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PureLink™ Quick Plasmid Miniprep Kit

USER GUIDE

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

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0003721 C (English)

Revision	Date	Description
C	11 June 2025	• Updated to the current document template, with associated updates to the limited license information, warranty, trademarks, and logos. • The contents and storage table was updated to include additional instructions for storage of purification columns.
B.0	11 March 2019	Baseline.

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

Contents

1 Product information

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

Product description

The PureLink™ Quick Plasmid Miniprep Kit enables isolation of high-quality plasmid DNA (up to 40 µg) from E. coli cells in 30-45 minutes. Purified plasmid DNA is suitable for all routine downstream applications including bacterial cell transformation, mammalian cell transfection, DNA sequencing, restriction enzyme digestion, cloning, and PCR. The kit can be used with a centrifuge or a vacuum manifold.

Contents and storage

All components of the PureLink™ Quick Plasmid Miniprep Kit are shipped at room temperature.

Table 1 Components of the PureLink™ Quick Plasmid Miniprep Kit

Component	Cat. No. K210010 (50 reactions)	Cat. No. K210011 (250 reactions)	Storage
Resuspension Buffer (R3)	15 mL	72 mL	15–25°C
RNase A (20 mg/mL)	0.11 mL	0.65 mL	15–25°C (unopened vial) -20°C (opened vial)
Lysis Buffer (L7)	15 mL	72 mL	15–25°C
Precipitation Buffer (N4)	20 mL	100 mL	
Wash Buffer (W9)	12 mL	60 mL	
Wash Buffer (W10)	12 mL	60 mL	
TE Buffer (TE)	30 mL	30 mL	

Table 1 Components of the PureLink Quick Plasmid Miniprep Kit *(continued)*

Component	Cat. No. K210010 (50 reactions)	Cat. No. K210011 (250 reactions)	Storage
PureLink™ Quick Spin Columns and Wash Tubes	1 pack	5 × 1 pack	15–25°C For better long-term performance store at 2°C to 8°C.
Elution Tubes	1 pack	5 × 1 pack	15–25°C

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Compatible vacuum manifold with vacuum source capable of –600 to –800 mbar (vacuum protocol only)	**MLS**
Microcentrifuge capable of ≥12,000 × *g* at room temperature	**MLS**
Ethanol, 100%	**MLS**
Isopropanol, 100%	**MLS**
(Optional) Qubit™ DNA Assay Kits	Q32853, Q32854

2 Methods

This manual provides protocols for plasmid DNA purification using centrifugation or vacuum manifolds.

Procedural guidelines

IMPORTANT! Ensure the bag containing the PureLink™ Quick Spin Columns is closed tightly after each use.

- Buffers contain hazardous reagents. Use caution when handling buffers.
- Warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter.
- If using a vacuum manifold, set up and attach the manifold to a vacuum source.
- Perform all centrifugation steps at room temperature using a microcentrifuge.
- Grow transformed E. coli in 1–5 mL LB medium overnight.

Before first use of the kit

Prepare resuspension buffer

1. Add RNase A to Resuspension Buffer (R3) according to the instructions on the label. Mix well.
2. Mark the bottle label after adding RNase A.

Store Buffer R3 with RNase A at 4°C for up to 6 months.

Prepare wash buffers

Add 96–100% ethanol to Wash Buffer (W9) and Wash Buffer (W10) according to instructions on each label. Mix well.

Store wash buffers with ethanol at room temperature.

Prepare bacterial culture

1. Centrifuge 1–5 mL of the overnight LB-culture. Remove all medium.

   Note: Use 1–2 × 10^9 E. coli cells for each sample.

2. Add 250 µL Resuspension Buffer (R3) with RNase A to the cell pellet, then resuspend the pellet until it is homogeneous.

Lyse cells

1. Add 250 µL Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is homogeneous.

   Note: Do not vortex.

2. Incubate the tube at room temperature for 5 minutes.

Precipitate plasmid DNA

1. Add 350 µL Precipitation Buffer (N4).

2. Mix immediately by inverting the tube, or for large pellets, vigorously shaking the tube, until the mixture is homogeneous.

   Note: Do not vortex.

3. Centrifuge the lysate at >12,000 × *g* for 10 minutes.

Purify plasmid DNA using a centrifuge

Follow this procedure to purify plasmid DNA using a centrifuge. Use a microcentrifuge capable of centrifuging at >12,000 × *g*. For processing a large number of samples simultaneously, see "Purify plasmid DNA using a vacuum manifold" on page 11.

Bind plasmid DNA

1. Load the supernatant from step 3 on page 8 onto a spin column in a 2 mL wash tube.
2. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the flowthrough, then place the column back into the wash tube.

(Optional) Wash plasmid DNA

Recommended for endA+ strains.

1. Add 500 µL Wash Buffer (W10) with ethanol to the column.
2. Incubate the column for 1 minute at room temperature.
3. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the flowthrough, then place column back into the wash tube.

Wash and remove ethanol

1. Add 700 µL Wash Buffer (W9) with ethanol to the column.
2. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the flowthrough, then place the column into the wash tube.
3. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the wash tube with the flowthrough.

Elute plasmid DNA

1. Place the Spin Column in a clean 1.5 mL elution tube.
2. Add 75 µL of TE Buffer (TE) to the center of the column.

   Note: *(Optional)* Preheat an aliquot of TE Buffer (TE) to 65–70°C for eluting DNA. Heating is optional for eluting 1–30 kb plasmid DNA but is recommended for eluting DNA >30 kb.

3. Incubate the column for 1 minute at room temperature.

Recover plasmid DNA

Centrifuge the column at 12,000 × g for 2 minutes. Discard the column.

Note: The elution tube contains the purified plasmid DNA.

Store plasmid DNA at 4°C (short term) or store the DNA in aliquots at –20°C (long term).

Purify plasmid DNA using a vacuum manifold

Follow this procedure to purify plasmid DNA using a vacuum manifold. Use a microcentrifuge, capable of centrifuging at >12,000 × *g*, a vacuum manifold, and a vacuum source. Follow the supplier's instructions to set up the vacuum manifold.

The vacuum manifold protocol starts after completing step 3 in "Precipitate plasmid DNA" on page 8.

Bind plasmid DNA

1. Remove the spin column from the wash tube (retain tube for later use), then attach the spin column to the luer extension.
2. Transfer the lysate from step 3 in "Precipitate plasmid DNA" on page 8. Apply vacuum.
3. After all of the supernatant has passed through the column, turn off the vacuum.

(Optional) Wash plasmid DNA

Recommended for endA+ strains.

1. Add 500 µL Wash Buffer (W10) with ethanol to the column.
2. Incubate the column for 1 minute at room temperature.
3. Apply vacuum. After all of the liquid has passed through the column, turn off the vacuum.

Wash plasmid DNA

1. Add 700 µL Wash Buffer (W9) with ethanol to the column.
2. Apply vacuum. After the liquid has passed through the column, turn off the vacuum.

Remove ethanol

1. Place the column into a 2 mL wash tube.
2. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the wash tube and flowthrough.

Elute plasmid DNA

1. Place the spin column in a clean 1.5 mL elution tube.
2. Add 75 µL of TE Buffer (TE) to the center of the column.

   Note: *(Optional)* Preheat an aliquot of TE Buffer (TE) to 65–70°C for eluting DNA. Heating is optional for eluting 1–30 kb plasmid DNA but is recommended for eluting DNA >30 kb.

3. Incubate the column for 1 minute at room temperature.

Recover plasmid DNA

Centrifuge the column at 12,000 × g for 2 minutes. Discard the column.

Note: The elution tube contains the purified plasmid DNA.

Store plasmid DNA at 4°C (short term) or store the DNA in aliquots at –20°C (long term).

Analyze DNA yield and quality

Measure DNA concentration

Measure DNA concentration using UV absorbance at 260 nm or Qubit™ DNA Assay Kits. The Qubit™ DNA Assay Kits provide a rapid, sensitive, and specific fluorescent method for measuring dsDNA concentration. The kits provide a state of the art quantitation reagent, DNA standards for standard curve, and pre-made buffer.

Measure DNA quality

Typically, DNA purified using the PureLink™ Quick Plasmid Miniprep Kit has an A_{260}/A_{280} of >1.80 when samples are diluted in Tris-HCl (pH 7.5), indicating that the DNA is reasonably clean of proteins that could interfere with downstream applications. Confirm absence of contaminating genomic DNA and RNA using agarose gel electrophoresis.

A Troubleshooting

Problem	Solution
Low plasmid DNA yield	• Use high copy number plasmids and appropriate growth conditions. For low copy number plasmids, increase the amount of culture and process as separate samples, if needed. • Carefully remove all media before resuspending the bacterial cell pellet. Ensure complete suspension of the pellet. • If the lysate is viscous, reduce the amount of cells used.
Denatured plasmid DNA	Do not incubate the lysate at room temperature for more than 5 minutes before adding Precipitation Buffer (N4). Denatured DNA appears as a band just above the supercoiled plasmid DNA. Restriction enzymes will not digest denatured DNA.
Contaminating Genomic DNA	Gently invert the tubes to mix the solution after adding Buffer L7. Do not exceed 5 minutes incubation before adding Precipitation Buffer (N4).
Contaminating RNA	Make sure that RNase A is added to Resuspension Buffer (R3). Store Buffer R3 with RNase A at 4°C for no longer than 6 months.
Enzymatic reactions are inhibited	Centrifuge the column to completely dry the column and remove any residual Wash Buffer (W9). Discard the flow-through.
Slow column flow (using vacuum)	Ensure that the vacuum manifold is attached to a vacuum source and that unused luer extensions are closed.

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.