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PureLink® HiPure Plasmid DNA Purification Kits

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

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Miniprep procedure

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.
- 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.

Isolating Miniprep Plasmid DNA

1. **Equilibrate.** Apply 2 mL Equilibration Buffer (EQ1) to the HiPure Mini Column. Allow the solution in the column to drain by gravity flow.
2. **Harvest.** Sediment the cells by centrifuging the overnight LB culture at 4,000 × g for 5–10 minutes. Remove all medium.
3. **Resuspend.** Add 0.4 mL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the pellet until it is homogeneous.
4. **Lyse**. Add 0.4 mL Lysis Buffer (L7). Mix gently by inverting the capped tube five times. Do not vortex. Incubate the tube at room temperature for 5 minutes.
5. **Precipitate.** 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 × g for 10 minutes at room temperature.
6. **Bind.** Load the supernatant onto the equilibrated column with a pipette. Allow the solution in the column to drain by gravity flow.
7. **Wash.** Add 2.5 mL Wash Buffer (W8) **twice** to the column. Discard the flow-through after the Wash Buffer (W8) drains from the column each time.
8. **Elute.** 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 × g for 30 minutes at 4°C. Discard the supernatant. Add 1 mL 70% ethanol to the pellet. Centrifuge the tube at >12,000 × g for 5 minutes at 4°C. Remove the supernatant.
10. **Resuspend.** Air-dry the pellet for 10 minutes, then resuspend the purified DNA in 50 µL TE Buffer (TE). Store plasmid DNA at –20°C.

Intended Use

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

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Midiprep procedure

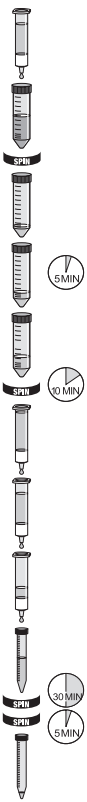
Before starting

- Add RNase A to the Resuspension Buffer (R3) according to the instructions on the label. Mix well. Indicate that RNase A has been added on the bottle label. Store the bottle at 4°C.
- If the Lysis Buffer (L7) contains salt precipitates, warm the buffer in a 37°C water bath until the solution clears. Do not shake the Lysis Buffer (L7).
- 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) to rapidly precipitate 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.

Isolating Midiprep Plasmid DNA



1. **Equilibrate.** Apply 10 mL Equilibration Buffer (EQ1) to the HiPure Midi Column. Allow the solution in the column to drain by gravity flow.
2. **Harvest.** Sediment the cells by centrifuging the overnight LB culture at 4,000 × g for 10 minutes. Remove all medium.
3. **Resuspend.** Add 4 mL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the pellet until it is homogeneous.
4. **Lyse.** Add 4 mL Lysis Buffer (L7). Mix gently by inverting the capped tube five times. Do not vortex. Incubate the tube at room temperature for 5 minutes.
5. **Precipitate.** 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 × g for 10 minutes at room temperature.
6. **Bind.** Load the supernatant onto the equilibrated column with a pipette. Allow the solution in the column to drain by gravity flow.
7. **Wash.** Add 10 mL Wash Buffer (W8) **twice** to the column. Discard the flow-through after the Wash Buffer (W8) drains from the column each time.
8. **Elute.** 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 × g for 30 minutes at 4°C. Discard the supernatant. Add 3 mL 70% ethanol to the pellet. Centrifuge the tube at >12,000 × g for 5 minutes at 4°C. Remove the supernatant.
10. **Resuspend.** 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.

Maxiprep procedure

Before starting

- Add RNase A to the Resuspension Buffer (R3) according to instructions on the label. Mix well. Indicate that RNase A has been added on the bottle label. Store at 4°C.
- If the Lysis Buffer (L7) contains salt precipitates, warm the buffer in a 37°C water bath until the solution clears. Do not shake the Lysis Buffer (L7).
- 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) to rapidly precipitate 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 all column purification steps.

Isolating Maxiprep Plasmid DNA

1. **Equilibrate.** Apply 30 mL Equilibration Buffer (EQ1) to the HiPure Maxi Column. Allow the solution in the column to drain by gravity flow.
2. **Harvest.** Sediment the cells by centrifuging the overnight LB culture at 4,000 × g for 10 minutes. Remove all medium.
3. **Resuspend.** Add 10 mL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the pellet until it is homogeneous.
4. **Lyse.** Add 10 mL Lysis Buffer (L7). Mix gently by inverting the capped tube five times. Do not vortex. Incubate the tube at room temperature for 5 minutes.
5. **Precipitate.** 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 × g for 10 minutes at room temperature.
6. **Bind.** Load the supernatant onto the equilibrated column with a pipette. Allow the solution in the column to drain by gravity flow.
7. **Wash.** Add 60 mL Wash Buffer (W8) to the column. Discard the flow-through after Wash Buffer (W8) drains from the column.
8. **Elute.** 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.** Add 10.5 mL isopropanol to the eluate. Mix well. Centrifuge the tube at >12,000 × g for 30 minutes at 4°C. Remove and discard the supernatant. Wash the DNA pellet in 5 mL 70% ethanol. Centrifuge the tube at >12,000 × g for 5 minutes at 4°C. Remove the supernatant.
10. **Resuspend.** 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 × g for 5 minutes to remove any remaining debris.
Low plasmid DNA yield	• 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	• 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.

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit www.invitrogen.com/support or email techsupport@invitrogen.com
www.lifetechnologies.com

life technologies™