PureLink™ HQ Mini Plasmid Purification Kit

QUICK REFERENCE CARD

Catalog no. K2100-01

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Instructions are provided below for isolating high-quality plasmid DNA from bacterial cells using the PureLink™ HQ Mini Plasmid Purification Kit.

For detailed instructions, refer to the manual supplied with the kit or download the manual from www.invitrogen.com.

Preparing Lysate

Before Starting

Prepare the Resuspension Solution with RNase A. Resuspend the lyophilized RNase A (12 mg) in 200 µl of Resuspension Solution, and then add the resuspended mixture to the remaining Resuspension Solution for a final concentration of 0.1 mg/ml RNase A. After mixing, Resuspension Solution should be stored at +4°C.

Prepare the Wash Buffer with ethanol. Add 64 ml of 96–100% ethanol to the entire volume of Wash Buffer (16 ml).

Check the Neutralization Buffer and Lysis Buffer before use for salt precipitate. If present, place each buffer in a 37°C water bath for 5 minutes until the salts redissolve and the solution clears. Do not shake the Lysis Buffer, as this can lead to foaming.

Preparing Cell Lysate

To prepare the cell lysate:

1. In a microcentrifuge tube, pellet 1–3 ml (1–2 × 10^9) of *E. coli* cells from overnight cultures by centrifugation in a tabletop centrifuge at 1,500 × *g* for 15 minutes.
2. Completely resuspend the pellet in 240 µl of Resuspension Solution, prepared with RNase A as described above.
3. Add 240 µl of Lysis Buffer to the above solution. Mix gently by inverting the tube 4–8 times.
4. Incubate for 3–5 minutes at room temperature. Do not exceed 5 minutes.
5. Add 340 µl of Neutralization/Binding Buffer, and mix gently by inverting the tube 4–8 times.
6. Centrifuge for 10 minutes at maximum speed in a tabletop centrifuge to clarify the cell lysate.

Proceed to **Binding DNA.**

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See our website: **www.invitrogen.com**

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continued

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Isolating DNA

Binding DNA

1. Place a PureLink™ spin column inside a 2 ml collection tube.
2. Pipette or decant the supernatant from step 6, previous page, into the spin column.
3. Centrifuge the column at room temperature at 10,000–14,000 × *g* for 1 minute. Discard the flowthrough, and place the column back in the tube.

   If loading multiple samples on the same column (up to 1.5×10^{10} cells), repeat Steps 2–3 for each lysate.
4. Add 650 µl of Wash Buffer, prepared with ethanol as described on the previous page, to the column.
5. Centrifuge the column at room temperature at 10,000–14,000 × *g* for 1 minute. Discard the flowthrough from the collection tube, and place the column back in the tube.
6. Centrifuge the column at maximum speed for 1–3 minutes to remove the residual wash buffer.

Proceed to **Eluting DNA.**

Eluting DNA

1. Place the spin column in a clean 1.7 ml elution tube. Add Elution Buffer or sterile, distilled water as specified below
   - Add 50 µl of Elution Buffer or water to the center of the column if the expected DNA yield is <30 µg.
   - Add 100 µl of Elution Buffer or water to the center of the column if the expected DNA yield is >30 µg.
2. Incubate the column at room temperature for 1 minute, then centrifuge at maximum speed for 1 minute.
3. The elution tube contains your purified DNA. Remove and discard the column.

Trouble-shooting

Problem	Solution
Low yield	Check the growth conditions of the cell culture to ensure plasmid propagation. Use a high copy-number plasmid if possible. For low copy-number plasmids, increase the amount of cell culture used to 4–6 ml and process as three separate samples. If the cell lysate is too viscous, reduce the amount of cells used.
Plasmid DNA degradation	Avoid using endA+ plasmid strains if possible. If you are using an endA+ strain, we recommend adding an additional wash step before Step 4, Binding DNA, using a solution of 40% isopropanol, 4.0 M guanidine-HCl (ph 6.9–7.1).
Genomic DNA contamination in the eluate	Perform Steps 3–5, Preparing Cell Lysate, with gentle mixing to avoid shearing genomic DNA. Genomic DNA must be intact to be efficiently precipitated away from the plasmid DNA. Do not exceed 5 minutes of cell lysis (Step 4, Preparing Cell Lysate).

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