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PowerProtect DNA/RNA Handbook

For stabilization of the microbial community profile and expression profile in stool samples

Sample to Insight

QIAGEN

Contents

Kit Contents

PowerProtect DNA/RNA **Catalog no.**	**(500 ml)** **14800**	**(1000 ml)** **14810**
PowerProtect DNA/RNA	500 ml	1000 ml
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Shipping and Storage

PowerProtect DNA/RNA is shipped at room temperature (15–25°C) and should be stored upon receipt at room temperature. PowerProtect DNA/RNA is stable under these conditions.

Intended Use

PowerProtect DNA/RNA 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.

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.

Quality Control

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

Introduction

Analysis of the microbiome of stool samples can provide insight into health and metabolism of individuals. However, the sample encounters a different environment outside the body, with significant effects on the microbial community within the sample. For example, significantly higher oxygen content, differing temperature, and other factors will all cause some bacterial species to grow more rapidly while hindering others. Stabilizing the sample in some way is essential to get the most accurate picture of the microbiome as it exists within the organism. This is particularly important for RNA analytes, which react more quickly and with a higher dynamic range than that of DNA. A common solution is deep freezing the samples, but this has significant logistical and cost issues.

PowerProtect DNA/RNA preserves bacterial DNA and RNA in stool samples after sampling and maintains the microbial community and functional profile during transport and storage. PowerProtect DNA/RNA is a reagent that penetrates cells and halts biological activity, keeping the microbial information content intact from the time it is sampled until it can be analyzed.

PowerProtect DNA/RNA preserves bacterial DNA indefinitely at temperatures between 4°C and 25°C and bacterial RNA for at least 2 months at those temperatures. PowerProtect DNA/RNA also keeps samples stabilized at elevated temperatures, such as those encountered during transport, for shorter periods. PowerProtect DNA/RNA keeps DNA stable for at least 14 days at 35°C and RNA for 3 days at 35°C. This feature, combined with the long-term stability at ambient temperatures, enables transportation, storage, and shipping of samples without ice or dry ice. For the most secure stabilization for long-term storage (i.e., years), we recommend storing stabilized samples at 2–8°C or frozen at –30 to –15°C or –90 to –65°C in the reagent. During storage or transport in PowerProtect DNA/RNA, even at elevated temperatures (e.g., room temperature or 35°C), the microbial profile remains intact.

Table 1. PowerProtect DNA/RNA stability of RNA and DNA in stool samples

	At 4–25°C)	Elevated temperature (35°C)	Frozen at –30 to –15°C or –90 to –65°C
DNA	Indefinite	14 days	Indefinite
RNA	At least 2months	3 days	Indefinite

Note: PowerProtect DNA/RNA has not been tested for stabilization of DNA and RNA in bacterial cultures, whole blood, plasma, or serum samples.

This handbook provides a detailed protocol for stabilization of microbial DNA and RNA in stool samples. Purification of DNA and/or RNA from the stabilized stool sample can then be performed using QIAGEN kits (see Table 2).

Table 2. QIAGEN Kits for nucleic acid purification from stabilized stool samples

Nucleic acid purified	Procedure	QIAGEN kit total RNA
Total RNA	Manual	RNeasy® PowerFecal® Pro Kit*
Genomic DNA	Manual	QIAamp® PowerFecal Pro Kit* DNeasy® PowerSoil® Pro Kit*
Genomic DNA	Automated (high-throughput)	QIAsymphony® PowerFecal Pro DNA Kit MagAttract® PowerSoil Pro Kit DNeasy 96 PowerSoil Pro QIAcube HT Kit
Genomic DNA	Manual (high-throughput)	DNeasy 96 PowerSoil Pro Kit†
Total RNA and genomic DNA	Manual	AllPrep® PowerFecal Pro DNA/RNA Kit

* These kits can also be automated on QIAcube instruments.

† Requires a centrifuge capable of handling two 96-well blocks (13 cm x 8.5 cm x 6 cm) at 4500 x *g*.

The range of QIAGEN kits for microbial DNA and RNA purification is continuously expanding. Visit **www.qiagen.com/applications/microbiome** to find out about the latest kits.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- PBS
- Stainless Steel Beads, 5 mm (200) (cat. no. 69989) or 7 mm (200) (cat. no. 69990)
- Container
- Centrifuge capable of handling container for sample storage at 5000 x *g*

Important Notes

DNA and RNA stabilization

Bacterial DNA and RNA in stool samples are not protected until the stool material is completely submerged in a sufficient volume of PowerProtect DNA/RNA. After sampling, the stool material should be **immediately** placed in **at least 4 volumes of PowerProtect DNA/RNA (or approx. 2 ml reagent per 500 mg stool)**. Larger volumes can be used if necessary or desired. We recommend using up to 9 volumes of reagent for dry stool samples. In general, use of higher volumes of PowerProtect DNA/RNA (up to 9 volumes) facilitates mixing of the stool material with the reagent and submerging the stool material completely in the reagent. Containers for sample storage should be large enough to hold the entire volume of sample and PowerProtect DNA /RNA as well as the volume of PBS that needs to be added to the sample after storage. Furthermore, the chosen container should be suitable for centrifugation. The procedures for stool sampling and nucleic acid stabilization should be carried out as quickly as possible.

Nucleic acid purification

Before using a QIAGEN kit (see Table 2, page 6) to purify bacterial DNA and/or RNA from stool stabilized with PowerProtect DNA/RNA, carefully read the handbook. The handbook provides guidelines about the amount of starting material that can be used and appropriate methods for disruption of bacterial cells. Optimal DNA and RNA yield and purity depend on using the correct amount of starting material and on efficient disruption of bacterial cells.

Protocol: Stabilization of Bacterial DNA and RNA in Stool Samples

This protocol describes how to stabilize and store stool in PowerProtect DNA/RNA for preservation of the microbial community profile and gene expression profile. For bacterial DNA and/or RNA purification from the stabilized stool using a QIAGEN kit (see Table 2, page 6), refer to the handbook supplied with the kit.

Important points before starting

- PowerProtect DNA/RNA reagent may form precipitate during storage below room temperature (15–25°C). Before using the reagent, redissolve the precipitate by heating to 37°C with agitation. Please note that this only applies before using the reagent for sample stabilization.
- **Note**: When choosing a container for sample storage, also consider the volume of PBS that needs to be added to the sample after storage (see Step 1 under "To proceed with nucleic acid extraction").
- **Note**: Ensure that the container chosen can be centrifuged for later purification.

Procedure

To stabilize the sample

1. Place the stool material in an appropriately sized container.

2. Add at least 4 volumes of PowerProtect DNA/RNA to 1 volume of sample (e.g., for 500 mg sample, add 2 ml PowerProtect DNA/RNA).

   Note: We recommend using up to 9 volumes of reagent for dry stool samples.

Note: In general, use of higher volumes of PowerProtect DNA/RNA (up to 9 volumes) facilitates mixing the stool material with the reagent and submerging the stool material completely in the reagent.

3. Add up to 3 stainless steel beads (5 or 7 mm) to the container.

4. Mix by shaking and inverting until sample forms a slurry.

The sample is ready to be stored. Stainless steel beads are inert and do not need to be removed for storage. PowerProtect DNA/RNA stabilizes nucleic acids at room temperature, 4°C, or –20°C for extended periods. PowerProtect DNA/RNA also stabilizes nucleic acids at higher temperatures for shorter durations. See pages 5–6 for additional details.

To proceed with nucleic acid extraction

1. Add PBS in a volume that is 1.5 times greater than that of PowerProtect DNA/RNA (e.g., if 2 ml of PowerProtect DNA/RNA reagent was used, add 3 ml PBS). Invert to mix.

2. Centrifuge for 10–20 min at 5000 x *g*. Remove the supernatant completely.

   Note: In general, centrifugation for 10 min is sufficient. If the sample is not sufficiently pelleted, this process can be repeated.

   Note: The use of wide-bore pipette tips or regular tips from which the end was cut off might facilitate removal of the supernatant.

3. Use pellet for nucleic acid extraction.

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 in our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook (for contact information, visit **support.qiagen.com**).

		Comments and suggestions
RNA degraded		
a)	Stool sample not immediately stabilized	Submerge the stool material in the appropriate volume of PowerProtect DNA/RNA reagent immediately after sampling.
b)	Too much stool material for proper stabilization	Reduce the amount of stool material, increase the amount of PowerProtect DNA/RNA,.
c)	stool sample not fully submerged in Reagent	Ensure that the stool material remains fully submerged in the reagent. Particles may tend to stick to the lid or the side of the container.
d)	Storage duration is exceeded	See pages 5–6 for detailed information. We recommend lower temperatures whenever possible.
e)	RNA degradation during RNA purification	Although all QIAGEN buffers for RNA purification have been tested and are guaranteed RNase free, RNases can be introduced during use. Be certain not to introduce any RNases during RNA purification or later handling.

Ordering Information

Product	Contents	Cat. no.
RNeasy PowerFecal Pro Kit	For purification of total RNA from stool	78404
QIAamp PowerFecal Pro Kit	For purification of total DNA from stool	51804
AllPrep PowerFecal Pro DNA/RNA Kit	For the isolation of total DNA and RNA from stool and gut material	80254
DNeasy 96 PowerSoil Pro QIAcube® HT Kit	for automated high-throughput isolation of microbial genomic DNA from soil and stool	47021
DNeasy 96 PowerSoil Pro Kit	For manual high throughput extraction of microbial genomic DNA from soil and stool sample	47017
MagAttract PowerSoil Pro DNA Kit	For automated high throughput isolation of microbial genomic DNA from soil and stool on the KingFisher instruments	47109
MagAttract PowerSoil Pro EP Accessories	Accessories for the automation of cat.no. 47109 on the epMotion instruments	47119
Stainless Steel Beads, 5 mm	200 stainless steel beads (5 mm diameter)	69989
Stainless Steel Beads, 7 mm (200)	200 stainless steel beads (7 mm diameter), suitable for use with TissueLyser systems	69990

Products for stabilization and purification of RNA from human saliva		
RNeasy Protect Saliva Mini Kit (50)	RNAprotect® Saliva Reagent (50 ml) and RNeasy Micro Kit (50)	74324
Products for stabilization and purification of RNA from cultured cells		
RNeasy Protect Cell Mini Kit (50)	RNAprotect Cell Reagent (50 ml) and RNeasy Plus Mini Kit (50)	74624

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
04/2022	Initial revision

Limited License Agreement for PowerProtect DNA/RNA

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

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