

BAC/PAC Large Plasmid Extraction Kit

Cat. No.: B1521017 | Pack size: 20T | Storage: Room temperature; -20°C for designated component(s)

Product Introduction

This kit uses an alkaline lysis method to extract plasmid DNA from bacterial cultures. It employs unique solution formulations and an endotoxin removal reagent. Only a few simple centrifugation steps are required to remove impurities such as proteins, polysaccharides, endotoxins, and RNA, yielding high-quality plasmid DNA. The OD_{260/280} ratio of the purified DNA is typically around 1.8. The obtained plasmid can be directly used in applications requiring high DNA purity, such as cell transfection and even in vivo animal experiments. The later purification steps are carried out in Eppendorf tubes – the procedure is simple, requires no special equipment, no column purification, and no phenol/chloroform extraction. It recovers essentially all of the plasmid DNA released from bacterial lysis, so there is no need to worry about plasmid loss. This method causes minimal damage to plasmid DNA and can effectively purify even large plasmids (>10 kb) or very large BAC/PAC plasmids (>100 kb), provided they can be extracted by alkaline lysis.

Product Features

No need for toxic reagents such as phenol or chloroform. Fast and convenient: from 100–140 mL of *E. coli* LB culture, 0.2–0.5 mg of pure high-copy plasmid DNA can be rapidly extracted with an extraction efficiency of 80–90%.

High yield, high proportion of supercoiled form, and high purity. The obtained plasmid can be directly used in various molecular biology experiments, including restriction enzyme digestion, transformation, PCR, in vitro transcription, and sequencing.

Extremely low endotoxin level (<0.1 EU/μg DNA), suitable for direct use in cell transfection.

Kit Contents & Storage

Cat. No.	Component	20T	Storage
B1521017A	RNase A (powder)	1 EA	RT.
B1521017B	Solution P1	130 mL	RT.
B1521017C	Solution P2	100 mL	RT.

Cat. No.	Component	20T	Storage
B1521017D	Solution PIII	100 mL	RT.
B1521017E	Impurity Removal Solution A	3 mL	RT.
B1521017F	Impurity Removal Solution B	30 mL	RT.
B1521017G	Endotoxin Removal Reagent	10 mL	-20°C
B1521017H	DNA Dissolution Buffer	10 mL	RT.

Store each component at the indicated temperature. Storage for 18 months does not affect performance.

Precautions

For first-time use, add the entire vial of RNase A (powder) to Solution P1 (final concentration 100 µg/mL) and store at 4°C. If RNase A in Solution P1 becomes inactivated, trace RNA contamination may appear in the extracted plasmid – simply add additional RNase A to Solution P1.

The Endotoxin Removal Reagent can be stored at 4°C for one month. For long-term storage, it is recommended to store at -20°C.

When ambient temperature is low, SDS in Solution P2 may precipitate, causing cloudiness or sediment. Warm the solution in a 37°C water bath for a few minutes to restore clarity. Do not shake vigorously to avoid excessive foaming.

The amount of plasmid extracted depends on factors such as bacterial culture concentration and plasmid copy number. For low-copy plasmids or large plasmids (>10 kb), increase the amount of bacterial culture and proportionally increase the volumes of Solutions P1, P2, and PIII.

When extracting large plasmids, handle gently and use pipette tips with widened openings to avoid mechanical shearing damage to the DNA.

After centrifugation of the DNA precipitation solution, a visible pellet may not be observed. If no pellet is seen and you are concerned about DNA loss, retain the supernatant until the entire procedure is completed, then verify by electrophoresis whether the final product has been obtained (hundreds of micrograms of DNA may pellet on the side wall of the tube and may not be visible as a distinct clump).

The concentration and purity of the extracted plasmid DNA can be assessed by agarose gel electrophoresis and UV spectrophotometry. An OD260 of 1 corresponds to approximately 50 µg/mL DNA. Electrophoresis may reveal a single band, or two or more bands, which is primarily due to different migration positions of various supercoiled plasmid conformations and depends on factors

such as culture duration and the vigor of operation. Under normal operating conditions with this product, the proportion of supercoiled plasmid typically exceeds 95%.

For research use only. Not for clinical diagnosis or other uses.

Materials Required but Not Provided

Isopropanol, 70% ethanol.

Procedure

Note: Pour the RNase A (powder) into Solution P1, mix well, and store at 2–8°C after use.

Transfer overnight bacterial culture (<140 mL) to a suitable centrifuge bottle. Centrifuge at 4,500–6,000×g for 10 min at 4°C to pellet the cells. Remove the supernatant completely.

Add 5 mL of Solution P1. Vigorously vortex or shake to completely resuspend the cell pellet until no clumps remain. Transfer the bacterial suspension to a 50 mL centrifuge tube. Allow to stand at room temperature for 3–5 min.

Add 5 mL of Solution P2. Gently invert the tube 6–8 times and let stand at room temperature for 5 min to completely lyse the bacteria. The solution should become clear.

Add 5 mL of Solution PIII. Immediately invert the tube gently 6–8 times to mix thoroughly until a white flocculent precipitate forms. Place on ice for 5–10 min. Centrifuge the lysate at 12,000–16,000×g for 15 min at 4°C. Carefully aspirate the supernatant and transfer it to a new 50 mL centrifuge tube.

Add 10 mL of isopropanol. Invert the tube to mix thoroughly (optional: let stand at room temperature for 10 min).

Centrifuge at 12,000–16,000×g for 10 min at 4°C. Carefully discard the supernatant, invert the tube to drain residual liquid, add 3–5 mL of 70% ethanol to wash the pellet once. Centrifuge at maximum speed for 5 min, discard the supernatant, and air-dry the pellet.

Add 1.4 mL of Solution P1 to completely dissolve the pellet (gently pipette with a wide-bore tip to aid dissolution). Transfer to a new 1.5 mL centrifuge tube. Place in a 60°C water bath for 10–20 min.

Centrifuge at maximum speed for 2 min. Transfer the supernatant into two new 1.5 mL centrifuge tubes (700 µL each). Add 55 µL of Impurity Removal Solution A to each tube, invert to mix thoroughly.

Add approximately 0.1 volume (about 80 µL) of ice-cold Endotoxin Removal Reagent. Invert and rotate 7–10 times (about 30 seconds) to mix thoroughly. Place on ice or in an ice bath for ≥10 min, occasionally inverting to mix. After adding the Endotoxin Removal Reagent, the supernatant will become turbid, but should become clear again after ice-incubation.

Place in a 37°C water bath; the solution will become turbid again. Mix by inversion and incubate at 37°C for 5 min.

Centrifuge at room temperature at 16,000×g for 5–10 min to separate phases (if the temperature is low, the Endotoxin Removal Reagent will not separate phases; therefore, centrifugation must be performed at room temperature above 20°C).

The solution should separate into two phases: the upper aqueous phase contains DNA, and the lower oily phase contains endotoxins and other impurities. Transfer the upper aqueous phase (containing DNA) to a new tube (be careful not to aspirate the oily layer). Discard the oily layer.

Optional step: repeat the extraction 1–2 times according to the desired endotoxin level (i.e., repeat steps 9–12). This will increase purity but will result in some loss of plasmid DNA; choose whether to perform this step based on your specific needs.

Add an equal volume of Impurity Removal Solution B (approximately 750 µL) to the upper aqueous phase obtained in the previous step. Mix gently and place on ice for 10–30 min. Centrifuge at 16,000×g for 10 min at 4°C. Discard the supernatant (be careful not to lose the DNA pellet). Gently add 1 mL of 70% ethanol to wash. Centrifuge, discard the supernatant, and invert the tube to air-dry for 5–10 min until the ethanol has completely evaporated.

Add an appropriate volume of DNA Dissolution Buffer (200–500 µL) to dissolve the pellet (incubate in a 37°C water bath with shaking to aid dissolution if desired). Note that much of the plasmid DNA may adhere to the wall of the centrifuge tube; even if not visible, be sure to pipette vigorously against the wall to dissolve and recover the plasmid DNA.

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