

Hieff™ Pro Endo-free Plasmid Maxi Kit

Product description

The Hieff™ Pro Endo-free Plasmid Maxi Kit enables rapid purification of 0.5–4 mg of high-copy plasmid DNA from 50–200 mL bacterial culture (100–200 mL LB medium; 50–100 mL 2YT medium), with recovery rates reaching 80–90%. The kit is characterized by high efficiency, speed, and ease of use. The spin column utilizes a proprietary silica-based matrix material developed in-house, combined with a unique lysis buffer formulation, to maximize recovery of high-purity DNA. The inclusion of a push filter simplifies operation. Purified DNA exhibits high purity and extremely low endotoxin levels (<0.01 EU/μg DNA), making it suitable for standard and sensitive cell transfections, as well as direct applications in restriction digestion, transformation, PCR, in vitro transcription, sequencing, and other molecular biology experiments.

Specifications

Cat NO.	19038ES02 / 19038ES10
Size	2 T / 10 T

Components

Part	Components No.	Name	19038ES02	19038ES10
Part I	19038-A	RNase A (10 mg/mL)	250 μL	1200 μL
Part II	19038-B	Hieff™ DNA Column E2	2	10
	19038-C	50-mL Collection Tube	2	10
	19038-D	Push Filter	2	10
	19038-E	Buffer BS (Balance Solution)*	10 mL	50 mL
	19038-F	Solution SI	25 mL	120 mL
	19038-G	Solution SII	25 mL	120 mL
	19038-H	Solution SIII	20 mL	100 mL
	19038-I	Solution ER (Endotoxin Removal Solution)	8 mL	40 mL
	19038-J	Solution BD (Binding Solution)*	1.6 mL	8 mL
	19038-K	Wash Solution (Concentrated)*	12 mL	48 mL
	19038-L	Elution Buffer (EB)	10 mL	30 mL
	19038-M	Blue Buffer	200 μL	600 μL

Storage

Part I: 19038-A (RNase A, 10 mg/mL), store at -15°C to -25°C. Stable for 18 months.

Part II: All other components, store at room temperature. Stable for 18 months

Notes

1. Buffer BS: Used to activate the silica membrane in the column and enhance plasmid binding efficiency.
2. Wash Solution (WS): Add 4 volumes of absolute ethanol before use. For 19038ES02, add 48 mL ethanol; for 19038ES10, add 192 mL ethanol. Mix thoroughly.

3. Binding Solution (BD): Add 25 volumes of isopropanol before use. For 19038ES02, add 40 mL isopropanol; for 19038ES10, add 200 mL isopropanol. Due to the viscosity of Solution BD and high density of isopropanol, mix vigorously to ensure homogeneity.
4. Excessive bacterial culture volume may reduce plasmid yield and quality. After adding Solution SII, invert and mix until the lysate becomes clear and transparent. If not, the culture volume may be too high.
5. After adding Solution SIII, mix thoroughly by inversion, applying more force if necessary. Incomplete mixing may result in weak or viscous pellets during centrifugation, making it difficult to separate the supernatant.
6. All centrifugation steps below are based on use of a fixed-angle rotor. Parameters for swing-bucket rotors are indicated in parentheses. Ensure liquid volume does not exceed the specified limit to avoid spillage, imbalance, and potential damage to the centrifuge.
7. This product is intended for research use only!

Instructions

Preparation Before Use

1. Confirm that Wash Solution (WS) has been mixed with ethanol at a 1:4 ratio. Confirm that Binding Solution (BD) has been mixed with isopropanol at a 1:25 ratio.
2. If Solution SII precipitates at low room temperature, heat at 37°C to dissolve, then cool to room temperature before use.
3. Use fresh overnight cultures (2YT medium recommended), or briefly frozen cell pellets stored at -20°C.

Procedure

I. Cell Harvesting, Lysis, and Clarification

1. Cell Harvesting: Pellet cells by centrifugation. Collect 50–200 mL culture (100–200 mL in LB; 50–100 mL in 2YT). Centrifuge at 6,000×g for 3 min (swing-bucket rotor: 4,000 rpm, 10 min). Discard supernatant.
2. Resuspension: Add 10 mL of Solution SI and vortex until no visible cell clumps remain. (Optional) Add 50 µL of Blue Buffer at this step to monitor cell lysis.
3. Lysis: Add 10 mL of Solution SII and gently invert 4–10 times to form a clear, viscous lysate (lysis time ≤5 min).
4. Neutralization and Clarification: Add 8 mL of Solution SIII and 100 µL of RNase A. Invert 8–10 times, incubate at room temperature for 10 min, then centrifuge at 10,000×g for 5 min (swing-bucket rotor: 4,000 rpm, 5 min).

[Note]: Adding RNase A immediately after Solution SIII significantly improves RNA degradation efficiency.

II. Endotoxin Removal

5. Filter the supernatant through the push filter into a 50 mL conical tube (yield: ~25–27 mL). Add 3 mL of Solution ER (Endotoxin Removal Solution) and invert to mix.
6. Optional: Incubate on ice/water bath for 3–5 min (can slightly improve endotoxin removal).
7. Incubate at 42°C for 3–5 min until the solution becomes cloudy and phase separation occurs. Centrifuge at 10,000×g for 5 min at room temperature (swing-bucket rotor: 4,000 rpm, 5 min). Let stand for 10–20 min to allow clear phase separation.
8. Carefully pour the upper phase into a new 50 mL centrifuge tube (yield: ~20 mL). Accurately add 40% volume of Solution BD (e.g., ~8 mL), mix well, and set aside.

III. Plasmid Purification

9. Column Equilibration: (Recommended during supernatant processing) Add 4 mL of Buffer BS to the Hieff™ DNA Column E2, let stand for 2 min, centrifuge at 6,000×g for 2 min, and discard the flow-through.
10. DNA Binding: Load the mixture from Step 8 onto the column in multiple aliquots (≤ 9 mL per load), centrifuging at 6,000×g for 2 min (swing-bucket rotor: ≤ 15 mL per load, 4,000 rpm, 1 min) to bind DNA to the column.
11. Washing: Wash once with 8 mL of Solution BD, followed by two washes with 8 mL of Wash Solution (WS) each. Centrifuge at 6,000×g for 2 min after each wash (swing-bucket rotor: 4,000 rpm, 1 min).
12. Drying: Centrifuge the empty column at 10,000×g for 5 min, then air-dry at room temperature for 5 min to completely remove residual ethanol (swing-bucket rotor: 4,000 rpm, 5 min, 50°C for 10 min).

IV. Elution of Plasmid DNA

13. Add 1.2 mL of Elution Buffer (EB), incubate at room temperature for 5 min, then centrifuge at 10,000×g for 3 min to collect the eluate.
(For swing-bucket rotor: Add 1.5 mL EB, incubate 5–10 min at room temperature, centrifuge at 4,000 rpm for 5 min. Re-elute by applying the eluate back to the membrane to improve recovery.)
14. Second Elution: If needed, perform a second elution as in Step 13 and collect the eluate separately for downstream use.