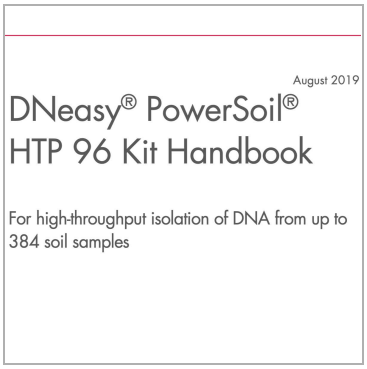

APR 13, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.8epv5j526l1b/v1

Protocol Citation: QIAGEN 2023. Qiagen DNeasy PowerSoil HTP 96 Kit. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv5j526l1b/v1

Protocol status: In development
We are still developing and optimizing this protocol

Created: Mar 20, 2023

Last Modified: Apr 13, 2023

PROTOCOL integer ID: 79105

Qiagen DNeasy PowerSoil HTP 96 Kit

QIAGEN[1]

[1]QIAGEN

Mark Louie Lopez

ABSTRACT

Introduction

The DNeasy PowerSoil HTP 96 Kit allows high-throughput isolation of DNA from up to 384 soil samples in less than one day.

Principle and procedure

This kit provides researchers with a high-throughput method for isolating genomic DNA from environmental samples using Inhibitor Removal Technology® (IRT) that efficiently removes humic substances that inhibit PCR. This procedure effectively removes PCR inhibitors from even the most difficult soil types, allowing for more successful PCR amplification of DNA. DNA isolated from many sample types, including compost, sediment and manure, was successfully used as template to amplify members of a wide range of microbial groups in soils. These include bacteria (gram-positive, gram-negative and spore-formers), actinomycetes, archaebacteria and fungi.

Environmental samples are added to a 96 well bead beating plate for rapid and thorough homogenization. Cell lysis occurs by a combination of mechanical and chemical methods. Humic substances are removed by a specialized precipitation process. Total genomic DNA is captured on a 96 well silica membrane in a spin-column plate format. DNA is then washed and eluted from the membrane. The eluted DNA is ready for PCR analysis and other downstream applications.

The estimated time from start to finish to process two 96 well plates for this protocol is approximately 8 hours. Stopping points at appropriate steps are mentioned in the protocol. The majority of the time is for weighing and loading the soil samples into the 96 well plates.

As of April, 2023 - no published studies successfully detecting fish sedDNA using this protocol, however research is still in development and we hope to see promising results in the future

ATTACHMENTS

HB-2258-003_HB_DNY_PowerSoil_96_0819_WW (1).pdf

GUIDELINES

The DNeasy PowerSoil HTP 96 Kit reagents and components can be stored at room temperature (15–25°C) until the expiry date printed on the box label.

This DNeasy PowerSoil HTP 96 Kit is designed to process 0.25 g of soil. Recommended starting amounts for different soil types are listed in Table 1.

MATERIALS

Equipment and Reagents to Be Supplied by User

- Centrifuge capable of handling two 96 well blocks (13 cm x 8.5 cm x 60 cm) at 4500 x g
  Note: If you have a centrifuge with a maximum speed less than 4500 x g, see the Troubleshooting Guide.
- Multi-channel pipettor (50–650 µl)
- Mechanical shaker for 96 well blocks and plate adaptors (cat. no. 11990)
- Vortex-Genie® 2 vortex with 3-inch platform
- 100% ethanol
- Reagent reservoirs (optional)
- Vacuum pump (optional)
- Vacuum manifold (optional)
- Plate seals (optional – if protocol is paused at step 15)

Kit Contents (384 preps; 12955-4)

- QIAamp® 96 Plates (4)
- PowerBead DNA Plates, Garnet (4)
- PowerBead Solution (2 x 200 ml)
- Solution C1 (45 ml)
- Solution C2 (128 ml)
- Solution C3 (106 ml)
- Solution C4 (2 x 330 ml)
- Solution C5-D* (120 ml)

*Note: *Before using for the first time, add 100% ethanol to Solution C5-D, as indicated in the protocol, to obtain a working solution.*

- Solution C6 (66 ml)
- Racked Elution Microtubes (4)
- Caps for Elution Microtubes (50 x 8)
- Collection Plates, 1 ml (4 x 4)
- Collection Plates, 2 ml (4)
- AirPore Tape Sheets (25)
- Sealing Tape, Polyester, 2 ml (16)
- S-Blocks (4)
- Square Well Mats (4)
- Quick Start Protocol (1)

SAFETY WARNINGS

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.

Solution C5-D is flammable after addition of ethanol.

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

PowerBead Solution and Solution C4 contain guanidine 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.

BEFORE START INSTRUCTIONS

Important points before starting:

- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- Prepare solution C5-D by adding an equal volume (120 ml) of 100% ethanol. Mix well.

Sample preparation & cell lysis

1h 12m 5s

1 **REMOVE** Spare Well Mat from a PowerBead Plate

ADD up to .25 g of soil sample

Note

Avoid cross contamination between sample wells. This is an appropriate stopping point. PowerBead Plate can be stored at 2-8 °C covered with the Square Well Mat

2 **ADD** 750 µL of PowerBead Solution to the wells of the PowerBead Plate

ADD 60 µL of Solution C1

3 **SECURE** the Square Well Mat tightly to the plate 20m

PLACE PowerBead Plate with the mat securely fastened between 2 Adapter Plates on a 96 well plate shaker or a TissueLyzer II

SHAKE at speed 20 Hz for 00:10:00

RE-ORIENT plates so the side that was closest to the machine body is now furthest from it and shake again at speed 20 Hz for 00:10:00

4 **CENTRIFUGE** at 4500 x g for 00:06:00 at Room temperature 6m

DISCARD the Square Well Mat

TRANSFER supernatant to a clean 1 mL Collection Plate

Note

Supernatant may still contain some soil particles

Inhibitor removal 1h 12m 5s

5 **ADD** 250 µL of Solution C2 10m 5s

APPLY Sealing Tape to the plate

VORTEX for 00:00:05

INCUBATE at 2-8 °C for 00:10:00

Note

You can skip the 10 min incubation. However, if you have already validated DNeasy PowerSoil extractions with the incubation, it is recommended to retain this step

6 **CENTRIFUGE** the plate at 4500 x g for 00:06:00 at Room temperature 6m

DISCARD Sealing Tape

AVOIDING the pellet, transfer the entire volume of supernatant to a new 1 mL Collection Plate

7 APPLY Sealing Tape to plate 10m 5s

VORTEX for 00:00:05

INCUBATE at 2-8 °C for 00:10:00

8 **CENTRIFUGE** the plate at 4500 x g for 00:06:00 at Room temperature 6m

DISCARD Sealing Tape

AVOIDING the pellet, transfer the entire volume of supernatant to a new 1 mL Collection Plate

Bind DNA 30m

9 **ADD** 200 µL of Solution C3

REPEAT steps 7-8 once

10 **APPLY** Sealing Tape to the plate 6m

CENTRIFUGE again at 4500 x g for 00:06:00 at Room temperature

TRANSFER no more than 650 µL of supernatant to a 2 mL Collection Plate

11 **ADD** 650 µL of Solution C4 to each well of the plate

REPEAT to add 1300 µL total

Note

You can pause here and store samples covered with Sealing Tape at 2-8 °C

12 **PIPET** samples up and down to mix

PLACE a spin plate onto an S-Block

13 **LOAD** approximately 650 µL into each well of the spin plate and seal the plate with an AirPore Tape Sheet 3m

CENTRIFUGE at 4500 x g for 00:03:00 at Room temperature

DISCARD flow-through and place the spin plate back on the same S-Block

DISCARD AirPore Tape Sheet

14 **REPEAT** step 13 until all the supernatant has been processed

DISCARD the final flow-through

PLACE the spin plate back on the same S-Block

Wash spin plate 30m

15 **ADD** 500 µL of Solution C5-D to each well of the spin plate and seal the plate with an AirPore Tape Sheet 3m

CENTRIFUGE at 4500 x g for 00:03:00 at Room temperature

DISCARD flow-through and place the spin plate back on the same S-Block

SEAL with an AirPore Tape Sheet

16 **CENTRIFUGE** again at 4500 x g for 00:05:00 at Room temperature 5m

DISCARD flow-through

CAREFULLY place the spin plate onto Racked Elution Microtubes

DISCARD AirPore Tape Sheet

17 **ALLOW** to air dry for 00:10:00 at Room temperature 10m

Elute the DNA 30m

18 **ADD** 100 µL of Solution C6 to the center of each well

SEAL plate with an AirPore Tape Sheet

19 **CENTRIFUGE** at 4500 x g for 00:03:00 at Room temperature 3m

DISCARD the AirPore Tape Sheet

20 **SEAL** Elution Microtubes with the Caps provided

DNA is now ready for downstream applications

Note

Qiagen recommends storing DNA frozen (-15 °C to 30 °C or -65 °C to 90 °C) as Solution C6 does not contain EDTA