PureLink™ Microbiome DNA Purification Kit

Purification of high-quality microbial and host DNA from buccal, vaginal, or skin swab samples

Catalog Number A29790
Pub. No. MAN0014268 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 0.1–5 μg from swab samples.

Procedure overview
This guide describes purification of DNA from buccal, vaginal, or skin swab samples. (For purification of DNA from rectal or environmental swab samples, refer to Pub. no. MAN0014333.) 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)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1—Lysis Buffer</td>
<td>40 mL</td>
<td>15°C to 30°C</td>
</tr>
<tr>
<td>S2—Lysis Enhancer</td>
<td>5 mL</td>
<td></td>
</tr>
<tr>
<td>S3—Cleanup Buffer</td>
<td>12.5 mL</td>
<td></td>
</tr>
<tr>
<td>S4—Binding Buffer</td>
<td>45 mL</td>
<td></td>
</tr>
<tr>
<td>S5—Wash Buffer Concentrate</td>
<td>13 mL</td>
<td></td>
</tr>
<tr>
<td>S6—Elution Buffer</td>
<td>5 mL</td>
<td></td>
</tr>
<tr>
<td>PureLink™ Spin Columns with Collection Tubes</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Required materials not included with the kit

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat block, dry bath, or water bath, 65°C</td>
<td>MLS</td>
</tr>
<tr>
<td>{Optional} For dry bath, Lab Armor™ Beads</td>
<td>Cat. no. A12543</td>
</tr>
<tr>
<td>Microcentrifuge capable of 14,000 × g</td>
<td>MLS</td>
</tr>
<tr>
<td>Vortex mixers, 2[1]</td>
<td>MLS</td>
</tr>
<tr>
<td>For vortex bead homogenization: hands-free adapter for vortex mixer, with horizontal tube orientation</td>
<td>Fisher Scientific NC0070788[2]</td>
</tr>
<tr>
<td>{Optional; alternative to vortex bead homogenization} Bead mill homogenizer</td>
<td>Omni 19-040, or equivalent</td>
</tr>
<tr>
<td>Adjustable pipettors, 100–1000 μL</td>
<td>MLS</td>
</tr>
<tr>
<td>Microcentrifuge tubes, DNase-free, 1.5 mL or 2.0 mL</td>
<td>MLS</td>
</tr>
<tr>
<td>4N6FLOQSwabs*, Regular Tip, or equivalent</td>
<td>Cat. no. 4473979, or equivalent</td>
</tr>
<tr>
<td>Ethanol, 96–100%</td>
<td>MLS</td>
</tr>
</tbody>
</table>

[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.

For Research Use Only. Not for use in diagnostic procedures.
Workflow

Prepare the lysate 30 min
+ S1 + beads + S2
Heat
Bead beat
Supernatant
Sample

Bind the DNA to the column 5 min
+ S4
Bind
Column

Wash and elute the DNA 5 min
+ S5
Wash
Elute
Column
Flow-through

Important procedural guidelines

Sample input requirements and handling
- The procedure was developed with 4N6FLOQSwabs™. Some testing was performed with Puritan™ PurFlock™ Ultra Flocked Swabs (Fisher Scientific Cat. no. 22-025-192).
- Collect swab samples according to your laboratory guidelines and experimental needs.
- Ensure that the swabs are broken to 1–3 cm in length, so that they fit inside the Bead Tube with the screw cap completely tightened.

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 \(\mu\)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 \(\mu\)L, either:
  - Perform two sequential elution steps with 50 \(\mu\)L of S6—Elution Buffer, or
  - Perform the first elution step with 100 \(\mu\)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 | a. Add 800 µL of S1—Lysis Buffer to the Bead Tube, then add one swab.  
|   |               | b. Add 100 µL of S2—Lysis Enhancer, cap securely, and vortex briefly.  
|   |               | c. Incubate at 65°C for 10 minutes.  
|   |               | d. Bead beat for 10 minutes at maximum speed on the vortex mixer.  
|   |               |   Use the hands-free adapter and horizontal agitation.  
|   |               | e. Centrifuge at 14,000 × g for 1 minute.  
|   |               | f. Transfer up to 500 µL of the supernatant to a clean microcentrifuge tube, avoiding the bead pellet and any debris.  
|   |               |   Some contact with the swab or swab debris will not affect DNA quality.  

| 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.  

| 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 50 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

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low yield</td>
<td>Inefficient lysis.</td>
<td>Heat samples at 95°C for 5–10 minutes instead of at 65°C for 10 minutes.</td>
</tr>
<tr>
<td></td>
<td>Low levels of DNA in the sample.</td>
<td>Heat at 95°C for 5–10 minutes, and bead beat for a longer time or using a higher power setting.</td>
</tr>
<tr>
<td>Inhibition of PCR or other downstream reactions</td>
<td>Presence of inhibitors in the recovered DNA.</td>
<td>Repeat the purification with more starting material.</td>
</tr>
</tbody>
</table>

### Documentation and support

**Revision history MAN0014268 (English)**

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.0</td>
<td>September 2015</td>
<td>New document.</td>
</tr>
</tbody>
</table>

**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](http://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](http://www.thermofisher.com/support).

The information in this guide is subject to change without notice.

**DISCLAIMER**

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

©2015 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

For support visit [thermofisher.com/support](http://thermofisher.com/support) or email techsupport@lifetech.com