October 2019

QIAsymphony® SP Protocol Sheet

DNASoilStool_600_V1

This document is the DNASoilStool_600_V1 *QIAsymphony SP Protocol Sheet*, R1, for QIAsymphony PowerFecal® Pro DNA Kit.

Sample to Insight

QIAGEN

General information

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

The QIAsymphony PowerFecal Pro DNA Kit is effective at removing PCR inhibitors from even the most difficult stool and soil types. Samples are added to a bead beating tube for rapid and thorough homogenization. Cell lysis occurs by mechanical and chemical methods. The binding of total genomic DNA takes place automatically on QIAsymphony by silica magnetic beads. DNA is then washed and eluted from the magnetic beads automatically and ready for NGS, PCR, and other downstream applications.

DNASoilStoolDNA_V1

Kit	QIAsymphony PowerFecal Pro DNA
Sample material	Stool and soil
Protocol name	DNASoilStool_600_V1
Default Assay Control Set	ACS_DNASoilStool600_V1
Elution volume	110 µl
Required software version	Buffer AVE
Kit	Version 4.0 or higher

Materials required but not provided

For all sample types

- Microcentrifuge (up to16,000 x *g*)
- Pipettor (50–1000 µl)
- Vortex-Genie® 2
- Vortex Adapter for 24 (1.5 or 2 ml) tubes (cat. no. 13000-V1-24)
- Optional: RNaseA (cat. no. 19101) and ProteinaseK (cat. no. 19131)

"Sample" drawer

Sample type	Lysates from stool or soil
Sample volume	600 µl
Primary sample tubes	See **www.qiagen.com/goto/QIAsymphony** for more information.
Secondary sample tubes	See **www.qiagen.com/goto/QIAsymphony** for more information.
Inserts	See **www.qiagen.com/goto/QIAsymphony** for more information.
Other	n/a

n/a = not applicable.

"Reagents and Consumables" drawer

Position A1 and/or A2	Reagent cartridge (RC)
Position B1	n/a
Tip rack holder 1–17	Disposable filter-tips, 200 µl
Tip rack holder 1–17	Disposable filter-tips, 1500 µl
Unit box holder 1–4	Unit boxes containing sample prep cartridges
Unit box holder 1–4	Unit boxes containing 8-Rod Covers

n/a = not applicable.

"Waste" drawer

Unit box holder 1–4	Empty unit boxes
Waste bag holder	Waste bag
Liquid waste bottle holder	Liquid waste bottle

"Eluate" drawer

Elution rack (we recommend using slot 1, cooling position)	See **www.qiagen.com/goto/QIAsymphony** for more information

Required plasticware

	One batch, 24 samples*	Two batches, 48 samples*	Three batches, 72 samples*	Four batches, 96 samples*
Disposable filter-tips, 200 µl†‡	96	96	128	128
Disposable filter-tips, 1500 µl†‡	128	192	224	288
Sample prep cartridges§	18	36	25	72
8-Rod Covers¶	3	6	9	12

* Use of less than 24 samples per batch decreases the number of disposable filter-tips required per run.
† There are 32 filter-tips/tip rack.
‡ Number of required filter-tips includes filter-tips for 1 inventory scan per reagent cartridge.
§ There are 28 sample prep cartridges/unit box.
¶ There are twelve 8-Rod Covers/unit box.

Preparation of sample material

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.

Important points before starting

- QIAsymphony magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample in step 5 indicated in the respective pre-treatment protocol.
- Before beginning the procedure, read "Important Notes" in the *QIAsymphony PowerFecal Pro DNA Handbook*.

Pretreatment protocol in single tubes

Things to do before starting

- Ensure that the PowerBead Pro Tubes rotate freely in the centrifuge without rubbing.
- Perform all centrifugation steps at room temperature (15–25°C).
- Refer to kit handbook for optimal homogenization method in step 3.
- Optional: Set a thermomixer or shaker–incubator to 56°C for use in step 5.

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.
2. Add 50–150 mg of stool or up to 250 mg of soil and 800 µl of Solution CD1. Vortex briefly to mix.
3. Secure the PowerBead Pro Tube horizontally on a vortex adapter for 1.5–2 ml tubes (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.

   Note: If using the Vortex Adapter for more than 12 preps simultaneously, increase the vortex time by 5–10 min.
4. Centrifuge the PowerBead Pro Tube at 15,000 x *g* for 1 min.
5. Transfer the supernatant to a clean 2 ml microcentrifuge tube (provided).

   Note: Expect a volume of 500–600 µl. The supernatant may still contain some stool or soil particles.

   Optional:

   5a. If RNA-free DNA is required, add 4 µl RNaseA and vortex shortly. Spin down and incubate the mixture for 5 min at room temperature.

   5b. Add 30 µl ProteinaseK and vortex shortly. Spin it down and incubate the mixture for 15 min at 56°C
6. Add 300 µl Solution CD2 and vortex for 5 s. Centrifuge at 15,000 x *g* for 1 min at room temperature.
7. Avoiding the pellet, transfer 600 µl of supernatant to a clean 2 ml micro tube (Sarstedt® cat. no. 72.694) (not provided).

Pretreatment protocol in 96-well format

Materials required but not provided

- PowerBead Pro Plate (cat. no. 19311)
- TissueLyser II (cat. no. 85300)
- Plate Adapters (cat. no. 11990)
- Collection Microtubes (CMTR) (cat. no. 19560)
- S-Blocks (cat.no. 19585)

Things to do before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Refer to *QIAsymphony PowerFecal Pro DNA Handbook*. for optimal homogenization method in step 7.

1. Spin the PowerBead Pro Plate (cat. no. 19311) briefly to ensure that the beads have settled at the bottom.
2. Remove the square well mat from a PowerBead Pro Plate. Add 50 to 150 mg of stool or up to 250 mg of soil sample.

   Note: Avoid cross contamination between sample wells.
3. Add 800 µl of Solution CD1 to the wells of the PowerBead Pro Plate.
4. Make sure to remove any residual liquid on top of the plate.

   Note: Liquid on top of the plate will prevent a tight sealing of the plate with the sealing film. This may result in leakage during disruption in the TissueLyser II (cat. no. 85300).
5. Secure the sealing film tightly onto the bead plate. Use a tool such as a scraper or plate roller to make the sealing film adhere firmly onto the plate.

   Note: A strong seal is essential to prevent leakage during disruption in the TissueLyser II.
6. Put the silicone compression mat on top of the bead plate that is sealed with the sealing film.

   Note: Two silicone compression mats are provided so that 2 plates can be processed in parallel in the TissueLyser II. The mats are reused for the remaining plates.
7. Place this entire assembly (from steps 1 to 6) between 2 plate adapters for disruption in the TissueLyser II.

   Important: When using this assembly, do not exceed the recommended disruption time and setting of 2 x 5 min at 25 Hz, because doing so might lead to leakage.
8. Shake at a speed of 25 Hz for 5 min. Re-orient plates so that the side that was closest to the machine body is now furthest from it and shake again at a speed of 25 Hz for 5 min.

   Note: This protocol uses a combination of mechanical and chemical lysis. Mechanical lysis is introduced at this step. By randomly shaking the beads, they collide with one another and with microbial cells causing them to break open.
9. Centrifuge at room temperature for 6 min at 4500 x *g*.
10. Discard the sealing film. Transfer the supernatant to the collection microtubes (CMTR).

    Note: Expect a volume of 500–600 µl. The supernatant may still contain some stool or soil particles.
11. Add 300 µl of Solution CD2. Seal collection microtubes with the caps provided and vortex.
12. Centrifuge the plate at room temperature for 6 min at 4500 x *g*.
13. Transfer up to 600 µl of supernatant to an S-Block (not provided) or 2 ml micro tube (Sarstedt 72.694) (not provided).

Installation procedure

1. Download zip file for protocol DNASoilStool600_V1 from .
   www.qiagen.com/QIAsymphony SP/AS
2. Unzip the file to main folder of the QIAsymphony USB drive.

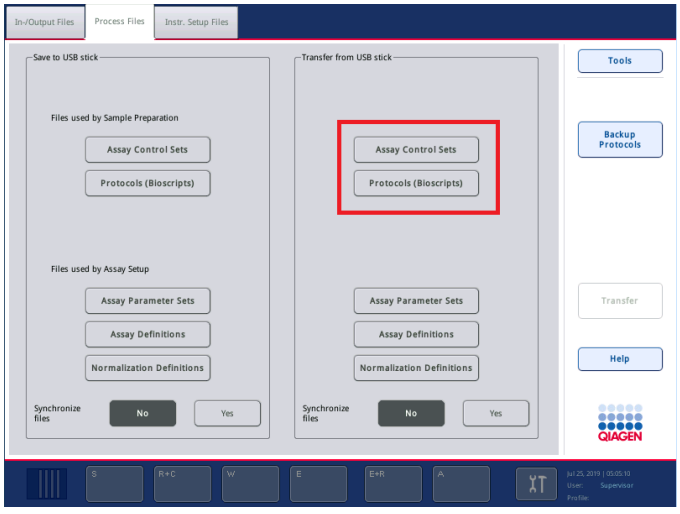
   Note: Make sure that the folder structure on the USB stick is as follows:

   /data/AssayControlSets/

   /data/BioScripts/

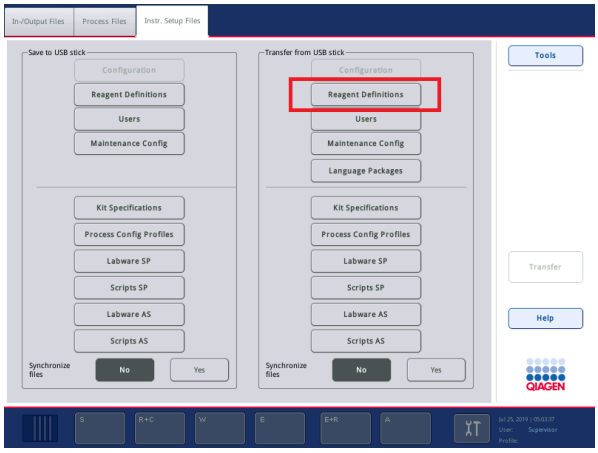
   /data/ReagentDefinitions
3. Start the QIAsymphony and login as a "Supervisor".
4. Plug the USB stick into one of the front USB ports of the QIAsymphony SP.
5. Select the "Tools" tab and press the "File Transfer" button.
6. Select the "Process Files" tab and press the "Protocols" and "Assay Control Sets" button. Press the buttons on the right-hand side.

   Note: There are two "Protocols" and "Assay Control Sets" buttons. The one on the left-hand side is used to save files to the USB stick and the one on the right-hand side is used to transfer files from the USB stick.



7. Transfer the files by pressing the "Transfer" button.
8. After successful data transfer, a message will appear confirming the data transfer.
9. Switch to the "Instr. Setup files" tab.

10. Select “Reagent Definitions” (SW5.0) or “Cartridge Information” (SW4.0) button.



11. Transfer the file by pressing the “Transfer” button.
12. A message will appear. Read the message and confirm.
13. Reboot the QIAsymphony (switch off and then on again).

Revision History

Date	Changes
10/2019	Initial release

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.

Ordering **www.qiagen.com/shop** | Technical Support **support.qiagen.com** | Website **www.qiagen.com**