at –20°C is an alternative. Freezing in small aliquots avoids subjecting the bulk sample to freeze–thaw cycles, which can increase the lysis of cells and degradation of nucleic acids. Frozen samples should be processed rapidly by adding lysis buffer CD1, inhibitor removal buffer CD2, and phenol–chloroform–isoamyl alcohol to the bead tube before the sample has fully thawed. Homogenize immediately to saturate the cellular nucleic acids in the protective lysis buffer. For fresh (non-frozen) samples, rapid homogenization in lysis buffer is especially critical to isolate the highest quality nucleic acids.

Disrupting and homogenizing starting material

Efficient disruption and homogenization of the staring material is an absolute requirement for all nucleic acid purification procedures. However, if the microorganism of interest requires stronger homogenization than provided by a vortex with the Vortex Adapter for 24 (1.5–2 mL) tubes (cat. no. 13000-V1-24), or if using a bead beater is desired, the EZ2 PowerFecal Pro DNA/RNA Kit contains bead tubes suitable for high-powered bead beating and may be used in conjunction with the TissueLyser III (cat. no. 9003240) using a 2 mL Tube Holder Set (cat. no. 11993).

For a convenient medium and high throughput 96-well homogenization, we offer the TissueLyser III (cat. no. 9003240) and Plate Adapter Set (cat. no. 11990). In conjunction with PowerBead Pro Plates (cat. no. 19301), the instrument provides high-throughput processing for simultaneous, rapid, and effective disruption of up to 2 x 96 samples in only a few minutes.

Preparation of buffers

Preparing DNase I stock solution for RNA-only protocol

Prepare DNase I stock solution by dissolving the lyophilized DNase I (1500 Kunitz units) in 550 µL RNase-free water. In some cases, the vial of DNase may appear to be empty. This is due to lyophilized enzyme sticking to the septum. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex.

Insoluble material may remain when dissolving DNase. This does not affect DNase performance. Due to the production process, insoluble material may be present in the lyophilized DNase. However, rigorous QC tests are carried out to ensure that DNase activity remains consistent from lot to lot.

Note: Do not vortex reconstituted DNase I as it is sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.

For long-term storage of DNase I, remove the stock solution from the vial, divide it into single-use aliquots, and store at –30 to –15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to four weeks. Do not refreeze the aliquots after thawing.

Working with the EZ2 Connect instrument

The main features of EZ2 Connect instruments include the following:

- Purification of high-quality nucleic acids from up to 24 samples per run
- Small footprint to save laboratory space
- Preprogrammed protocols for nucleic acid purification
- Prefilled, sealed reagent cartridges for easy, safe, and fast run setup
- Complete automation of nucleic acid purification, from opening of reagent cartridges to elution of nucleic acids, with no manual centrifugation steps
- Optional bar code reading and sample tracking
- Kit data tracking with the Q-Card provided in the kit
- UV LED to help eliminate sample carryover from run-to-run and to allow pathogen decontamination on the worktable surfaces

Note: UV decontamination helps to reduce possible pathogen contamination of the EZ2 Connect. The efficiency of inactivation has to be determined for each specific organism and depends, for example, on layer thickness and sample type. QIAGEN cannot guarantee complete eradication of specific pathogens.

EZ2 Connect reagent cartridges

Reagents for the purification of nucleic acids from a single sample are contained in a single reagent cartridge (Figure 1). Each well of the cartridge contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer. Positions 11 and 12 can be equipped individually. Details on preparation of these positions are displayed during the run setup on the LED display of the EZ2 Connect.

Figure 1. Ease of worktable setup using reagent cartridges. (A) A sealed, prefilled reagent cartridge. Fill levels vary, depending on the type of reagent cartridge. (B) Loading reagent cartridges into the cartridge rack. The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges must be loaded.

EZ2 Connect tip racks

The EZ2 Connect tip racks holds tips inserted into tip holders and tubes for samples or elution. Details on how to equip the tip racks are displayed during the run setup on the LED display of the EZ2 Connect.

Figure 2. The EZ2 Connect Tip Rack (A) has 4 positions label A–D by engravings. It is designed to hold sample and elution tubes as well as tips in their respective tip holders (B).

Worktable

The worktable of EZ2 Connect instruments is where the user equipped cartridge and tip racks (Figure 3).

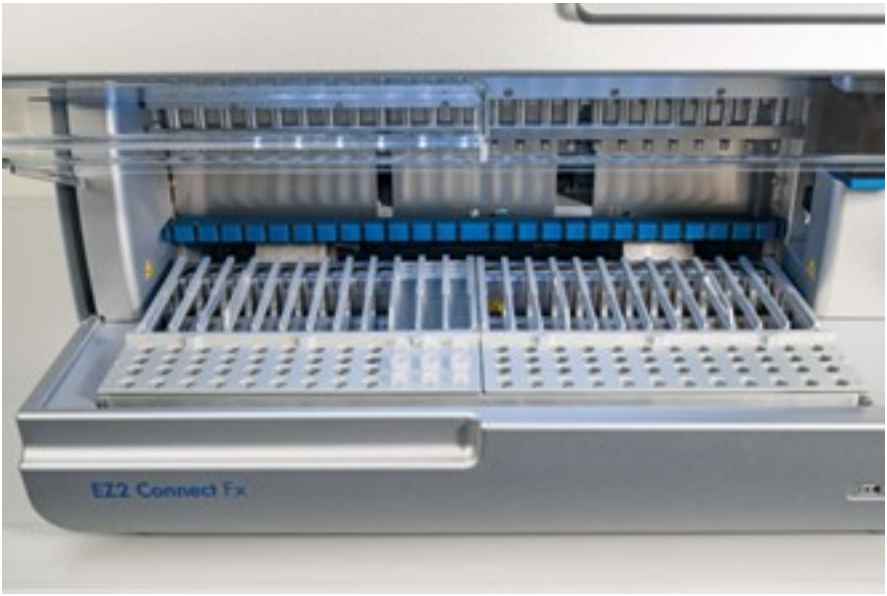


Figure 3. EZ2 Connect worktable.

1. EZ2 Connect Cartridge Rack – left
2. EZ2 Connect Cartridge Rack – right
3. EZ2 Connect Tip Rack – left
4. EZ2 Connect Tip Rack – right

Operation of the EZ2 Connect

The EZ2 Connect provides various features to support the sample preparation workflow. These include functions for remote access via QIAsphere®, data input via bar code reading, data storage and transfer, report generation, and guided instrument maintenance. For more information about these features, please refer to the *EZ2 Connect* and *EZ2 Connect Fx User Manual*.

Protocol: EZ2 PowerFecal Pro DNA/RNA Kit

Important notes before starting

- RNA-only protocol: Prepare DNase I stock enzyme by adding 550 µL RNase-free Water to the DNase I (RNase-free) lyophilized powder and mixing gently. Aliquot the DNase I stock enzyme in 100 µL portions and store at –30 to –15°C for long-term storage. Avoid freeze/thaw more than three times. To prepare DNase I Solution, thaw and combine 100 µL DNase I stock enzyme with 900 µL RDD Buffer per prep. DNase I is sensitive to physical denaturation; do not vortex resuspended DNase I.
- Perform the centrifugation step at room temperature (15–25°C).
- If preparing RNA for the first time, read "Important Notes" on page 10.

Procedure

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.
2. Add up to 100 mg of stool, 700 µL of Solution CD1, 100 µL Solution CD2, and 100 µL phenol–chloroform–isoamyl alcohol (25:24:1, pH 6.5–8.0) to the PowerBead Pro Tube (in that order), and vortex briefly to mix.

   Note: The absolute maximum of stool material that can be processed is 100 mg. We generally recommend to start with no more than 50 mg of stool which will give high amounts of nucleic acids for most kind of stool samples.

   Note: As an alternative to phenol chloroform isoamyl alcohol, a mix of benzyl alcohol and chloroform can be used. For more information about alternatives to phenol chloroform isoamyl alcohol, see "Appendix: Phenol-free Lysis and IRT" on page 23.
3. Mechanically disrupt the samples using one of the following methods:

- Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 24 (1.5–2 mL) tubes (cat. no. 13000-V1-24). Orient the tube caps to point toward the center of the vortex adapter. Vortex at maximum speed for 10 min.
- Use a TissueLyser III. Place the PowerBead Pro Tube into the TissueLyser Adapter Set 2 x 24 (cat. no. 69982) or 2 mL Tube Holder (cat. No. 11993) and Plate Adapter Set (cat. no.1190). Fasten the adapter into the instrument and shake for 5 min at 25 Hz speed. Re-orient the adapter so that the side that was closest to the machine body is now furthest from it. Shake again for 5 min at 25 Hz speed.

4. Centrifuge the PowerBead Pro Tube at 18,000 x g for 5 min.
5. Load reagent cartridges into the cartridge rack (invert cartridge 4 times to mix beads).

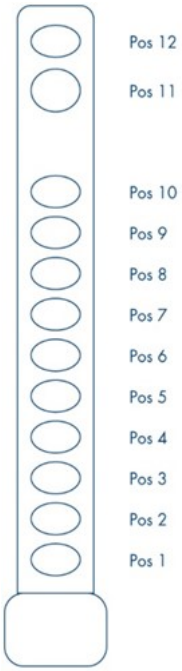


Figure 4. EZ2 PowerFecal Pro cartridge.

6. RNA-only protocol: Transfer 40 µL Buffer RDD and 10 µL resuspended DNase I into position 12 of the EZ2 PowerFecal Pro cartridge.
7. Remove caps of all tubes and prepare the Tip Rack as follows (see Figure 2):
    - Position 4/A: 2.0 mL sample tube (provided)
    - Position 3/B: empty
    - Position 2/C: Tip holder with Filter Tip (provided)
    - Position 1/D: 1.5 mL elution tube (provided)

Figure 5. Tip Rack.

8. Transfer 600 µL sample supernatant after centrifugation in step 4 into a 2 mL sample tube in position 4/A.

   Note: For DNA-only protocol, pipette 4µL RNase A into the sample supernatant (mixing is not required).

Procedure on the EZ2 Connect

9. Turn on the EZ2 Connect instrument.

   Important: Ensure the heating block of the EZ2 Connect instrument is at room temperature.

10. For all protocols tap **Microbe** on the Applications panel, then select **PowerFecal Pro**. Press **Next**.

11. Select one of the three protocols: **total NA**, **DNA-only**, or **RNA-only** protocol, then press **Next**.

12. Select positions on the work deck according to the number of samples to be processed, then press **Next**.

13. Enter sample IDs or press **Generate missing sample IDs**. Press **Next**.

14. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.

15. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument. Press **Next**.

16. Start the run according to the instructions on the instrument display.
17. When the run is completed, "Protocol finished" is displayed. Press **Finish**.
18. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position D of the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste.

    Optional: Follow onscreen instructions for UV decontamination of worktable surfaces.
19. Perform regular maintenance after each run. Press **Finish** to return to the home screen.

    Optional: Follow onscreen instructions for UV decontamination of worktable surfaces.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx** (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
General handling	
a. Insufficient reagent aspirated	After inverting the reagent cartridges to resuspend the magnetic particles, make sure to tap the cartridges to deposit the reagents at the bottom of the wells.
b. Magnetic particles not completely resuspended	Make sure to resuspend the magnetic particles thoroughly before loading the reagent cartridges into the holder.
c. Error message in instrument display	Refer to the user manual supplied with your EZ2 Connect instrument.
d. Clogging of tips	Too much starting material, some stool samples contain high amounts of nucleic acids), which might lead to clogging the tips. Please reduce the input amount to reduce clogging issues.
RNA degraded	
a. Inappropriate handling of starting material	Use phenol chloroform isoamyxl alcohol or as an alternative benzyl alcohol and chloroform in lysis to protect RNA from degradation by RNases. Furthermore, ensure to process the sample material as soon as possible after collection or store it either at –90 to –20°C or in an appropriate stabilization reagent such as PowerProtect DNA/RNA until nucleic acid extraction.
Low or no recovery of RNA and DNA	
a. Too much starting material	In subsequent preparations, reduce the amounts of starting material. It is essential to use the correct amount of starting material. See "Handling and storage of starting material" on page 10.
b. Inefficient disruption and/or homogenization	See "Disrupting and homogenizing starting material" on page 11 for a detailed description of homogenization methods.

		Comments and suggestions
c.	Lysis without phenol chloroform isoamyl alcohol	Lysis without phenol chloroform isoamyl alcohol can result in reduced RNA and DNA yield. In subsequent preparation use phenol chloroform isolamyl alcohol to ensure extraction of highest possible yields.

Appendix: Phenol-free Lysis and IRT

The use of phenol chloroform isoamyl alcohol in lysis is crucial for the depletion of downstream inhibitors and extraction of high amounts of nucleic acids with the EZ2 PowerFecal Pro workflow. At the same time, it also deactivates RNases which can be released during disruption, allowing the extraction of high-quality RNA.

However, if working with phenol is not desired, benzyl alcohol mixed with chloroform in a 9:1 ratio can be used as an alternative to phenol chloroform isoamyl alcohol. Another phenol-free alternative is to follow a two-step lysis and IRT procedure.

Please note that for both phenol-free protocols RNA quality might not match the one obtained with the protocol using phenol chloroform isoamyl alcohol and overall RNA and DNA yields can be reduced.

A. Procedure using benzyl alcohol and chloroform in lysis

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.
2. Add up to 100 mg of stool, 700 µL of Solution CD1, 100 µL Solution CD2, 90 µL benzyl alcohol, and 10 µL chloroform to the PowerBead Pro Tube (in that order), and vortex briefly to mix.

   Note: The absolute maximum of stool material that can be processed is 100 mg. We generally recommend to start with no more than 50 mg of stool which will give high amounts of nucleic acids for most kind of stool samples.
3. Proceed with step 3 of "Protocol: EZ2 PowerFecal Pro DNA/RNA Kit " on page 16.

B. Two-step lysis and IRT procedure

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.
2. Add up to 100 mg of stool and 800 µL of Solution CD1. Vortex briefly to mix.

   Note: The absolute maximum of stool material that can be processed is 100 mg. We generally recommend to start with no more than 50 mg of stool which will give high amounts of nucleic acids for most kind of stool samples.
3. Mechanically disrupt the samples using one of the following methods:
   - Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 24 (1.5–2 mL) tubes (cat. no. 13000-V1-24). Orient the tube caps to point toward the center of the vortex adapter. Vortex at maximum speed for 10 min.
   - Use a TissueLyser III. Place the PowerBead Pro Tube into the TissueLyser Adapter Set 2 x 24 (cat. no. 69982) or 2 mL Tube Holder (cat. no. 11993) and Plate Adapter Set (cat. no. 1190). Fasten the adapter into the instrument and shake for 5 min at 25 Hz speed. Re-orient the adapter so that the side that was closest to the machine body is now furthest from it. Shake again for 5 min at 25 Hz speed.
4. Perform the following steps:
   a. Centrifuge the PowerBead Pro Tube at 18,000 x g for 5 min.
   b. Transfer the supernatant to a clean 2 mL Microcentrifuge Tube (not provided).

      Note: Expect 500–600 µL. The supernatant may still contain some stool particles.
   c. Add 300 µL of Solution CD2 and vortex for 5 s.
   d. Centrifuge at 15,000 x g for 1 min.
5. Proceed with step 5 of "Protocol: EZ2 PowerFecal Pro DNA/RNA Kit " on page 16.

Ordering Information

Product	Contents	Cat. no.
EZ2 PowerFecal Pro DNA/RNA Kit (48)	For the isolation of microbial total NA, DNA only or RNA only from stool samples	954634
RELATED PRODUCTS		
PowerBead Pro Tubes (50)	Bead tubes ready for rapid and reliable biological sample lysis from a wide variety of starting materials, 2 mL	19301
RNase-Free DNase Set (50)	For the removal of genomic DNA contamination in RNA preparations	79254
RNase A (17,500 U)	For the removal of RNA contamination in DNA preparations	19101
TissueLyser III	Bead mill (100–120/220–240 V, 50/60 Hz) for medium- to high-throughput sample disruption for molecular analysis; requires the use of adapters.*	9003240
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2 mL microcentrifuge tubes on the TissueLyser	69982
Plate Adapter Set	2 sets of adapter plates for use with two 96-well plates on the TissueLyser III, compatible with the PowerBead Pro Plates and 2 mL Tube Holder Set	11990
2 mL Tube Holder	Only operational in conjunction with Plate Adpater Set	11993
EZ2 Connect		

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit Instructions for Use. QIAGEN kit Instructions for Use are available at

*The TissueLyser III must be used in combination with TissueLyser adapters.

www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Revision	Description
October 2024	Initial release

Limited License Agreement for EZ2® PowerFecal Pro DNA/RNA Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this Instructions for Use and for use with components contained in the panel only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this panel with any components not included within this panel except as described in the protocols provided with the product, this Instructions for Use, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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