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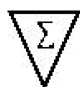
Huwel Plasmid Extraction Kit



HL-PLX-25 : 25 Exts
HL-PLX-50 : 50 Exts
HL-PLX-100 : 100 Exts

PI/HLPLX-00

Intended use

Huwel Plasmid Extraction Kits are intended for molecular biology applications. These kits are not intended for the diagnosis, prevention, or treatment of a disease.

Background Information

Huwel Plasmid Extraction Kits provided a simple, fast, and cost-effective plasmid DNA miniprep method for routine molecular biology applications. These kits provide high quality plasmid DNA eluted in a small volume of water or Tris buffer or TE buffer.

Kit components

Component	25 Exts	50 Exts	100 Exts	Storage
HPP Spin Columns with Collection Tubes	25 Nos.	50 Nos.	100 Nos.	RT
Buffer-1	7.5 mL	14 mL	28 mL	2–8°C
Buffer-2	7.5 mL	14 mL	28 mL	RT
Buffer-3	10.5 mL	20 mL	38 mL	RT
Wash Buffer-1	15 mL	28 mL	55 mL	RT
Wash Buffer-2	2 x 11 mL	39 mL	2 x 39 mL	RT
Elution Buffer	5 mL	10 mL	20 mL	RT
RNase A (20 mg/mL)	37.5 µL	70 µL	140 µL	2–8°C

Storage

Huwel Plasmid Extraction Kits can be stored at room temperature (15–25°C) or 2–8°C for long term storage, till the expiration date mentioned on the kit.

Notes before start

- To Buffer-1, add the entire RNase A solution provided, mix, and store at 2–8°C.
- Warm Buffer-2 briefly at 37°C to dissolve any particulate matter.

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Procedure

1. Pellet 1–5 mL overnight bacterial culture by centrifugation at 12,000 x g for 3 min at room temperature.

2. Resuspend the pellet in 250 µL **Buffer-1** and transfer to a microcentrifuge tube.

3. Add 250 µL **Buffer-2** and mix thoroughly by inverting the tube 4–6 times until the solution becomes clear. **Note:** Do not allow the lysis reaction to proceed for more than 5 min.

4. Add 350 µL **Buffer-3** and mix immediately and thoroughly by inverting the tube 4–6 times.

5. Centrifuge the tube for 10 min at >12,000 x g.

6. Carefully transfer the supernatant from step 5 to the HPP spin column-collection tube assembly by decanting or pipetting. Centrifuge at 12,000 x g for 1 min and discard the flow-through.

7. *(Optional: Recommended for endA+ strains)* Wash the HPP spin column by adding 0.5 mL **Wash buffer-1**. Centrifuge at 12,000 x g for 1 min and discard the flow-through.

8. Wash the HPP spin column by adding 0.7 mL **Wash buffer-2**. Centrifuge at 12,000 x g for 1 min and discard the flow-through. Transfer the HPP spin column to the collection tube.

9. Centrifuge at 12,000 x g for 1 min to remove residual wash buffer.

10. Place the HPP spin column in a clean 1.5 mL microcentrifuge tube. Add 50-100 µL **Elution Buffer** or water or TE buffer to the center of the HPP spin column, let stand for 1 min, and centrifuge at 12,000 x g for 1 min. *Optional: For better yields, preheat an aliquot of Elution buffer to 65-70°C before adding it to the spin column.*
    Store the eluent at -20°C.

Analysis of DNA quality and yield

Quality of DNA and absence of genomic DNA contamination can be visualized using agarose gel electrophoresis. Measure the eluted plasmid concentration using absorbance at 260 nm. Purity of the extracted plasmid can be assessed based on A_{260}/A_{280}. For the plasmids extracted using Huwel Plasmid Extraction Kits, A_{260}/A_{280} is typically ≥ 1.8 indicating that the DNA is devoid of protein contamination that could interfere with downstream application.

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Troubleshooting guide

Problem	Solution
Low plasmid DNA yield	Use high copy number plasmids and optimal bacterial growth conditions. For low copy number plasmids, increase the amount of culture and process as separate samples.
	Carefully remove media from cell pellet and ensure complete resuspension of the pellet.
	DNA is eluted only in the presence of low-salt buffer (e.g., Elution Buffer [10 mM Tris·Cl, pH 8.5] or TE Buffer or water. When using water for elution, make sure that the pH value is between pH 7.0 and 8.5. Store DNA at –30 to –15°C when eluted in water, because DNA may degrade in the absence of a buffering agent.
Denatured plasmid DNA	Do not allow the lysis reaction to proceed for more than 5 min. 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-2. Do not allow the lysis reaction to proceed for more than 5 min.
Contaminating RNA	Make sure to add RNase A to Buffer-1 and store the buffer with RNase A at 4°C for no longer than 6 months.
Inhibition of enzymatic reactions	After column wash with Wash Buffer-2, make sure to give additional 1 min spin to remove residual wash buffer and completely dry the spin column.