

PureLink™ HiPure Plasmid DNA Purification Kits

Catalog numbers K2100-02, K2100-03, K2100-04, K2100-05, K2100-06, K2100-07

Publication Part Number 25-0788

MAN0003643

Revision A.0

Before starting

- Add RNase A to Resuspension Buffer (R3) according to the instructions on the label.
- Warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter. Do not shake bottle.
- Use the Nucleic Acid Purification Rack (Cat. no. K2100-13) for column purification steps.
- Grow transformed *E. coli* in LB medium. Use 1–3 mL (high copy number plasmid) or 10–15 mL (low copy number plasmid) of an overnight culture.

Steps		Miniprep Procedure Details
1	Equilibrate	1. Add 2 mL Equilibration Buffer (EQ1) to the HiPure Mini Column. Allow the solution to drain by gravity flow.
2	Harvest	2. Sediment cells by centrifugation at 4,000 × <i>g</i> for 10 min. Discard all medium.
3	Resuspend	3. Add 0.4 mL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the pellet until it is homogeneous.
4	Lyse	4. Add 0.4 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is homogeneous. Do not vortex. Incubate at room temperature for 5 minutes.
5	Precipitate	5. Add 0.4 mL Precipitation Buffer (N3). Mix immediately by inverting the tube until the mixture is homogeneous. Do not vortex. Centrifuge the lysate at >12,000 × <i>g</i> for 10 minutes at room temperature.
6	Bind	6. Load the supernatant onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
7	Wash	7. Add 2.5 mL Wash Buffer (W8) to the column. Discard the flow-through after Wash Buffer (W8) drains from the column. Repeat wash step once.
8	Elute	8. Place a sterile microcentrifuge tube under the column. Add 0.9 mL Elution Buffer (E4) to the column. Allow the solution to drain by gravity flow. The elution tube contains the purified DNA.
9	Precipitate and Wash	Add 0.63 mL isopropanol to the eluate. Mix well. Centrifuge the tube at >12,000 × <i>g</i> for 30 minutes at 4°C. Discard the supernatant. Wash the pellet in 1 mL 70% ethanol. Centrifuge the tube at >12,000 × <i>g</i> for 5 minutes at 4°C. Discard the supernatant.
10	Resuspend	9. Air-dry the pellet for 10 minutes, then resuspend the purified plasmid DNA in 50 µL TE Buffer (TE). Store plasmid DNA at –20°C.

Before starting

- Add RNase A to Resuspension Buffer (R3) according to the instructions on the label.
- Warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter. Do not shake bottle.
- Use the Nucleic Acid Purification Rack (Cat. no. K2100-13) for column purification steps.
- Grow transformed *E. coli* in LB medium. Use 15–25 mL (high copy number plasmid) or 25–100 mL (low copy number plasmid) of an overnight culture.

Note

- Use the PureLink™ HiPure Precipitator Module (Cat. no. K2100-21) for rapid precipitation of DNA within 10 minutes without using a centrifuge.
- Use the Nucleic Acid Purification Rack (Cat. no. K2100-13) or Column Holders (included) placed in the mouth of an Erlenmeyer flask (or similar) for column purification steps.

Steps		Midiprep Procedure Details
1	Equilibrate	1. Add 10 mL Equilibration Buffer (EQ1) to the HiPure Midi Column. Allow the solution to drain by gravity flow.
2	Harvest	2. Sediment cells by centrifugation at 4,000 × <i>g</i> for 10 min. Discard all medium.
3	Resuspend	3. Add 4 mL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the pellet until it is homogeneous.
4	Lyse	4. Add 4 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is homogeneous. Do not vortex. Incubate at room temperature for 5 minutes.
5	Precipitate	5. Add 4 mL Precipitation Buffer (N3). Mix immediately by inverting the tube until the mixture is homogeneous. Do not vortex. Centrifuge the lysate at >12,000 × <i>g</i> for 10 minutes at room temperature.
6	Bind	6. Load the supernatant onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
7	Wash	7. Add 10 mL Wash Buffer (W8) to the column. Discard the flow-through after Wash Buffer (W8) drains from the column. Repeat wash step once.
8	Elute	8. Place a sterile 15-mL centrifuge tube under the column. Add 5 mL Elution Buffer (E4) to the column. Allow the solution to drain by gravity flow. The elution tube contains the purified DNA.
9	Precipitate and Wash	Add 3.5 mL isopropanol to the eluate. Mix well. Centrifuge the tube at >12,000 × <i>g</i> for 30 minutes at 4°C. Discard the supernatant. Wash the pellet in 3 mL 70% ethanol. Centrifuge the tube at >12,000 × <i>g</i> for 5 minutes at 4°C. Discard the supernatant.
10	Resuspend	9. Air-dry the pellet for 10 minutes, then resuspend the purified plasmid DNA in 100–200 µL TE Buffer (TE). Store plasmid DNA at –20°C.

Before starting

- Add RNase A to Resuspension Buffer (R3) according to the instructions on the label.
- Warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter. Do not shake bottle.
- Use the Nucleic Acid Purification Rack (Cat. no. K2100-13) for column purification steps.
- Grow transformed *E. coli* in LB medium. Use 100 mL (high copy number plasmid) or 250–500 mL (low copy number plasmid) of an overnight culture.

Note

- Use the PureLink™ HiPure Precipitator Module (Cat. no. K2100-21) for rapid precipitation of DNA within 10 minutes without using a centrifuge.
- Use the Nucleic Acid Purification Rack (Cat. no. K2100-13) or Column Holders (included) placed in the mouth of an Erlenmeyer flask (or similar) for column purification steps.

Steps		Maxiprep Procedure Details
1	Equilibrate	1. Add 30 mL Equilibration Buffer (EQ1) to the HiPure Maxi Column. Allow the solution to drain by gravity flow.
2	Harvest	2. Sediment cells by centrifugation at 4,000 × <i>g</i> for 10 min. Discard all medium.
3	Resuspend	3. Add 10 mL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the pellet until it is homogeneous.
4	Lyse	4. Add 10 mL Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is homogeneous. Do not vortex. Incubate at room temperature for 5 minutes.
5	Precipitate	5. Add 10 mL Precipitation Buffer (N3). Mix immediately by inverting the tube until the mixture is homogeneous. Do not vortex. Centrifuge the lysate at >12,000 × <i>g</i> for 10 minutes at room temperature.
6	Bind	6. Load the supernatant onto the equilibrated column. Allow the solution in the column to drain by gravity flow.
7	Wash	7. Add 60 mL Wash Buffer (W8) to the column. Discard the flow-through after Wash Buffer (W8) drains from the column.
8	Elute	8. Place a sterile 50-mL centrifuge tube under the column. Add 15 mL Elution Buffer (E4) to the column. Allow the solution to drain by gravity flow. The elution tube contains the purified DNA.
9	Precipitate and Wash	9. Add 10.5 mL isopropanol to the eluate. Mix well. Centrifuge the tube at >12,000 × <i>g</i> for 30 minutes at 4°C. Discard the supernatant. Wash the pellet in 5 mL 70% ethanol. Centrifuge the tube at >12,000 × <i>g</i> for 5 minutes at 4°C. Discard the supernatant.
10	Resuspend	10. Air-dry the pellet for 10 minutes, then resuspend the purified plasmid DNA in 200–500 µL TE Buffer (TE). Store plasmid DNA at –20°C.

Troubleshooting

Problem	Solution
Viscous, non-adherent cell debris pellet	After centrifuging the lysate, allow the tube to sit for 5 minutes to separate the clear lysate from the pellet (the pellet may float). Carefully transfer the clear lysate to a clean tube and centrifuge the lysate at $>12,000 \times g$ for 5 minutes to remove any remaining debris.
Low plasmid DNA yield	<ul style="list-style-type: none">• Increase the volume of starting material.• Use the correct volume of Precipitation Buffer (N3).• Carefully remove isopropanol and ethanol without disturbing the DNA pellet during alcohol precipitation and washing steps. Do not use a vacuum system to dry the DNA pellet.• Store the Lysis Buffer (L7) and Elution Buffer (E4) at room temperature. Ensure that the rotor and centrifuge are at room temperature for the lysate centrifugation step.
Slow column flow	Avoid transferring any particulate matter onto the column. (Pipet the lysate onto the column. Do not pour the lysate onto the column.)
Genomic DNA contamination	Gently invert the tubes to mix the solution after adding Buffers L7 and N3, respectively. Do not vortex.
Plasmid DNA is degraded	Incubate the lysate, after the addition of Lysis Buffer (L7), at room temperature for no longer than 5 minutes.
Contaminating RNA	<ul style="list-style-type: none">• 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. After 6 months, add fresh RNase A to Buffer R3.• Carefully remove all media before resuspending cells. Avoid adding excess Precipitation Buffer (N3).• Make sure that the lysate does not warm above room temperature while centrifuging the lysate.• Perform column washing and elution steps without any delays.• Wash droplets of lysate from the column wall with Wash Buffer.

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5 June 2015

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