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QIAsprint® PowerExtract IRT Application Guide

For automated purification of microbial DNA, RNA, and total NA from inhibitor-rich samples like stool or plant

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Required Kits and Components

The QIAasprint Connect offers the possibility of a modular kit system. The following combinations of QIAasprint PowerExtract IRT (Inhibitor Removal Technology) PrepSet and essential kits are recommended.

For purification of microbial genomic DNA, RNA, or total nucleic acids (depending on the chosen protocol) from sample materials that contain a high level of inhibitory substances such as stool samples, combine QIAasprint PowerExtract IRT PrepSet with QIAasprint Essential Kit C.

Required kit/component name	Cat. no.
QIAasprint PowerExtract IRT PrepSet (384)	587679
QIAasprint Essential Kit C (384)	587009
QIAasprint Prep Cover (8)	582103
QIAasprint Prep Plate Insert (32)	582226
QIAasprint Elution Plate (10)	583204
Optional kit/component name	Cat. no.
QIAasprint DNA Removal Set (384)	586409
RNase A	19101

For purification of DNA from inhibitor-rich plant sample material such as seed samples, combine QIAasprint PowerExtract IRT PrepSet with QIAasprint Essential Kit A.

Required kit/component name	Cat. no.
QIAasprint PowerExtract IRT PrepSet (384)	587679
QIAasprint Essential Kit A (384)	585009
QIAasprint Prep Cover (8)	582103
QIAasprint Prep Plate Insert (32)	582226
QIAasprint Elution Plate (10)	583204

Note: For plate setup, reagent dispensing scheme, and procedure descriptions for DNA extraction using the QIAasprint PowerExtract IRT PrepSet in combination with the QIAasprint Essential Kit A, refer to “Protocol: Extraction of DNA from Inhibitor-Rich Plants” to extract DNA from inhibitor-rich plants.

Kit Contents

QIAsprint PowerExtract IRT PrepSet	(384)
Catalog no.	587679
Number of preps	384
Solution CD1 *	200 mL
Solution CD2	60 mL
Buffer MVL [†] (concentrate)	125 mL

* Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 10 for Safety Information.

[†] Addition of isopropanol

QIAsprint Essential Kit A	(384)
Catalog no.	585009
Number of preps	384
Buffer AW1 * (concentrate)	95 mL
Buffer AW1 * (concentrate)	27 mL
Buffer AW2 (concentrate)	2 × 68 mL
RNase-free water	50 mL
RNase-free water	30 mL
MagG Bead Suspension	12 mL

* Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 10 for Safety Information.

QIAprep Essential Kit C**(384)****Catalog no.****587009****Number of preps****384**

Buffer MW1* (concentrate)

77 mL

RNase-free water

80 mL

MagG Bead Suspension

12 mL

* Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 10 for Safety Information.

QIAprep DNA Removal Set**(384)****Catalog no.****586409****Number of preps****384**

DNase I, RNase-Free (lyophilized)

8 × 1500 Kunitz units*

RNase-free water

10 mL

Buffer RDW

30 mL

* Kunitz units are the commonly used units for measuring DNase I, defined as the amount of DNase I that causes an increase in A₂₆₀ of 0.001 per minute per milliliter at 25°C, pH 5.0, with highly polymerized DNA as the substrate (Kunitz, M. [1950] J. Gen. Physiol. 33, 349 and 363).

RNase A**Catalog no.****19101****Number of preps****450**

RNase A

2.5 mL (100 mg/mL;
7000 units/mL, solution)*

* Sufficient for up to 450 DNA preparation, according to Protocol: Extraction of DNA from Inhibitor-Rich Plants (page 31)

Shipping and Storage

The QIAasprint PowerExtract IRT PrepSet and QIAasprint Essential Kits A and C are shipped at ambient temperature.

Solution CD2 should be stored at 2–8°C upon arrival. All other buffers and reagents should be stored at room temperature (15–25°C) until the expiration date printed on the box label.

For extraction of pure RNA, the QIAasprint DNA Removal Set (cat. no. 586409) is required. All components of this set should be stored at room temperature (15–25°C), except for DNase I, which must be stored at 4°C.

Intended Use

The QIAasprint PowerExtract IRT PrepSet 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 components.

CAUTION

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



Solution CD1 and Buffers MW1, MVL, and AW1 contain chaotropic salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with a suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIA Sprint PowerExtract IRT PrepSet is tested against predetermined specifications to ensure consistent product quality.

Introduction

The application guide describes processing of the QIAAsprint PowerExtract IRT PrepSet with the QIAAsprint Connect instrument. For a detailed description of the QIAAsprint Connect instrument, refer to the respective user manual (www.qiagen.com/HB-3708) and quick-start guide (www.qiagen.com/HB-3709). The QIAAsprint PowerExtract IRT application enables automated high-throughput purification of microbial DNA, RNA, and total NA from inhibitor-rich sample materials such as:

- Stool
- Wastewater
- Plant materials with, for example, high polyphenolic content

The kit can be used to purify nucleic acids from a broad range of inhibitor-rich samples. However, kit performance is not guaranteed for each sample material and must be validated by the user. Magnetic-particle technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. The purified nucleic acids are ready to use for highly sensitive detection in downstream assays, such as amplification, NGS analysis, and other enzymatic reactions. The QIAAsprint Connect can process up to 96 samples per run, and subsequent runs allow handling of up to 192 samples in a single session.

Automated purification on QIAasprint Connect

Nucleic acid purification is automated on the QIAasprint Connect platform. The advanced technology integrated into QIAasprint Connect ensures efficient purification of nucleic acids for a wide range of applications while offering exceptional flexibility to allow customers to customize their purification protocols according to specific requirements.

QIAasprint Connect provides comprehensive protocols and purification guides for plasmid DNA, gDNA, RNA, and viral/bacterial nucleic acids.



Figure 1. The QIAasprint Connect instrument.

Principle and procedure

The QIAprint PowerExtract IRT PrepSet comprises a new proprietary one-step lysis and inhibitor removal technology for a more streamlined automatic handling of inhibitor-rich sample material. The procedure allows fully automated processing of samples after lysis to reduce manual steps and hands-on time significantly for more convenience. It combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles. The procedure is intended to remove all inhibitory substances and efficiently extract nucleic acids from all microorganisms present in the sample. The purification comprises four steps: lyse, bind, wash, and elute.

The QIAprint PowerExtract IRT PrepSet efficiently lyses microbial cells and removes inhibitory substances commonly found in, for example, stool (e.g., 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 eventually an organic phase separation agent (e.g., phenol–chloroform–isoamyl alcohol, PCIA) ensure both effective mechanical and chemical lysis using bead beating tubes or plates and efficient inhibitor depletion during centrifugation after lysis and disruption. The supernatant is placed on the QIAprint Connect instrument and total NA, DNA-only, or RNA-only can be extracted in a fully automated workflow.

The workflow is optimized for microbial nucleic acid extraction, and elution is performed in RNase-free water. For DNA-only or RNA-only extraction, the corresponding enzyme—RNase A (cat. no. 19101) or the QIAprint DNA Removal Set (cat. no. 586409)—must be purchased separately.

Please note that the use of PCIA is beneficial for depleting downstream inhibitors. At the same time, it deactivates RNases that may be released during disruption, allowing the extraction of high-quality RNA. For detailed information about alternatives to PCIA, see Appendix A: Phenol-free Lysis and IRT.

The recommended amount of starting material is 50 mg of stool or up to 50 mg of fresh or frozen plant tissue.

For example, for typical stool samples, 50 mg of starting material provides yields of 10–25 µg DNA and 20–90 µg RNA. The maximum amount that can be processed with the QIA sprint PowerExtract IRT PrepSet is 100 mg. Processing substantially larger amounts may result in inefficient disruption or ineffective washing, which can compromise the accuracy of microbial abundance measurements and reduce nucleic acid yield and quality. These recommendations are based on the total amount of stool material, not the total volume; for example, 50 mg of stool in 200 µL of transport buffer can still be processed.

Automation

QIA sprint Connect performs all steps following sample lysis and disruption (see Figure 2). This automated workflow is based on magnetic-particle technology and includes nucleic acid binding, optional enzymatic digestion, washing, and elution. QIA sprint Connect can process up to 96 samples per run, and subsequent runs allow handling of up to 192 samples in a single session.

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 a single step through binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are subsequently separated from the lysates using a magnet, and an additional washing step removes residual contaminants. Finally, nucleic acids are efficiently eluted.

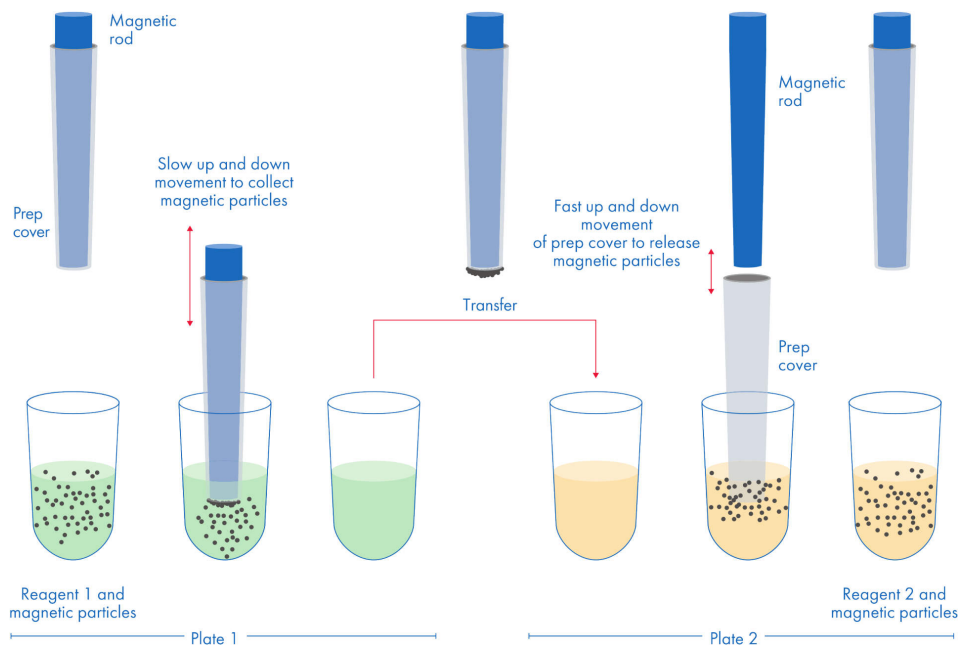


Figure 2. Handling of magnetic particles by the QIAprint Connect instrument.

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.

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

- **For RNA-only protocol:** QIAprint DNA Removal Set (384) (cat. no. 586409)
- **For DNA-only protocol:** RNase A (cat. no. 19101)
- **Recommended for all protocols:** Phenol–chloroform–isoamyl alcohol (PCIA) (25:24:1) pH 6.5–8.0
- 96–100% Ethanol*
- Isopropanol
- Centrifuge capable of handling two 96-well blocks at 4500 × g
- Multichannel pipettors (100–850 µL)
- Single-channel pipettors (5–1000 µL)
- Multichannel pipettor reagent reservoirs for up to 150 mL
- Vortexer

* Do not use denatured alcohol, which contains other substances such as methanol or methyl ethyl ketone.

- **Optional:** QIAasprint Frame Shield (100) (cat. no. 580010)

Note: If desired, use QIAasprint Frame Shield to minimize adhesions of beads to the QIAasprint Prep Cover Frame (cat. no. 582111). For this, place QIAasprint Frame Shield into the QIAasprint Prep Cover Frame before loading the QIAasprint Prep Cover. After the run, discard the QIAasprint Frame Shield.

- 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 × 24 [cat. no. 69982] or 2 mL Tube Holder [cat. no. 11993]) in conjunction with Plate Adapter Set (cat. no. 11990), or when working with PowerBead Pro Plates (cat. no. 19311), use two Plate Adapter Sets (cat. no. 11990)
- QIAasprint Connect consumables (see Table 1)

Table 1. QIAasprint Connect consumables for 1–96 samples

Item	Cat. no.	Pieces required per run for microbial DNA or total RNA extraction	Pieces required per run for microbial RNA extraction
QIAasprint Prep Cover (8)	582103	1	1
QIAasprint Prep Plate Insert (32)	582226	4	6
QIAasprint Elution Plate (10)	583204	1	1

Important Notes

Handling and storage of starting material

The QIAprint PowerExtract IRT PrepSet efficiently lyses cells and removes inhibitory substances and can be used to isolate nucleic acids from, for example, stool, wastewater, plant material, or comparable inhibitor-rich samples.

Stool and wastewater

The main components of stool are water (65–85%), bacterial cells, undigested food and fiber, bile, and bilirubin, which is derived from degraded red blood cells. To a lesser extent, cellular components shed from the gastrointestinal tract wall can also be present. Host DNA may also be detected in these samples but is typically a minor component compared with microbial nucleic acids.

Wastewater samples typically consist of a mixture of microbial cells, organic matter, suspended solids, and chemical contaminants originating from residential, industrial, and environmental sources. Similar to stool, wastewater contains a high proportion of dead and decaying microbial biomass, which can result in partial nucleic acid degradation. The complexity and heterogeneity of wastewater therefore require rapid processing after sample collection to preserve nucleic acid integrity.

Nucleic acids isolated from stool and wastewater may appear partially degraded when analyzed using standard methods because of relatively high content of dead and decaying cells. To optimize nucleic acid quality from these sample types, process samples as quickly as possible after collection. For stool samples, PowerProtect DNA/RNA Reagent enables stabilization at room temperature. Detailed processing recommendations are available (www.qiagen.com/HB-3043).

Freezing samples at -65°C to -90°C preserves nucleic acid quality. If freezing at ultra-low temperatures is not possible, storage at -20°C is an acceptable alternative. Freezing samples in small aliquots avoids repeated freeze–thaw cycles of the bulk material, which can increase cell lysis and nucleic acid degradation. Frozen samples should be processed rapidly by adding lysis buffer to the bead tube or plate before the sample has fully thawed.

Plants

The efficiency of DNA purification, reflected in the yield and integrity of the extracted DNA, can vary depending on the plant species and the quality of the plant material. Proper sampling and storage of plant samples are essential to ensure successful extraction. Homogenize samples immediately to saturate cellular nucleic acids in the protective lysis buffer. For fresh (non-frozen) samples, rapid homogenization in lysis buffer is especially critical to isolate high-quality nucleic acids.

Disruption and homogenization of starting material

Efficient disruption and homogenization of the starting material are essential for all nucleic acid purification procedures. This requires the use of a specialized plate shaker to facilitate the bead-beating process in PowerBead Pro Plates. QIAGEN recommends TissueLyser III (cat. no. 9003240) with adapter sets for use with PowerBead Pro Tubes, including TissueLyser Adapter Set 2 × 24 (cat. no. 69982) or the 2 mL Tube Holder (cat. no. 11993), in conjunction with Plate Adapter Set (cat. no. 11990). Homogenization may also be performed in 2 mL PowerBead Pro Tubes using a Vortex-Genie 2 or a high-powered bead beater with appropriate adapter sets.

Preparation of buffers

Preparation of DNase I stock solution for RNA-only protocol

Prepare the DNase I stock solution by dissolving the lyophilized DNase I (1500 Kunitz units) in 550 μL of RNase-free water. In some cases, the DNase I vial may appear empty because the lyophilized enzyme adheres 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, and mix gently by inverting the vial. Do not vortex.

Insoluble material may remain after dissolving DNase I; this does not affect DNase I performance. Owing to the production process, insoluble material may be present in the lyophilized enzyme. However, rigorous quality control tests are performed to ensure consistent DNase I activity from lot to lot.

Note: Do not vortex reconstituted DNase I, as it is sensitive to physical denaturation. Mix only by gently inverting the vial.

For long-term storage, remove the DNase I stock solution from the vial, divide it into single-use aliquots, and store at -30°C to -15°C for up to 9 months. Thawed aliquots can be stored at $2-8^{\circ}\text{C}$ for up to 4 weeks. Do not refreeze aliquots after thawing.

Preparation of Buffer AW1, Buffer AW2, Buffer MVL, and Buffer MW1

Add the required volume of ethanol (96–100%) to Buffer AW1* concentrate, Buffer AW2 concentrate, and Buffer MW1 concentrate, and add the required volume of isopropanol to Buffer MVL concentrate as described on the bottle. Tick the checkbox on the label to indicate that ethanol/isopropanol has been added. Store the reconstituted buffers at room temperature (15–25°C). The reconstituted buffers are stable for up to 1 year when stored at room temperature, but only until the kit expiration date.

Note: Always mix reconstituted Buffers AW1 and AW2 by shaking before starting the purification procedure.

Working with the QIA sprint Connect

Procedure on the QIA sprint Connect

1. Turn on the QIA sprint Connect instrument and log in.
 2. Navigate to the Protocols screen with the tab in the upper part of the home screen.
 3. Locate the desired protocol for the PowerExtract IRT application, and press  and  **Run protocol**.
- Optional:** Enter run information in the Run details screen. This information will be displayed in the run report.
4. Close the hood and press **Confirm** to get to the loading screen.

* Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 10 for Safety Information.

5. With the plate hotels located on the lab bench, insert the plates according to the screen instructions into the hotels.
 - The QIAAsprint Prep Cover must be located in the metal prep cover frame.

Note: If desired, use the QIAAsprint Frame Shield to minimize adhesions of beads to the QIAAsprint Prep Cover Frame. For this, place QIAAsprint Frame Shield into the QIAAsprint Prep Cover Frame before loading the QIAAsprint Prep Cover. After the run, discard the QIAAsprint Frame Shield.
 - All QIAAsprint Prep Plates need to sit firmly in their metal frames.
 - The QR codes need to face the user.
6. Open the hood and place the hotels on their platforms, taking care not to mix up the left and right hotels.
7. **Optional:** If you have heating or cooling steps during your protocol run and the thermal adapter is not already located on the heater-cooler, place the thermal adapter in one of the slots of the thermal adapter platform with the QR code facing the user. The instrument will install it automatically during the run.
8. Press **Start run** to start the run.
9. After the end of the run, remove the plate hotels from the instrument. Store the elution plate safely or use it directly in your downstream applications.

Protocol: Extraction of DNA, RNA, or Total NA from Stool or Wastewater Samples

Important notes before starting

- PCIA (25:24:1), pH 6.5–8.0, is required for this protocol
- When working with PowerBead Pro Tubes, ensure that they rotate freely in the centrifuge without rubbing.
- Add ethanol (96–100%) to Buffer MW1 concentrates and isopropanol to Buffer MVL concentrates before use (see the bottle label for volume).
- Prepare 80% ethanol for wash steps. Prepare sufficient solution for a minimum volume of 2 mL per sample when performing DNA or total nucleic acid extraction and 3 mL per sample when performing RNA extraction.
- Prepare the binding/bead mixture (Buffer MVL/MagG Bead Suspension) by mixing 470 μ L of Buffer MVL with 25 μ L of MagG Bead Suspension per sample. Use a 10% overage when preparing a master mix for multiple samples. Mix well by inversion and store at room temperature.
- **RNA-only protocol:** Prepare the DNase I stock enzyme by adding 550 μ L of RNase-free water to the lyophilized DNase I (RNase-free) and mixing gently. Aliquot the DNase I stock enzyme in 100 μ L portions and store at -30°C to -15°C for long-term storage. Avoid more than three freeze–thaw cycles. DNase I is sensitive to physical denaturation; do not vortex resuspended DNase I.
- Prepare a mix of 140 μ L of Buffer RDW and 10 μ L of DNase I stock enzyme per prep. Use a 10% overage when preparing a master mix for multiple samples. Mix well by inversion and store at room temperature.

- Perform centrifugation steps at room temperature.
- If preparing RNA for the first time, read “Important Notes” on page 19.

Procedure using PowerBead Pro Plates

1. Spin the PowerBead Pro Plate (cat. no. 19311) briefly to ensure that the beads have settled at the bottom of the wells. Remove and discard the square well mat from the bead plate.
2. **Stool samples:** Add 50–100 mg of fresh or frozen stool material and 450 µL of Solution CD1 to each well of the PowerBead Pro Plate.

Note: The absolute maximum amount of stool material that can be processed is 100 mg. QIAGEN recommends starting with no more than 50 mg of stool, which typically yields high amounts of nucleic acids for most stool samples.

Wastewater samples: Centrifuge a 40 mL wastewater sample at $4500 \times g$ for 2 h and discard the supernatant. Add 450 µL of Solution CD1 to the wastewater pellet. Resuspend the pellet by pipetting up and down, and transfer the sample to one well of the PowerBead Pro Plate.

3. Add 150 µL of Solution CD2 to each well of the PowerBead Pro Plate.
4. Ensure that no residual liquid remains on top of the plate.

Note: Liquid on top of the plate prevents tight sealing with the sealing film and may result in leakage during disruption in TissueLyser III (cat. no. 9003240).

5. Secure the sealing film (provided with the PowerBead Pro Plates) tightly onto the bead plate. Use a tool such as a scraper or plate roller to ensure firm adhesion of the sealing film to the plate.

Note: A strong seal is essential to prevent leakage during disruption in TissueLyser III. Use of a mechanical plate sealer may be advantageous to achieve a consistent and uniform seal.

6. Place the silicone compression mat (provided with the PowerBead Pro Plates) on top of the sealed bead plate.

Note: Two silicone compression mats are provided so that two plates can be processed in parallel in TissueLyser III. The mats are reusable.

7. Place the entire assembly (steps 1–6) between two Plate Adapter Sets (cat. no. 11990) and disrupt samples in TissueLyser III for 2 × 5 min at 25 Hz.

Important: Do not exceed the recommended disruption time or settings, as extended processing may lead to leakage.

8. After the first 5 min cycle, remove the adapter block and rotate it so that the side closest to the instrument body is now furthest away. Disrupt again for 5 min at 25 Hz.
9. Spin the plate down briefly and carefully to avoid splashing. Remove the sealing film (do not discard and have it ready for resealing at step 10). Add 150 µL of PCIA (25:24:1, pH 6.5–8.0) to each well of the PowerBead Pro Plate.

Note: PCIA is essential to prevent RNA degradation and to enable efficient inhibitor removal. It is not compatible with the sealing film and must not be added before sample disruption.

After addition, avoid contact between the liquid in the wells and the inner surface of the sealing film.

Note: As an alternative to PCIA, a benzyl alcohol–chloroform mixture can be used. For details, see Appendix A: Phenol-free Lysis and IRT on page 39.

10. Reseal the plate with the same sealing film and centrifuge the PowerBead Pro Plate at 4500 × g for 6 min at room temperature.
11. Use the supernatant for DNA, total nucleic acid, or RNA extraction.

For DNA extraction, add 5 µL of RNase A (ordered separately) to each well before adding the supernatant.

For RNA or total nucleic acid extraction, proceed without RNase A.

12. Carefully remove and discard the sealing film. Transfer 420 μL of the supernatant to a clean QIAprint Prep Plate Insert.

Note: The supernatant may still contain residual stool particles.

Procedure using PowerBead Pro Tubes

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.
2. **Stool samples:** Add 50–100 mg of fresh or frozen sample material and 450 μL of Solution CD1 to the PowerBead Pro Tube.

Note: The absolute maximum amount of stool material that can be processed is 100 mg. QIAGEN recommends starting with no more than 50 mg of stool, which typically yields high amounts of nucleic acids for most stool samples.

Wastewater samples: Centrifuge a 40 mL wastewater sample at $4500 \times g$ for 2 h and discard the supernatant. Add 450 μL of Solution CD1 to the wastewater pellet. Resuspend the pellet by pipetting up and down, and transfer the sample to the PowerBead Pro Tube.

3. Add 150 μL of Solution CD2 and 150 μL of PCIA (25:24:1, pH 6.5–8.0) to the PowerBead Pro Tube. Vortex briefly to mix.

Note: As an alternative to PCIA, a benzyl alcohol–chloroform mixture can be used. For more information, see Appendix A: Phenol-free Lysis and IRT on page 39.

4. Mechanically disrupt the samples using one of the following methods:
 - **Vortex adapter:** Secure the PowerBead Pro Tube horizontally on a vortex adapter for 24 tubes (1.5–2 mL) (cat. no. 13000-V1-24). Orient the tube caps toward the center of the adapter. Vortex at maximum speed for 10 min.
Note: If processing more than 12 preps simultaneously, increase the vortex time by 5–10 min.
 - **TissueLyser III:** Place the PowerBead Pro Tube into TissueLyser Adapter Set 2 × 24 (cat. no. 69982) or the 2 mL Tube Holder (cat. no. 11993) in conjunction with Plate Adapter Set (cat. no. 11990). Secure the adapter in the instrument and disrupt samples for 5 min at 25 Hz. Reorient the adapter so that the side closest to the instrument body is now furthest away, and disrupt again for 5 min at 25 Hz.
5. Centrifuge the PowerBead Pro Tube at 15,000 × g for 5 min.
6. Use the supernatant for DNA, total nucleic acid, or RNA extraction.

For DNA extraction, add 5 µL of RNase A (not included in the QIAprint PowerExtract IRT PrepSet) to each well before adding the supernatant.

For RNA or total nucleic acid extraction, proceed without RNase A.
7. Carefully transfer 420 µL of the supernatant to the appropriate wells of a QIAprint Prep Plate Insert.

Note: The supernatant may still contain residual stool particles.

Plates preparation

- 1. Thoroughly mix the binding/bead mixture (Buffer MVL/MagG Bead Suspension), and add 495 µL to each well containing 420 µL supernatant.
- 2. Prepare the plate as described in the tables below:

Table 2. For total nucleic acid or DNA extraction

Plate	Content	Volume (µL)
Binding Plate	For DNA extraction: RNase A	5
	Lysate supernatant	420
	MagG Bead Suspension	25
	Buffer MVL	470
Wash Plate 1	Buffer MW1	800
Wash Plate 2	80% EtOH	800
Wash Plate 3	80% EtOH	800
Elution Plate	RNase-free Water	100

Table 3. For RNA extraction

Plate	Content	Volume (µL)
Binding Plate	Lysate supernatant	420
	MagG Bead Suspension	25
	Buffer MVL	470
Wash Plate 1	80% EtOH	800
DNase Plate	Buffer RDW	140
	DNase I	10
Wash Plate 2	Buffer MW1	800
Wash Plate 3	80% EtOH	800
Wash Plate 4	80% EtOH	800
Elution Plate	RNase-free Water	100

Note: For plate setup, reagent dispensing scheme, and procedure descriptions for DNA extraction using the QIAasprint PowerExtract IRT PrepSet in combination with the QIAasprint Essential Kit A, refer to Protocol: Extraction of DNA from Inhibitor-Rich Plants.

Note: Residual adhesive can lead to instrument crash. If you cover plates with adhesive foil after plate preparation, ensure that no adhesive remains on the plate surface after removal of the foil.

3. Proceed to the purification protocol.

Using the following protocols is recommended: “QIAasprint RNA from Stool” or “QIAasprint DNA/total NA from Stool”. Before loading, remove the cover from the Elution Plate unless the cover is specified in the protocol.

Protocol: Extraction of DNA from Inhibitor-Rich Plants

Important points before starting

- If using the QIA sprint PowerExtract IRT PrepSet and QIA sprint Essential Kit A for the first time, read "Important Notes".
- After receiving the QIA sprint PowerExtract IRT PrepSet and QIA sprint Essential Kit A, check all kit components for damage. If any components are damaged, contact QIAGEN Technical Support or your local distributor. In the event of liquid spillage, refer to the Safety Information. Do not use damaged PrepSet or essential kit components, as this may lead to poor performance.
- Buffer MVL and Buffer AW1 contain guanidine salts and are not compatible with disinfecting reagents containing bleach.

Things to do before starting

- If necessary, redissolve any precipitate in Buffers MVL and AW1 by warming.
- Perform all steps at room temperature and work quickly.
- Add isopropanol or ethanol (96–100%) to Buffer MVL, Buffer AW1, and Buffer AW2 concentrates before use, as indicated on the bottle labels.
- **Optional:** Prepare a fresh CD1/RNase solution (2 μ L of RNase per 450 μ L of Solution CD1).
- For a 100 μ L elution volume, add 120 μ L of RNase-free water.

Procedure

Homogenization and lysis of plant samples

Plant samples can be disrupted and homogenized using various methods, which may differ between laboratories. For optimal results, QIAGEN recommends using TissueLyser III.

1. Add up to 50 mg of fresh or frozen plant tissue and 450 μ L of Solution CD1 to a collection microtube or a 2 mL tissue disruption tube, depending on the number of samples.

Note: Cut tissue into small pieces before loading into the tube.

Note: When ground seeds are used as starting material, bead beating of the seed powder can improve extraction efficiency and DNA yield.

Note: The volume of Solution CD1 may be increased to up to 500 μ L for some sample types, such as beans.

Note: For DNA extraction from plant-associated bacteria or fungi, use PowerBead Pro Tubes (cat. no. 19301) or PowerBead Pro Plates (cat. no. 19311) with TissueLyser Adapter Set 2 $\times$ 24 (cat. no. 69982) or the Plate Adapter Sets (cat. no. 11990). In this case, the amount of Solution CD1 to be added in this case may be increased up to 800 μ L.

2. Disruption using TissueLyser III:
 - Use the TissueLyser Plate Adapter Set 2 $\times$ 96.
 - Place one or two 3 mm stainless steel beads in each tube.
 - Load the samples into TissueLyser III and disrupt at 30 Hz for 2 min.
 - After the first cycle, remove the adapter block and rotate it so that the side closest to the instrument body is now furthest away. Disrupt again for 2 min at 30 Hz.
 - Repeat the procedure if intact tissue is still visible in the lysate.

3. Centrifuge the tissue disruption tubes at $\geq 6000 \times g$ for 5 min.
4. Transfer approximately 300–350 μL of the clear supernatant to a fresh collection microtube.

Note: Expect 300–350 μL of supernatant. Residual plant particles may still be present.

Note: For protein-rich samples such as beans, addition of 25 μL of QIAGEN Proteinase K (cat. no. 19134) may increase yield. In this case, incubate samples at 65°C for 15 min.

5. Add 150 μL of Solution CD2 and vortex for 5 s.

Note: For problematic samples, increase the volume of Solution CD2 to 200–250 μL .

6. Centrifuge the tubes at $\geq 6000 \times g$ for 5 min, then transfer up to 400 μL of the supernatant to the Lysate Binding Plate.

For additional guidance on plant homogenization, refer to the *QIAprint DNA Plant Application Guide* (www.qiagen.com/HB-3716).

Plates preparation

- 7. Ensure sufficient resuspension of the MagG Bead Suspension) by pipetting or vortexing.
- 8. Prepare the plate as described in the tables below:

Plate	Content	Volume (µL)
Binding Plate	Lysate supernatant	400
	MagG Bead Suspension	20
	Buffer MVL	400
Wash Plate 1	Buffer AW1	700
Wash Plate 2	Buffer AW2	650
Wash Plate 3	Buffer AW2	500
Elution Plate	RNase-free Water	100 (+20 µL to recover a 100 µL eluate)

- 9. To start the run, follow the instructions from “Working with the QIAprint Connect” on page 19.

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 support.qiagen.com).

Comments and suggestions	
Low nucleic acid yield	
Sample stored under unfavorable conditions	Samples should be stored at –65°C to –90°C.
Insufficient disruption and homogenization	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample, PCIA, Solution CD1, and Solution CD2 until the sample is thoroughly homogenized.
Too much starting material	Overloading the nucleic acid binding system significantly reduces nucleic acid yields. Reduce the amount of starting material.
Stool processing	
Amount of stool to process	The QIAprint PowerExtract IRT PrepSet, in combination with QIAprint Essential Kit C, is designed to process up to 0.1 g of stool. For inquiries regarding the use of larger sample amounts, contact QIAGEN Technical Support for guidance.
Stool sample has high water content	Add an appropriate amount of stool to a centrifuge tube and centrifuge at room temperature for 30 s at 10,000 × g. Remove as much liquid as possible using a pipette tip. Transfer 50–100 mg of stool to a PowerBead Pro Tube or PowerBead Pro Plate.
Stool sample is stabilized	If using stool samples preserved in PowerProtect DNA/RNA, refer to the <i>PowerProtect DNA/RNA Handbook</i> .

Comments and suggestions

DNA/RNA

Inefficient removal of inhibitory substances	In most cases, no inhibition is observed when amplifying nucleic acids purified using QIAprep PowerExtract IRT PrepSet in combination with QIAprep Essential Kit C. If template nucleic acids perform poorly in downstream applications, dilute the template before downstream processing (suggested dilution 1:10). If dilution does not improve performance, repeat the nucleic acid purification procedure using less starting material. Take care not to transfer any of the pellet after centrifugation.
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Storing DNA/RNA	DNA and RNA are eluted in RNase-free water and must be stored at –30°C to –15°C or –90°C to –65°C to prevent degradation.
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DNA fragmented

Sample stored under unfavorable conditions	Samples should be stored at –65°C to –90°C.
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Homogenization too vigorous	The length of purified DNA depends strongly on the homogenization conditions. If longer DNA fragments are required, minimize homogenization time or use a gentler homogenization method if possible (e.g., use a vortex adapter instead of a TissueLyser system).
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RNA degraded

Sample stored under unfavorable conditions	Samples should be stored at –65°C to –90°C.
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RNase contamination	RNases are ubiquitous and stable laboratory contaminants and can be introduced during sample processing. Avoid introducing RNases during RNA extraction or subsequent handling. Refer to Appendix B: General Remarks on Handling RNA for guidelines.
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PCIA not added to lysate	PCIA is essential to prevent RNA degradation and to enable efficient inhibitor removal. Add PCIA to the lysate when working with PowerBead Pro Tubes, or add PCIA directly after disruption when using PowerBead Pro Plates. PCIA is not compatible with the sealing foil and must not be added to the PowerBead Pro Plate before sample disruption.
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Comments and suggestions

Plant samples: Low DNA content recovery

Low DNA content of the plant tissue	Increase the amount of starting material, but do not use more than 100 mg.
Insufficient sample disruption	Ensure that the starting material is completely disrupted. See "Important Notes", <i>QIAasprint DNA Plant Application Guide</i> (www.qiagen.com/HB-3716).
Insufficient sample lysis	Ensure that the starting material is resuspended thoroughly in Solution CD1. Frozen samples should be resuspended immediately after disruption. Do not allow samples to thaw. Before the bead-beating step, the yield of DNA from some plant tissues may be improved by heating at 65°C for 10 min in the presence of Solution CD1. This effect is dramatically changed among species

Plant samples: Green-, red-, or yellow-colored eluates

Insufficient washing of the MagG Bead Suspension	Chlorophyll or carotenoids can be efficiently removed by adding one additional wash step using Buffer AW2 or a 100% ethanol in future preparations. Use QIAasprint PowerExtract IRT PrepSet with QIAasprint Essential Kit C. Buffer MW1 can help reduce coloration in the eluate and enhance DNA yield in some plant materials such as beans.
Too much starting material	Reducing the amount of starting material to the recommended amounts. Slight color in the eluate does not necessarily interfere with downstream applications.

Contact Information

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or contact one of the QIAGEN Technical Service Departments or local distributors (visit support.qiagen.com).

Appendix A: Phenol-free Lysis and IRT

The use of PCIA during lysis is crucial for the depletion of downstream inhibitors and for the extraction of high yields of nucleic acids when using the QIA Sprint PowerExtract IRT workflow. In addition, PCIA deactivates RNases that may be released during disruption, enabling 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 PCIA. A second phenol-free option is to perform a two-step lysis and IRT procedure.

Note: For both phenol-free protocols, RNA quality may not match that obtained using PCIA, and overall RNA and DNA yields may be reduced.

Procedure using benzyl alcohol and chloroform during lysis

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.
2. When using PowerBead Pro Tubes, add up to 100 mg of stool, 450 µL of Solution CD1, 150 µL of Solution CD2, 135 µL of benzyl alcohol, and 15 µL of chloroform to the PowerBead Pro Tube in that order, and vortex briefly to mix.

Note: The absolute maximum amount of stool that can be processed is 100 mg. QIAGEN recommends starting with no more than 50 mg of stool, which yields high amounts of nucleic acids for most stool sample types.

Proceed with step 4 of “Procedure using PowerBead Pro Tubes” on page 28.

3. When using PowerBead Pro Plates, add up to 100 mg of stool, 450 µL of Solution CD1, and 150 µL of Solution CD2 to each well of the PowerBead Pro Plate in that order.

Note: The absolute maximum amount of stool that can be processed is 100 mg. We recommend starting with no more than 50 mg of stool, which will give high amounts of nucleic acids for most kind of stool sample.

4. Proceed with steps 4–8 of “Procedure using PowerBead Pro Plates” on page 25.
5. Spin the plate briefly, carefully and without splashing. Remove the sealing film and add 135 μ L of benzyl alcohol and 15 μ L of chloroform to each well.
6. Proceed with step 10 of “Procedure using PowerBead Pro Plates” on page 26.

Two-step lysis and IRT procedure (PowerBead Pro Tubes)

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.

Add up to 100 mg of stool and 800 μ L of Solution CD1. Vortex briefly to mix.

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

2. Mechanically disrupt the samples using one of the following methods:
 - Secure the PowerBead Pro Tube horizontally on Vortex Adapter for 24 (1.5–2 mL) Tubes (cat. no. 13000-V1-24). Orient the tube caps toward the center of the adapter and vortex at maximum speed for 10 min.
 - Use a TissueLyser III. Place the PowerBead Pro Tube into the TissueLyser Adapter Set 2 $\times$ 24 (cat. no. 69982), 2 mL Tube Holder (cat. no. 11993), or Plate Adapter Set (cat. no. 11990). Shake for 5 min at 25 Hz. Reorient the adapter so that the side closest to the machine body is now furthest from it and shake again for 5 min at 25 Hz.

3. Perform the following steps:
 - a. Centrifuge the PowerBead Pro Tube at $18,000 \times g$ for 5 min.
 - b. Transfer the supernatant to a clean 2 mL microcentrifuge tube (not provided).
 - c. **Note:** Expect 500–600 μL . The supernatant may still contain some stool particles.
 - d. Add 300 μL of Solution CD2 and vortex for 5 s.
 - e. Centrifuge at $15,000 \times g$ for 1 min.
4. Proceed with step 6 of “Procedure using PowerBead Pro Tubes” on page 26.

Two-step lysis and IRT procedure (PowerBead Pro Plates)

1. Spin the PowerBead Pro Plate briefly to ensure that the beads have settled at the bottom. Remove and discard the square well mat.
2. Add up to 100 mg of stool and 800 μL of Solution CD1 to each well of the PowerBead Pro Plate.

Note: The absolute maximum amount of stool that can be processed is 100 mg. We recommend starting with no more than 50 mg of stool.
3. Remove any residual liquid from the top of the plate.

Note: Residual liquid can prevent tight sealing of the plate and may cause leakage during disruption in the TissueLyser III (cat. no. 9003240).
4. Secure the sealing film tightly onto the bead plate using a scraper or plate roller.

Note: A strong seal is essential to prevent leakage. Use of a mechanical plate sealer may improve seal consistency.
5. Place the silicone compression mat on top of the sealed bead plate.

Note: Two reusable silicone compression mats are provided so that two plates can be processed in parallel.

6. Place the assembled plate between two Plate Adapter Sets (cat. no. 11990) and disrupt in the TissueLyser III for 2×5 min at 25 Hz.

Important: Do not exceed the recommended disruption time or speed, as extended processing may cause leakage.

7. After the first 5 min cycle, remove and rotate the assembly so that the side closest to the machine body is now furthest from it. Shake again for 5 min at 25 Hz.
8. Centrifuge the sealed PowerBead Pro Plate at $4500 \times g$ for 6 min.
9. Transfer the supernatant to a clean 96-well block (not provided).

Note: Expect 500–600 μL . The supernatant may still contain some stool particles.

10. Add 300 μL of Solution CD2 and vortex for 5 s.
11. Proceed with step 10 of “Procedure using PowerBead Pro Plates” on page 26.

Appendix B: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Because RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. To create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non-disposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds, which are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Non-disposable plasticware

Non-disposable plasticware should be treated before use to ensure that it is RNase free. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA,* followed by RNase-free water. Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

Glassware

Glassware should be treated before use to ensure that it is RNase free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with diethyl pyrocarbonate (DEPC)*. Fill glassware with 0.1% DEPC* (0.1% in water), allow to stand overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS*), thoroughly rinsed with RNase-free water, and then rinsed with ethanol† and allow to dry.

* 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.

† Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC.* DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 mL DEPC to 100 mL of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO₂. When preparing Tris buffers, treat water with DEPC first and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues has been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

* 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.

Ordering Information

Product	Contents	Cat. no.
QIA sprint PowerExtract IRT PrepSet (384)	For 384 DNA/RNA or total NA preps for inhibitor-rich samples like stool or plant samples: Solutions CD1 and CD2 and Buffer MVL	587679
QIA sprint Essential Kit A (384)	For 384 preps for purification of nucleic acids: MagG Bead Suspension, Buffer AW1, Buffer AW2, and nuclease-free water	585009
QIA sprint Essential Kit C (384)	For 384 preps for purification of nucleic acids: purified water, Buffer MW1, and MagG Bead Suspension	587009
QIA sprint DNA Removal Set (384)	DNase, RNase-free, 8 vials, 30 mL Buffer RDW, and 10 mL RNase-free water	586409
QIA sprint Prep Cover (8)	Set of 8 prep covers for use with QIA sprint Connect	582103
QIA sprint Prep Plate Insert (32)	Set of 32 prep plate inserts for use with QIA sprint Connect	582226
QIA sprint Elution Plate (10)	Set of 10 elution plates for use with QIA sprint Connect	583204
Related products		
QIA sprint Connect	Benchtop instrument for automated isolation of nucleic acids; includes 1 year warranty on parts and labor	9001234
QIA sprint Frame Shield (100)	Set of 100 frame shields for use with QIA sprint Connect	580010
QIA sprint Prep Cover Frame (2)	Set of 2 prep cover frames for use with QIA sprint Connect	582111
QIA sprint Prep Plate Frame (2)	Set of 2 prep plate frames for use with QIA sprint Connect	582211
RNase A (17,500 U)	2.5 mL solution for removal of RNA	19101
PowerBead Pro Tubes (2 mL) (50)	For disruption of stool and all soil samples in 2 mL format	19301
PowerBead Pro Plates (4)	Bead plates ready for rapid and reliable biological sample lysis from a wide variety of starting materials	19311
Collection Microtubes (racked, 10 × 96)	Nonsterile polypropylene tubes (1.2 mL), 960 in racks of 96 for mechanical disruption and homogenization of the plant material	19560

Product	Contents	Cat. no.
Collection Microtube Caps (120 × 8)	Nonsterile polypropylene caps for collection microtubes (1.2 mL, 960 in strips of 8 for sealing the collection microtubes to prevent leakage	19566
Tissuelyser III	Bead mill, 100–120/220–240 V, 50/60 Hz; requires Tissuelyser Adapters (available separately)	9003240
Vortex Adapter for 24 (1.5–2.0 mL) tubes	For vortexing 1.5 and 2 mL tubes using the Vortex-Genie 2 Vortex.	13000-V1-24
Tissuelyser Adapter Set 2 × 24	Two sets of adapter plates and two racks for use with 2 × 24 samples in 2 mL microcentrifuge tubes on the Tissuelyser III	69982
Tissuelyser Adapter Set 2 × 96	Two sets of adapter plates for use with Collection Microtubes (racked) on the Tissuelyser III	69984
Plate Adapter Set	Two sets of adapter plates and two racks for use with two 96-well plates on the Tissuelyser III	11990
2 mL Tube Holder Set	For sample homogenization of up to 2 × 48 samples in 2 mL bead tubes on the Tissuelyser III	11993
PowerWater DNA Bead Tube (50)	Bead tubes for processing filter membranes	14900-50-NF-BT
PowerProtect DNA/RNA (500 mL)	For the stabilization of the microbial community profile and expression profile in stool samples	14800
PowerProtect DNA/RNA (1000 mL)	For the stabilization of the microbial community profile and expression profile in stool samples	14810

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 Support or your local distributor.

Document Revision History

Date	Description
03/2026	Initial release
05/2026	Updated to match the Essential Kit configuration.

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