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PureLink® HiPure Plasmid Filter DNA Purification Kits

Catalog numbers K2100-14, K2100-15, K2100-16, K2100-17, K2100-26, K2100-27
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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, if necessary.

Midiprep Procedure

Notes

- For all column steps, use Column Holders (included) or a Nucleic Acid Purification Rack.
- 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.

Isolate midiprep plasmid DNA

1. **Equilibrate.** Apply 15 mL Equilibration Buffer (EQ1) directly into the Filtration Cartridge, which is inserted in the PureLink® HiPure Midi Column. Allow the solution in the column to drain by gravity flow.
2. **Harvest.** Centrifuge the overnight LB culture at 4000 × g for 10 minutes in a 50-mL disposable centrifuge tube. Remove all medium.
3. **Resuspend.** Add 10 mL Resuspension Buffer (R3) with RNase A to the cell pellet and gently shake the pellet until the cell suspension is homogenous.
4. **Lyse**. Add 10 mL 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.
5. **Precipitate.** Add 10 mL Precipitation Buffer (N3). Mix by inverting the tube until the mixture is homogeneous.
6. **Clarify.** Transfer the precipitated lysate into the column. Allow the lysate to drain by gravity flow. *Optional:* Wash the column with 10 mL Wash Buffer (W8). Allow the buffer to flow through the column by gravity flow.
7. **Wash.** Discard the inner filtration cartridge. Add 20 mL Wash Buffer (W8) to the column. Discard the flow-through after Wash Buffer (W8) drains from the column.
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. Discard the column. *The elution tube contains the purified DNA.* Proceed to **Precipitate DNA.**

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Midiprep Procedure, Continued

Precipitate DNA

1. **Precipitate.** Add 3.5 mL isopropanol to the eluate. Incubate DNA with isopropanol for 2 minutes at room temperature. Centrifuge the tube at >12,000 × g for 30 minutes at 4°C.
2. **Wash.** 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.
3. **Resuspend.** Air-dry the pellet for 10 minutes. Resuspend the purified DNA in 100–200 µL TE Buffer (TE). Store plasmid DNA at –20°C.

Maxiprep Procedure

Notes

- For all column steps, use Column Holders (included) or a Nucleic Acid Purification Rack.
- Grow transformed *E. coli* in LB medium. Use 100–200 mL (high copy number plasmid) or 250–500 mL (low copy number plasmid) of an overnight culture.

Isolate maxiprep plasmid DNA

1. **Equilibrate.** Apply 30 mL Equilibration Buffer (EQ1) directly into the Filtration Cartridge, which is inserted in the PureLink® HiPure Maxi Column. Allow the solution in the column to drain by gravity flow.
2. **Harvest.** Centrifuge the overnight LB culture at 4000 × g for 10 minutes. Remove medium.
3. **Resuspend.** Add 10 mL Resuspension Buffer (R3) with RNase A to the cell pellet and gently shake the pellet until the cell suspension is homogenous.
4. **Lyse**. Add 10 mL 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.
5. **Precipitate.** Add 10 mL Precipitation Buffer (N3). Mix immediately by inverting the tube until the mixture is homogeneous. Do not vortex.
6. **Clarify.** Transfer the precipitated lysate into the column. Allow the lysate to filter through the column by gravity flow. *Optional:* Wash the column with 10 mL Wash Buffer (W8). Allow the buffer to flow through the column by gravity flow.
7. **Wash.** Discard the inner filtration cartridge. Wash the column with 50 mL Wash Buffer (W8). Discard the flow-through after the buffer drains.
8. **Elute.** Place a sterile 50-mL centrifuge tube under the HiPure Filter Column. Add 15 mL Elution Buffer (E4) to the column. Allow the solution to drain by gravity flow. Discard the column. *The elution tube contains the purified DNA.*

   Proceed to **Precipitate DNA using a Centrifuge** (for Cat. nos. K2100-16 and K2100-17) or **Precipitate DNA with a PureLink® HiPure Precipitator Module** (for Cat. nos. K2100-26 and K2100-27).

Precipitate DNA using a Centrifuge (for Cat. nos. K2100-16 and K2100-17)

1. **Precipitate.** Add 10.5 mL isopropanol to the eluate. Mix well. Centrifuge the tube at >12,000 × g for 30 minutes at 4°C. Discard the supernatant.
2. **Wash.** Add 5 mL 70% ethanol to the pellet. Centrifuge the tube at >12,000 × g for 5 minutes at 4°C. Remove the supernatant.
3. **Resuspend.** Air-dry the pellet for 10 minutes. Add 500 µL (for high copy number plasmids) or 200 µL (for low copy number plasmids) TE Buffer (TE) to the purified DNA. Store plasmid DNA at –20°C.

Precipitate DNA with a PureLink® HiPure Precipitator Module (for Cat. nos. K2100-26 and K2100-27)

Figure 1

Figure 2

Figure 3

1. **Precipitate.** Add 10.5 mL isopropanol to the eluate. Mix well. Incubate DNA with isopropanol for 2 minutes at room temperature.
2. **Prepare.** Remove the plunger from a 30-mL syringe (included) and attach the PureLink® HiPure Precipitator through the luer lock inlet to the syringe nozzle (see Figure 1).
3. **Load.** Add the precipitated DNA mixture to the syringe. Use a slow, constant force to push the plunger to pass the DNA mixture through the precipitator (see Figure 2). Discard the flow-through.
4. **Wash.** Detach the precipitator from the syringe, remove the plunger, then reattach the precipitator to the syringe. Add 5 mL 70% ethanol into the syringe and push the plunger. Detach the precipitator and remove the plunger.
5. **Remove ethanol.** Reattach the precipitator to the syringe. Insert the plunger into the syringe and push the plunger to pass air through the precipitator two times.
6. **Dry.** Blot any ethanol droplets on the precipitator nozzle with a paper towel. Detach the precipitator and discard the 30-mL syringe.
7. **Prepare.** Remove the plunger from a 5-mL syringe (included) and attach the precipitator to the 5-mL syringe.
8. **Elute.** Add 0.75–1.0 mL TE buffer to the 5-mL syringe. Insert the plunger, and place the precipitator over a clean, sterile microcentrifuge tube. Push the plunger to elute the plasmid DNA into the new tube (see Figure 3).
9. ***Optional* Elution:** Detach the precipitator, remove the plunger, and reattach the precipitator to the syringe nozzle. Load the entire volume of eluate from step 8 into the syringe. Place the precipitator nozzle over a new microcentrifuge tube and elute the DNA into the tube.
10. **Store.** Proceed to a downstream application, or store plasmid DNA at –20°C.

Troubleshooting

Problem	Solution
Low plasmid DNA yield	• Store Lysis Buffer (L7) and Equilibration Buffer (EQ1) at room temperature. Store Resuspension Buffer (R3) with RNase A at 4°C. • Increase the volume of starting material. Doubling the volumes of Resuspension Buffer (R3), Lysis Buffer (L7) and Precipitation Buffer (N3) may increase plasmid yield and quality. • Attach the precipitator to the syringe nozzle using the luer lock mechanism without applying excessive force. Before removing the plunger from the syringe, always remove the precipitator to avoid damaging the membrane. Do not apply excessive pressure while pushing the solution through the precipitator.
Contaminating Genomic DNA	Gently invert tubes to mix after adding Buffers L7 and N3, respectively. Do not vortex.
Precipitator is clogged (FP Maxiprep Kit)	• Load the eluate from **one** anion exchange column onto the precipitator. • Ethanol-precipitated DNA consists of fine particles that may clog the precipitator. Always use isopropanol to precipitate plasmid DNA.
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). If necessary, increase RNase A concentration to 400 µg/mL. • Carefully remove all media before resuspending cells. • Perform column washing and elution steps without any delays. • Wash droplets of lysate from the column wall with Wash Buffer (W8).
Bacterial lysate is slowly filtered with columns	• Reduce volume of culture used. • Remove precipitated cell debris from overgrown cultures by centrifuging the bacterial lysate at 12,000 × g for 5 minutes.
Enzymatic reactions are inhibited	Remove ethanol by air-drying as described in the protocol.

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