

Hieff™ Endo-free Plasmid Midi Kit

Product description

The Hieff™ Endo-free Plasmid Midi Kit enables rapid purification of 100–500 µg of high-purity, high-copy plasmid DNA from 30–70 mL of *E. coli* LB (Luria-Bertani) culture. The spin column utilizes a proprietary silica-based matrix developed by our company, combined with a unique lysis buffer formulation, to maximize recovery of high-quality plasmid DNA. A specialized endotoxin-removal process ensures extremely low endotoxin levels (<0.1 EU/µg DNA), resulting in excellent performance in cell transfection. The purified plasmid is also directly suitable for a wide range of downstream molecular biology applications, including restriction digestion, transformation, PCR, in vitro transcription, sequencing, and more.

Specifications

Cat NO.	19023ES02 / 19023ES20
Size	2 T / 20 T

Components

Components No.		Name	19023ES02	19023ES20
Part I	19023-A	RNase A (10 mg/mL)	50 µL	500 µL
Part II	19023-B	Endotoxin Removal Solution ER (ER Solution E2)	1.8 mL	18 mL
	19023-C	RS Buffer* (RS Buffer E2*)	5 mL	50 mL
Part III	19023-D	AC Buffer (AC Buffer E2)	1 mL	10 mL
	19023-E	Hieff™ DNA Column E2	2 columns	20 columns
	19023-F	15 mL Collection Tube E2	2 tubes	20 tubes
	19023-G	Lysis Buffer LB (LB Buffer E2)	5 mL	50 mL
	19023-H	Binding Buffer BD (BD Buffer E2)	5 mL	50 mL
	19023-I	Protein Removal Buffer PL* (PL Buffer E2*)	4 mL	40 mL
	19023-J	Wash Buffer W*	2.5 mL	25 mL
	19023-K	Elution Buffer	2 mL	20 mL

Storage

Part I: Store at –25°C to –15°C

Part II: Store at 2–8°C

Part III: Store at room temperature

Valid life: 2 years

Notes

1. To prevent evaporation, oxidation, or pH shifts, keep all reagent bottles tightly closed after use.
2. At low ambient temperatures, SDS in Lysis Buffer LB may precipitate, causing cloudiness or visible particles. Warm the buffer at 37°C for several minutes until clear, then mix gently. Avoid vigorous shaking to prevent excessive foaming.

3. Plasmid yield depends on bacterial culture density and plasmid copy number. For low-copy plasmids or large plasmids (>10 kb), increase the amount of bacterial pellet and proportionally scale up RS Buffer*, Lysis Buffer LB, and Binding Buffer BD. Pre-warm the Elution Buffer to 70°C, and consider extending binding and elution incubation times to improve recovery.
4. Purified plasmid DNA can be assessed by agarose gel electrophoresis and UV spectrophotometry. An OD₂₆₀ of 1.0 ≈ 50 µg/mL DNA. Electrophoresis may show a single band or multiple bands—this reflects varying degrees of supercoiled conformation and is influenced by culture duration and handling intensity. Under standard operating conditions, >90% of the plasmid is typically in the supercoiled form.
5. The exact molecular size of plasmid DNA can only be determined after linearization by restriction enzyme digestion and comparison with a DNA molecular weight marker. Circular or supercoiled plasmids migrate unpredictably in gels and cannot be sized accurately by electrophoresis alone.
6. For your safety and health, wear a lab coat and disposable gloves during operation.
7. This product is for research use only.

Instructions

Pre-Experiment Preparation

1. User-supplied equipment and reagents: Benchtop centrifuge, vortex mixer or shaker, water bath or heat block, isopropanol, centrifuge tubes, etc.
2. Unless otherwise specified, all centrifugation steps are performed at room temperature using a benchtop centrifuge capable of 8,000 ×g (~9,000 rpm) with rotors compatible with ≥15 mL tubes.
3. Before first use, add the entire volume of RNase A (10 mg/mL, 19023-A) into the RS Buffer (19023-C)* bottle. Mix thoroughly, label clearly, and store at 4°C (stable for ~3 months). If RNase A activity declines over time, trace RNA contamination may occur; in such cases, supplement additional RNase A directly into the sample during resuspension.
4. Before first use, add the specified volume of absolute ethanol (as indicated on the label) to *Protein Removal Buffer PL (19023-I)**, mix well, and label. Before use, add 0.625 times the volume of anhydrous ethanol as indicated on the label:
 - For 19023ES02, add 2.5 mL of anhydrous ethanol.
 - For 19023ES20, add 25 mL of anhydrous ethanol.
5. Before first use, add 4 volumes of absolute ethanol to *Wash Buffer W (19023-J)**, mix thoroughly, and label. If the buffer volume appears inaccurate due to shipping or storage issues, use a graduated cylinder to adjust to the correct volume before adding ethanol. Always tightly cap the bottle after use to maintain ethanol concentration.

Procedure

I. Sample Pretreatment

1. Transfer 30–50 mL (maximum 75 mL) of overnight bacterial culture into a 50 mL centrifuge tube. Centrifuge at 8,000 ×g (About 9000 rpm) for 1 min, decant supernatant completely, and collect the bacterial pellet.

▲ If using 15 mL tubes, repeat centrifugation and supernatant removal in the same tube with additional culture until sufficient biomass is collected.
2. Resuspend the pellet in 2.3 mL RS Buffer* by pipetting or vortexing until fully homogeneous.

▲ Incomplete resuspension will impair lysis, reducing yield and purity.

3. Add 2.3 mL Lysis Buffer LB, gently invert the tube 6–8 times to lyse cells, and incubate at room temperature for 4–5 min.

▲ Mix gently—do not vortex or shake vigorously to avoid genomic DNA shearing! Total lysis time must not exceed 5 min to prevent plasmid damage. The lysate should become clear and viscous. Persistent turbidity suggests excessive biomass; reduce input in future preps.

4. Add 2.3 mL Binding Buffer BD, immediately invert 6–8 times to mix thoroughly. A white flocculent precipitate will form. Centrifuge at 8,000 $\times$ g for 10 min, then carefully transfer the supernatant to a new tube, avoiding the floating white precipitate.

▲ Mix immediately after adding BD Buffer to prevent localized SDS precipitation.

▲ Minor carryover of white precipitate does not affect results, as it will be removed during subsequent wash steps.

5. Column equilibration: Add 0.5 mL AC Buffer to the DNA column, centrifuge at 8,000 $\times$ g for 1 min, discard flow-through, and set aside.

▲ Equilibration enhances nucleic acid binding to the silica membrane. Use columns prepared on the same day.

6. Add 0.1 volume (10%) of Endotoxin Removal Solution ER (e.g., ~0.7 mL per 7 mL supernatant) to the cleared lysate from Step 4. Mix by gentle inversion.

7. Add 0.5 volume of isopropanol (e.g., ~3.3 mL) to the mixture from Step 6, mix thoroughly by inversion, and load the solution onto the equilibrated column in $\leq$ 3.5 mL aliquots. Centrifuge at 8,000 $\times$ g for 1 min after each load, discarding flow-through. Repeat until all solution has passed through the column.

▲ For centrifuges with steep rotor angles, limit each load to $\leq$ 3.5 mL to prevent leakage.

II. Plasmid DNA Purification

8. Add 3 mL Protein Removal Buffer PL (confirm ethanol has been added!), centrifuge at 8,000 $\times$ g for 1 min, discard flow-through.

9. Add 3 mL Wash Buffer W* (confirm ethanol has been added!), centrifuge at 8,000 $\times$ g for 1 min, discard flow-through. Repeat with another 3 mL of Wash Buffer W*.

10. Place the empty column back into its collection tube and centrifuge at 8,000 $\times$ g for 3 min to remove residual ethanol from the membrane.

▲ Residual ethanol inhibits downstream enzymatic reactions and reduces elution efficiency.

11. Transfer the column to a new 15 mL collection tube. Air-dry at room temperature for 2–3 min. Add 0.5 mL Elution Buffer (adjustable between 0.3–0.6 mL) directly onto the center of the membrane. Pre-warming the buffer to 65–75°C improves yield. Incubate at room temperature for 3 min, then centrifuge at 8,000 $\times$ g for 1 min to elute. Discard the column.

▲ For higher recovery, reapply the eluate to the column, incubate 1 min, and re-centrifuge. A second elution increases concentration by ~10%.

▲ Larger elution volumes improve recovery but dilute DNA. For higher concentration, reduce volume—but do not go below 0.3 mL, as this significantly reduces yield.

Appendix: Enhanced Endotoxin Removal Protocol

Recommended for transfection of sensitive primary cells or fragile cell lines to achieve optimal transfection efficiency.

1. After Step 6 above, place the ER-treated lysate on ice (or in a freezer) for 5 min until the solution becomes clear (or slightly cloudy). Invert once during incubation to mix.

▲ Initial cloudiness after ER addition is normal; clarity should return after chilling.

2. Return to room temperature for 3–5 min until the solution turns cloudy again. Mix by inversion.

▲ At low ambient temperatures, warming at 37–42°C accelerates cloudiness formation.

3. Centrifuge at 8,000 ×g for 10 min at room temperature to separate phases. The upper aqueous phase contains plasmid DNA; the lower blue oily phase contains endotoxins and impurities. Carefully transfer the upper phase to a new tube, avoiding the blue layer. Discard the oily phase.

4. Add 0.5 volume of isopropanol (~3.3 mL) to the recovered aqueous phase, mix thoroughly, and proceed with Step 7 (loading onto the column) and all subsequent steps.