invitrogen

PureLink™ Microbiome DNA Purification Kit

Purification of high-quality microbial DNA from soil samples

Catalog Number A29790

Pub. No. MAN0014331 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Invitrogen™ PureLink™ Microbiome DNA Purification Kit enables fast purification of high-quality microbial and host DNA from a wide variety of sample types. The kit uses proven PureLink™ spin-column technology for robust yields of purified DNA that is ready for downstream PCR, sequencing, or other applications.

Typical DNA recovery is 0.5–15 µg from 0.2 g of soil sample.

Procedure overview

This guide describes purification of of microbial DNA from soil samples. In this procedure, the microorganisms are efficiently lysed by a combination of heat, chemical, and mechanical disruption with specialized beads. Inhibitors are eliminated by precipitation using a proprietary cleanup buffer. The sample is then applied to a PureLink™ spin column, and the DNA that is bound to the column undergoes a single wash step before elution.

Kit contents

Table 1 PureLink™ Microbiome DNA Purification Kit (Cat. no. A29790, 50 reactions)

Component	Quantity	Storage
S1—Lysis Buffer	40 mL	15°C to 30°C
S2—Lysis Enhancer	5 mL	
S3—Cleanup Buffer	12.5 mL	
S4—Binding Buffer	45 mL	
S5—Wash Buffer Concentrate[1]	13 mL	
S6—Elution Buffer	5 mL	
PureLink™ Spin Columns with Collection Tubes	50	
PureLink™ Collection Tubes	100	15°C to 30°C
Bead Tubes[2]	50	

[1] Add 13 mL of 96–100% ethanol before use. See "Before you begin" on page 2.

[2] Ships separately.

Required materials

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**www.fisherscientific.com**) or other major laboratory supplier.

Table 2 Required materials not included with the kit

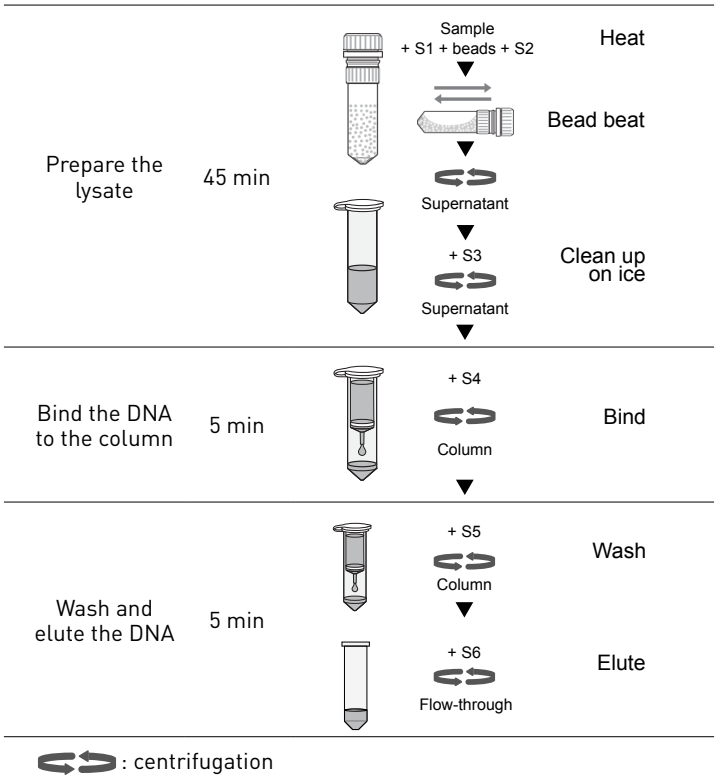
Item	Source
Heat block, dry bath, or water bath, 65°C	MLS
(*Optional*) For dry bath, Lab Armor™ Beads	Cat. no. A12543
Microcentrifuge capable of 14,000 × *g*	MLS
Vortex mixers, 2[1]	MLS
For vortex bead homogenization: hands-free adapter for vortex mixer, with horizontal tube orientation	Fisher Scientific NC0070788[2]
(*Optional*; alternative to vortex bead homogenization) Bead mill homogenizer	Omni 19-040, or equivalent
Adjustable pipettors, 100–1000 µL	MLS
Microcentrifuge tubes, DNase-free, 1.5 mL or 2.0 mL	MLS
Ethanol, 96–100%	MLS

[1] For vortex bead homogenization: we recommend using two mixers, one dedicated to the hands-free adapter.

[2] Cat. no. AM10024 (not available for sale) can also be used.

ThermoFisher SCIENTIFIC

Workflow



Important procedural guidelines

Sample input requirements and handling

- Collect samples according to your laboratory guidelines and experimental needs.
- Ensure that samples are mixed thoroughly with S1—Lysis Buffer and S2—Lysis Enhancer to create a homogenous sample.

  One way to ensure thorough mixing is to vortex the tube with the cap down.

Alternatives to the optimized procedure

- This procedure is optimized for homogenization by bead beating on the vortex mixer with horizontal agitation. This is a cost-effective method for recovery of high-quality microbial DNA. Ensure that the vortex adapter enables horizontal agitation; adapters with a vertical tube orientation may not agitate adequately.

  Note: Balance the vortex adapter to ensure proper movement of the adapter and optimal homogenization.

  If you use a bead mill homogenizer, follow the manufacturer's instructions to optimize sample disruption.
- This procedure is optimized for centrifugations at 14,000 × *g*. The PureLink™ Spin Columns with Collection Tubes can withstand up to 16,000 × *g*.

  If your microcentrifuge is not capable of 14,000 × *g*, adjust the centrifugation times to ensure that all of the sample passes through the column.

Options for elution

- The DNA can be eluted from the column with 50–200 µL of S6—Elution Buffer, to optimize the concentration of the recovered DNA.
- Two sequential elution steps with S6—Elution Buffer might increase the yield slightly. For example, for a total elution volume of 100 µL, either:
  - Perform two sequential elution steps with 50 µL of S6—Elution Buffer, or
  - Perform the first elution step with 100 µL of S6—Elution Buffer, then apply the flow-through (containing the eluted DNA) to the same column and repeat for a second elution.
- If desired, perform the final elution spin into nuclease-free 1.5-mL microcentrifuge tubes, instead of the collection tubes supplied with the kit, which do not have caps. Position the cap of the microcentrifuge tubes opposite the direction of rotation.

Before you begin

Before first use of the kit: prepare S5—Wash Buffer

Add 13 mL of 96–100% ethanol to S5—Wash Buffer Concentrate , mix well, and store at room temperature.

Before each use of the kit

If precipitate is visible in S1—Lysis Buffer or S4—Binding Buffer, warm the buffers at 37°C for 5 minutes and shake well to dissolve the precipitate.

Methods

Perform the procedure at room temperature (20–25°C), unless otherwise indicated.

1 Prepare the lysate

a. Add 600 µL of S1—Lysis Buffer to the Bead Tube.

b. Add 0.2±0.05 g of soil, cap securely, then vortex.

c. Add 100 µL of S2—Lysis Enhancer, cap securely, and vortex briefly.

d. Incubate at 65°C for 10 minutes.

e. Homogenize by bead beating for 10 minutes at maximum speed on the vortex mixer.

Use the hands-free adapter and horizontal agitation.

f. Centrifuge at 14,000 × *g* for 5 minutes.

g. Transfer up to 400 µL of the supernatant to a clean microcentrifuge tube.

IMPORTANT! A layer of debris may be present on top of the bead pellet. Avoid transfer of this debris with the supernatant.

h. Add 250 µL of S3—Cleanup Buffer, and vortex immediately.

Vortex immediately to ensure even dispersion of S3—Cleanup Buffer and uniform precipitation of inhibitors.

i. Incubate on ice for 10 minutes.

j. Centrifuge at 14,000 × *g* for 1 minute.

k. Transfer up to 500 µL of the supernatant to a clean microcentrifuge tube, avoiding the pellet.

2 Bind the DNA to the column

a. Add 900 µL of S4—Binding Buffer, and vortex briefly.

b. Load 700 µL of the sample mixture onto a spin column-tube assembly, and centrifuge at 14,000 × *g* for 1 minute.

c. Discard the flow-through, and repeat step 2b with the remaining sample mixture.

Ensure that the entire sample mixture has passed into the collection tube by inspecting the column. If sample remains in the column, centrifuge again at 14,000 × *g* for 1 minute.

3 Wash and elute the DNA

a. Place the spin column in a clean collection tube, add 500 µL of S5—Wash Buffer, then centrifuge the spin column-tube assembly at 14,000 × *g* for 1 minute.

b. Discard the flow-through, then centrifuge the spin column-tube assembly at 14,000 × *g* for 30 seconds.

The second centrifugation optimizes removal of S5—Wash Buffer, which could interfere with downstream applications.

c. Place the spin column in a clean tube, add 100 µL of S6—Elution Buffer, then incubate at room temperature for 1 minute.

d. Centrifuge the spin column-tube assembly at 14,000 × *g* for 1 minute, then discard the column.

The purified DNA is in the tube.

The DNA is ready for immediate use. Alternatively, store the purified DNA:

- At 4°C for up to 1 week.
- At –20°C for long-term storage.

Troubleshooting

Observation	Possible cause	Recommended action
There is less than 400 µL of supernatant after lysis spin	For some samples, it can be difficult to withdraw 400 µL of supernatant at step 1g, while avoiding debris.	If <250 µL of supernatant is transferred, add S1—Lysis Buffer to the transferred supernatant to bring the volume to 400 µL.
Low yield	Inefficient lysis.	Heat samples at 95°C for 5–10 minutes instead of at 65°C for 10 minutes.
		Heat at 95°C for 5–10 minutes, and bead beat for a longer time or using a higher power setting.
	Low levels of DNA in the sample.	Repeat the purification with more starting material. Do not exceed 0.25 g.
	For some challenging samples, too much starting material can result in low yield.	Repeat the purification with less starting material, and increase the volume of S1—Lysis Buffer so that the total volume of sample/S1—Lysis Buffer is 800 µL.
Inhibition of PCR or other downstream reactions	Presence of inhibitors in the recovered DNA.	Dilute the DNA 10- to 100-fold for PCR.
		Repeat the purification with less starting material, and increase the volume of S1—Lysis Buffer so that the total volume of sample/S1—Lysis Buffer is 800 µL.

Documentation and support

Revision history MAN0014331 (English)

Revision	Date	Description
A.0	September 2015	New document.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.