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PureLink® Quick Plasmid Miniprep Kits

Catalog numbers K2100-10 and K2100-11

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Introduction

Use the PureLink® Quick Plasmid Miniprep Kit to isolate high quality plasmid DNA (up to 30 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 PureLink® Quick Plasmid Miniprep Kit can be used with a centrifuge or a vacuum manifold.

Contents

Component	Catalog number	
	K2100-10 50 reactions	K2100-11 250 reactions
Resuspension Buffer (R3; 50 mM Tris-HCl, pH 8.0; 10 mM EDTA)	13 mL	65 mL
RNase A (20 mg/mL in Resuspension Buffer R3)	100 µL	550 µL
Lysis Buffer (L7; 200 mM NaOH, 1% w/v SDS)	13 mL	65 mL
Precipitation Buffer (N4)	18 mL	90 mL
Wash Buffer (W9)	12 mL	55 mL
Wash Buffer (W10)	12 mL	56 mL
TE Buffer (10 mM Tris-HCl, pH 8.0; 0.1 mM EDTA)	15 mL	30 mL
Wash and recovery tubes	50 each	250 each
Spin columns	50 each	250 each

Before Starting

- Add RNase A to Resuspension Buffer (R3) according to the instructions on the label. Mix well. Mark the bottle label after adding RNase A. Store Buffer R3 with RNase A at 4°C.
- Warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter.
- 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.
- If you are using a vacuum manifold, set up and attach the manifold to a vacuum source.
- Grow transformed *E. coli* in 1–5 mL LB medium overnight.

Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

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Purification Procedure Using Centrifugation

Introduction

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 **Purification Procedure Using Vacuum**.

Notes

- Perform all centrifugation steps at room temperature using a microcentrifuge.
- *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.
- **Caution:** Buffers contain hazardous reagents. Use caution when handling buffers.

Isolate miniprep plasmid DNA

1. **Harvest.** Centrifuge 1–5 mL of the overnight LB-culture. (Use 1–2 × 10^9 *E. coli* cells for each sample.) Remove all medium.
2. **Resuspend.** Add 250 µL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the pellet until it is homogeneous.
3. **Lyse.** Add 250 µL Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is homogeneous. Do not vortex. Incubate the tube at room temperature for 5 minutes.
4. **Precipitate.** Add 350 µL Precipitation Buffer (N4). Mix immediately by inverting the tube, or for large pellets, vigorously shaking the tube, until the mixture is homogeneous. Do not vortex. Centrifuge the lysate at >12,000 × *g* for 10 minutes.
5. **Bind.** Load the supernatant from step 4 onto a spin column in a 2-mL wash tube. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the flow-through and place the column back into the wash tube.
6. ***Optional* Wash.** (Recommended for endA+ strains). Add 500 µL Wash Buffer (W10) with ethanol to the column. Incubate the column for 1 minute at room temperature. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the flow-through and place column back into the wash tube.
7. **Wash and remove ethanol.** Add 700 µL Wash Buffer (W9) with ethanol to the column. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the flow-through and place the column into the wash tube. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the wash tube with the flow-through.
8. **Elute.** Place the Spin Column in a clean 1.5-mL recovery tube. Add 75 µL of preheated TE Buffer (TE) to the center of the column. Incubate the column for 1 minute at room temperature.
9. **Recover.** Centrifuge the column at 12,000 × *g* for 2 minutes. *The recovery tube contains the purified plasmid DNA.* Discard the column. Store plasmid DNA at 4°C (short-term) or store the DNA in aliquots at –20°C (long-term).

Purification Procedure Using Vacuum

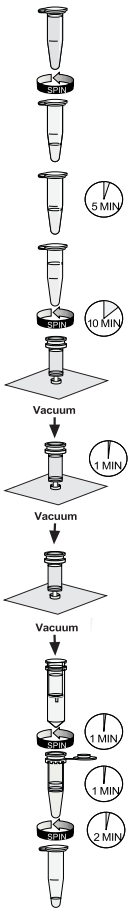
Introduction

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 your vacuum manifold.

Notes

- 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.
- **Caution:** Buffers contain hazardous reagents. Use caution when handling buffers.

Isolate miniprep plasmid DNA



1. **Harvest.** Centrifuge 1–5 mL of the overnight LB-culture. (Use 1–2 × 10^9 *E. coli* cells for each sample.) Remove all medium.
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4. **Precipitate.** Add 350 µL Precipitation Buffer (N4). Mix immediately by inverting the tube, or for large pellets, vigorously shaking the tube, until the mixture is homogeneous. Do not vortex. Centrifuge the lysate at >12,000 × *g* for 10 minutes.
5. **Bind.** Attach the spin column with the supernatant from step 4 to a luer extension of the vacuum manifold. Apply vacuum. After all of the supernatant has passed through the column, turn off the vacuum.
6. ***Optional* Wash.** (Recommended for endA+ strains). Add 500 µL Wash Buffer (W10) with ethanol to the column. Incubate the column for 1 minute at room temperature. Apply vacuum. After all of the liquid has passed through the column, turn off the vacuum.
7. **Wash.** Add 700 µL Wash Buffer (W9) with ethanol to the column. Apply vacuum. After the liquid has passed through the column, turn off the vacuum.
8. **Remove ethanol.** Place the column into a 2-mL wash tube. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the wash tube and flow-through.
9. **Elute.** Place the spin column in a clean 1.5-mL recovery tube. Add 75 µL of preheated TE Buffer (TE) to the center of the column. Incubate the column for 1 minute at room temperature.
10. **Recover.** Centrifuge the column at 12,000 × *g* for 2 minutes. *The recovery tube contains the purified plasmid DNA.* Discard the column. Store plasmid DNA at 4°C (short-term) or store the DNA in aliquots at –20°C (long-term).

DNA Analysis and Troubleshooting

Analyzing DNA yield and quality

Measure DNA concentration using UV absorbance at 260 nm or Qubit® DNA Assay Kits (Cat. nos. Q32853 and Q32854). 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.

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.

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.

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