

PureLink™ Microbiome DNA Purification Kit

Purification of high-quality microbial and host DNA from saliva and urine samples

Catalog Number A29790

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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 0.5–2 µg from 1 mL of saliva or 0.1–1 µg from 1 mL of urine.

Procedure overview

This guide describes purification of DNA from saliva and urine samples. In this procedure, the microorganisms are efficiently lysed by a combination of heat, chemical, and mechanical disruption with specialized beads. 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 ^[1]	12.5 mL	
S4—Binding Buffer	45 mL	
S5—Wash Buffer Concentrate ^[2]	13 mL	
S6—Elution Buffer	5 mL	
PureLink™ Spin Columns with Collection Tubes	50	

Component	Quantity	Storage
PureLink™ Collection Tubes	100	15°C to 30°C
Bead Tubes ^[3]	50	

^[1] Not used in this procedure.

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

^[3] 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
<i>(Optional)</i> For dry bath, Lab Armor™ Beads	Cat. no. A12543
Microcentrifuge capable of 14,000 × <i>g</i>	MLS
Vortex mixers, 2 ^[1]	MLS
For vortex bead homogenization: hands-free adapter for vortex mixer, with horizontal tube orientation	Fisher Scientific NC0070788 ^[2]
<i>(Optional; alternative to vortex bead homogenization)</i> 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

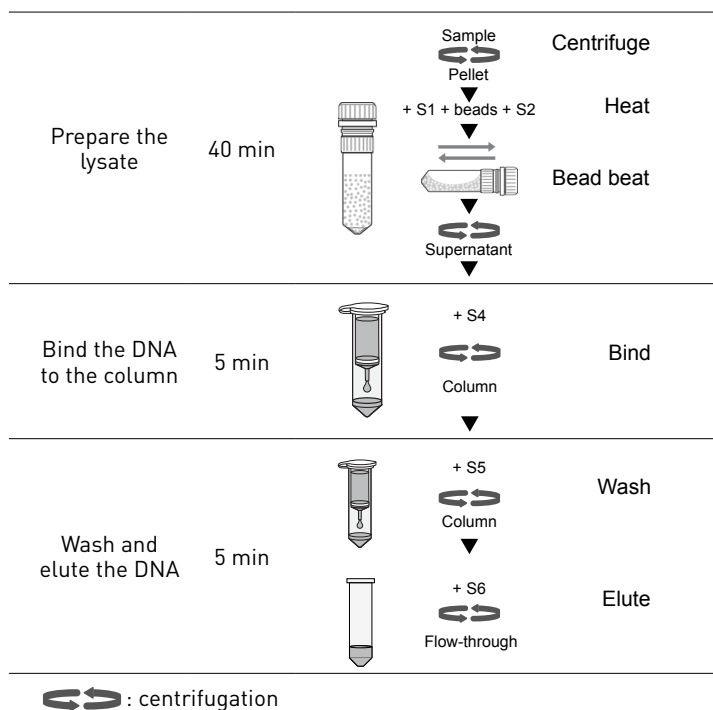
^[1] For vortex bead homogenization: we recommend using two mixers, one dedicated to the hands-free adapter.

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

Table 3 Additional materials required for sample volumes >2.0 mL

Item	Source
Conical tubes, 15 mL, DNase-free	Cat. no. AM12500
Benchtop centrifuge for 15-mL conical tubes	MLS

Workflow



Important procedural guidelines

Sample input requirements and handling

- Collect samples according to your laboratory guidelines and experimental needs.
- Recommended input amount:

Sample type	Amount
Saliva	1–2 mL
Urine	1–10 mL

For either sample type, volumes as low as 0.2 mL can be used. For 0.2-mL samples, you may add the sample directly to 600 μ L of S1—Lysis Buffer, instead of centrifuging and resuspending the microbial pellet in 800 μ L of S1—Lysis Buffer.

- Ensure that samples are mixed thoroughly with S1—Lysis Buffer and S2—Lysis Enhancer to create a homogenous sample.

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 $14,000 \times g$. The PureLink™ Spin Columns with Collection Tubes can withstand up to $16,000 \times g$.

If your microcentrifuge is not capable of $14,000 \times g$, 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 μ L 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 μ L, either:
 - Perform two sequential elution steps with 50 μ L of S6—Elution Buffer, or
 - Perform the first elution step with 100 μ L 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**
- Centrifuge the sample at $14,000 \times g$ for 10 minutes to pellet the microorganisms.
Samples larger than 5 mL may require longer centrifugation times for optimal recovery of microorganisms.
Use an appropriately sized centrifuge tube; do not use a Bead Tube.
 - Carefully remove the supernatant and discard.
Avoid disturbing the microbial pellet during this step. Loss of pellet will result in decreased yield.
 - Resuspend the microbial pellet in 800 μL of S1—Lysis Buffer, pipet up and down or vortex to resuspend, then transfer the sample to the Bead Tube.
 - Add 100 μL of S2—Lysis Enhancer, cap securely, and vortex briefly.
 - Incubate at 65°C for 10 minutes.
 - Homogenize by bead beating for 10 minutes at maximum speed on the vortex mixer.
Use the hands-free adapter and horizontal agitation.
 - Centrifuge at $14,000 \times g$ for 2 minutes.
 - Transfer up to 500 μL of the supernatant to a clean microcentrifuge tube, avoiding the bead pellet.
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- 2 Bind the DNA to the column**
- Add 900 μL of S4—Binding Buffer, and vortex briefly.
 - Load 700 μL of the sample mixture onto a spin column-tube assembly, and centrifuge at $14,000 \times g$ for 1 minute.
 - Discard the flow-through, and repeat step 2b with the remaining sample mixture.
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- 3 Wash and elute the DNA**
- Place the spin column in a clean collection tube, add 500 μL of S5—Wash Buffer, then centrifuge the spin column-tube assembly at $14,000 \times g$ for 1 minute.
 - Discard the flow-through, then centrifuge the spin column-tube assembly at $14,000 \times g$ for 30 seconds.
The second centrifugation optimizes removal of S5—Wash Buffer, which could interfere with downstream applications.
 - Place the spin column in a clean tube, add 50 μL of S6—Elution Buffer, then incubate at room temperature for 1 minute.
 - Centrifuge the spin column-tube assembly at $14,000 \times g$ 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.
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Troubleshooting

Observation	Possible cause	Recommended action
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. Larger sample volumes may require longer centrifugation times in the initial step to pellet the microorganisms.
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 an additional cleanup step: <ol style="list-style-type: none"> 1. After bead beating and centrifugation, transfer up to 400 µL of the supernatant to a clean microcentrifuge tube. 2. Add 250 µL of S3—Cleanup Buffer, and vortex immediately. 3. Centrifuge at 14,000 × <i>g</i> for 2 minutes, then transfer up to 500 µL of the supernatant to a clean microcentrifuge tube, avoiding the pellet. 4. Proceed to “Bind the DNA to the column” on page 3.

Documentation and support

Revision history MAN0014267 (English)

Revision	Date	Description
A.0	September 2015	New document.

Limited product warranty

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