

Hieff™ Endo-free Plasmid Maxi Kit V2

Product description

The Hieff™ Endo-free Plasmid Maxi Kit V2 is designed for 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 a high recovery rate of 80–90%. The kit features high efficiency, speed, and convenience. The spin columns utilize a proprietary silica-based matrix developed by our company, which, in combination with our unique lysis buffer formulation, enables maximum recovery of high-purity DNA. The inclusion of push filters simplifies operation. The purified DNA exhibits high purity with low endotoxin levels (<1 EU/μg DNA), making it suitable for routine cell transfection, as well as direct use in restriction digestion, transformation, PCR, in vitro transcription, sequencing, and other molecular biology applications.

Specifications

Cat NO.	19037ES02 / 19037ES10 / 19037ES20
Size	2 T / 10 T / 20 T

Components

Category	Components No.	Name	19037ES02	19037ES10	19037ES20
Part I	19037-A	RNase A (10 mg/mL)	250 μL	1200 μL	1200 μL×2
Part II	19037-B	Binding Column E2 (Hieff™ DNA Column E2)	2	10	10×2
	19037-C	50 mL Collection Tube	2	10	10×2
	19037-D	Push Filter	2	10	10×2
	19037-E	Equilibration Buffer BS (Buffer BS)*	10 mL	50 mL	100 mL
	19037-F	Solution SI (Solution I)	25 mL	120 mL	240 mL
	19037-G	Solution SII (Solution II)	25 mL	120 mL	240 mL
	19037-H	Solution SIII (Solution III)	20 mL	100 mL	200 mL
	19037-I	Binding Solution PB (Solution PB)*	7 mL	32 mL	64 mL
	19037-J	Solution W1 (Solution W1)	20 mL	100 mL	200 mL
	19037-K	Wash Solution WS (concentrate)*	12 mL	48 mL	48 mL x 2
	19037-L	Elution Buffer EB (Elution Buffer)	10 mL	30 mL	60 mL
	19037-M	Blue Buffer	200 μL	600 μL	1200 μL

Storage

Part I: 19037-A RNase A (10 mg/mL), store at -15°C ~ -25°C, stable for 18 months.

Part II: Store at room temperature, stable for 18 months.

Notes

1. **Equilibration Buffer BS:** Used to activate the silica membrane in the binding column and enhance plasmid binding efficiency.
2. **Wash Solution WS:** Add 4 volumes of absolute ethanol before use. For 19037ES02, add 48 mL ethanol; for 19037ES10, add 192 mL ethanol. Mix thoroughly. 19037ES20 contains two bottles of Wash Solution (WS). Add 192 mL of absolute ethanol to each bottle and mix thoroughly.
3. **Binding Solution PB:** Add 3 volumes of isopropanol before use. For 19037ES02, add 21 mL isopropanol; for 19037ES10, add 96 mL isopropanol. For 19037ES20, add 192 mL of isopropanol to the Binding Buffer (PB). As PB is relatively viscous and isopropanol has a higher density, be sure to mix vigorously and thoroughly to ensure complete homogenization.
4. Excessive bacterial culture volume will reduce plasmid yield and quality. After adding Solution SII, invert and mix until the solution becomes clear and transparent. If not, the bacterial load is excessive.
5. After adding Solution SIII, mix thoroughly; shake more vigorously if necessary. Otherwise, the subsequent centrifugation may result in loose or gelatinous pellets, making it difficult to separate the supernatant.
6. The following protocols are based on fixed-angle rotors. Parameters for swinging-bucket rotors are indicated in parentheses. Ensure the solution volume does not exceed the tube capacity during centrifugation to avoid spillage, imbalance, and potential damage to the centrifuge. 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 and that Binding Solution PB has been mixed with isopropanol at a 1:3 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 the required amount of bacteria through multiple centrifugations. Transfer 50–200 mL culture (100–200 mL LB medium; 50–100 mL 2YT medium) and centrifuge at 6000×g for 3 min (swinging-bucket rotor: 4000 rpm, 10 min). Discard supernatant.
2. **Resuspension:** Add 10 mL Solution SI and vortex until no visible cell clumps remain.
(Optional) Add 50 µL Blue Buffer at this step to monitor cell lysis.
3. **Lysis:** Add 10 mL Solution SII and gently invert 4–10 times to mix, forming a clear, viscous lysate (lysis time ≤5 min).
4. **Neutralization and Clarification:** Add 8 mL Solution SIII and 100 µL RNase A, invert 8–10 times to mix, incubate at room temperature for 10 min, then centrifuge at 10000×g for 5 min (swinging-bucket rotor: 4000 rpm, 5 min).

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

5. Filter the supernatant through a push filter into a 50 mL conical tube (yielding approximately 25–27 mL supernatant). Add 40% volume of **Binding Solution PB** to the supernatant (e.g., 10 mL Binding Solution PB for 25 mL supernatant), mix well, and set aside.

[Note]: If prior small-scale plasmid prep indicates expected yield exceeds 2 mg, increase the proportion of Binding Solution PB to 45%.

II. Plasmid Purification

1. **Column Equilibration:** (Recommended during supernatant processing) Load **4 mL Equilibration Buffer BS** onto the Binding Column E2, let stand for 2 min, then centrifuge at 6000×g for 2 min. Discard flow-through.

2. **DNA Binding:** Load the mixture from Step 5 onto the Binding Column E2 in multiple centrifugation steps. Load ≤9 mL per spin, centrifuge at 6000×g for 2 min (swinging-bucket rotor: ≤15 mL per spin, 4000 rpm, 1 min).

3. **Washing:** Wash once with **8 mL Solution W1**, then twice with **8 mL Wash Solution WS**. Centrifuge at 6000×g for 2 min after each wash (swinging-bucket rotor: 4000 rpm, 1 min).

4. **Drying:** Centrifuge the empty column at 10000×g for 5 min, then air-dry at room temperature for 5 min to completely remove ethanol (swinging-bucket rotor: 4000 rpm, 5 min; place at 50°C for 10 min).

III. Plasmid Elution

1. Place the E2 column into a new 50 mL collection tube. Add **1.2 mL of Elution Buffer EB** and let it stand at room temperature for 5 min. Centrifuge at 10,000 ×g for 3 min to collect the eluate.

[Note]: When using a horizontal rotor: add 1.5 mL of EB, incubate at room temperature for 5–10 min, and centrifuge at 4,000 rpm for 5 min. Then, apply the eluate back onto the membrane and repeat the elution step to improve efficiency.

2. **Second Elution:** Depending on experimental needs, perform a second elution and collect the eluate separately for use.