Instruction for Use

SteadyPure Endo-free Plasmid DNA Extraction Kit

AG 21028
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product can extract various endotoxin-free plasmids (Plasmid, Cosmid, etc.) from bacterial culture. Utilizing an optimized alkaline lysis method combined with anion exchange resin effectively removes endotoxins. Subsequently, desalting and concentration are achieved through isopropanol precipitation, followed by dissolution in Endo-free TE Buffer or Endo-free water, resulting in high-yield, high-purity plasmid DNA. Various purification methods (gravity flow, vacuum, push-column) can be employed to obtain plasmid DNA, all of which effectively reduce endotoxin levels (< 0.1 EU/μg). The purified plasmid DNA can be directly utilized in molecular biology experiments such as transfection, in vitro transcription translation, and enzyme modification.

2. Kit Information

Kit Name	Cat. No	Specification
SteadyPure Endo-free Plasmid DNA Extraction Kit	AG 21028	10 rxns

3. Transportation and Storage

	Package 2-1	Package 2-2
Storage	Store at -20°C	Store at Room Temperature
Transportation	Transport at -20°C	Transport at Room Temperature

4. Kit Components

Package 2-1 (-20 °C Storage)

RNase A (10 mg/ml)	1.2 ml

Package 2-2 (Room Temperature Storage)

Buffer PEL	200 ml
Buffer PRS	120 ml
Buffer PLS	100 ml
Buffer PBS	100 ml
Buffer PWB	300 ml
Endo-free Elution Buffer	100 ml
Endo-free water for 70% ethanol	18 ml
Endo-free TE Buffe	10 ml
Column Filter	10 pcs
Plasmid DNA Midi Columns	10 pcs
RNase Free Tubes	10 pcs

*1: Before first use, RNase A must be added to Buffer PRS at a volume ratio of 1:100 (RNase A : Buffer PRS). Mix thoroughly and mark the bottle accordingly. Buffer PRS supplemented with RNase A should be stored at 2-8 °C and is stable for up to 6 months.

*2: Before first use, 42 ml of absolute ethanol (user-supplied) should be added to Endo-free water for 70% ethanol to prepare 70% ethanol for use.

*3: Plasmid DNA Midi Columns are labeled as "Plasmid Maxi Column" on the actual product.

5. Experimental Preparation

1) Prepare the following items: Endo-free water, pre-chilled isopropanol, pyrogen-free and endotoxin-free centrifuge tubes, pipettes, pipette tips, etc.
2) If vacuum-based purification will be used in subsequent steps, please prepare the vacuum manifold/system in advance.
3) When stored at low temperatures, Buffer PLS and Buffer PBS may form precipitates. Before use, warm at 37 °C until the precipitate is completely dissolved, then proceed.

4) Do not vortex Buffer PLS vigorously, as this may generate bubbles.
5) For elution of plasmid DNA bound to Plasmid DNA Midi Columns, preheat the Endo-free Elution Buffer to 50-65 °C to improve DNA elution efficiency.
6) Before first use, RNase A must be added to Buffer PRS at a volume ratio of 1:100 (RNase A : Buffer PRS). Mix thoroughly and label the bottle. Buffer PRS supplemented with RNase A should be stored at 2-8 °C and is stable for up to 6 months.
7) Before first use, add 42 ml of user-supplied absolute ethanol to Endo-free water for 70% ethanol to prepare 70% ethanol for use.

6. Recommendation and Experimental Precautions

1) Plasmid copy number and yield

When using the same amount of bacterial biomass, differences in plasmid copy number (high-copy vs. low-copy plasmids) will result in different plasmid DNA yields after purification.

- ◆ In general, for high-copy plasmids (e.g., pUC19), it is recommended to use 100–200 ml of overnight culture, which typically yields 200–400 µg of high-purity plasmid DNA.
- ◆ For low-copy plasmids (e.g., pBR322), it is recommended to use 100–300 ml of overnight culture, yielding approximately 50–150 µg of high-purity plasmid DNA.

2) Effect of host strain on plasmid quality

The bacterial strain used for plasmid preparation has a significant impact on the quality of purified plasmid DNA. Host strains such as DH1, DH5α, and C600 produce high-quality plasmid DNA when used with this product.

Strains from the HB101, TG1, and JM100 series contain large amounts of secondary metabolites (e.g., carbohydrates), which may interfere with downstream applications such as restriction digestion and ligation. In addition, some strains (e.g., JM101, JM110, and HB101) exhibit high endonuclease activity, which can reduce plasmid yield and decrease the proportion of supercoiled plasmid DNA. If the purity or integrity of the purified plasmid DNA does not meet expectations, switching to a different host strain is recommended.

3) Bacterial growth state

The growth state of the bacteria significantly affects the purity, yield, and integrity of purified plasmid DNA. We recommend harvesting cells during the logarithmic growth phase, typically after 12–16 hours of cultivation. For bacterial cells stored long-term at low temperatures, it is recommended to re-streak on agar plates and select fresh colonies for liquid culture before plasmid preparation.

4) Sample loading limits

This product allows plasmid DNA purification from bacterial cells with a total loading of OD600 ≤ 600 (for example, 300 ml culture at an OD600 of 2.0). The sample input should not exceed the recommended maximum starting amount (OD600 ≤ 600). Excessive biomass may impair cell lysis and plasmid release, resulting in reduced plasmid purity.

If the starting material exceeds OD600 > 600, increase the volumes of Buffer PRS, Buffer PLS, and Buffer PBS proportionally. After lysis, transfer the entire supernatant to the Plasmid DNA Midi Columns. The maximum capacity of a Plasmid DNA Midi Column is 30 ml. If the sample volume exceeds this limit, load the sample in multiple rounds (load 30 ml supernatant, allow it to pass through, then load the remaining sample), or use multiple Plasmid DNA Midi Columns for purification.

5) Column Filter usage

The supplied Column Filter, which resembles a syringe, removes impurities by pushing liquid through the filter (see Figure A). After resuspension, lysis, neutralization, and centrifugation, most white precipitates are removed; however, small amounts of white flocculent material may remain in the supernatant and can be removed using the Column Filter.

6) Flow rate variation

The packing density of the resin in the supplied Plasmid DNA Midi Columns (anion-exchange columns) may vary slightly,

resulting in differences in flow rate during purification. This variation does not affect experimental results.

7) Purification methods

This product supports multiple purification methods, including gravity flow, vacuum, and pressure-driven (plunger) methods.

① Gravity method:

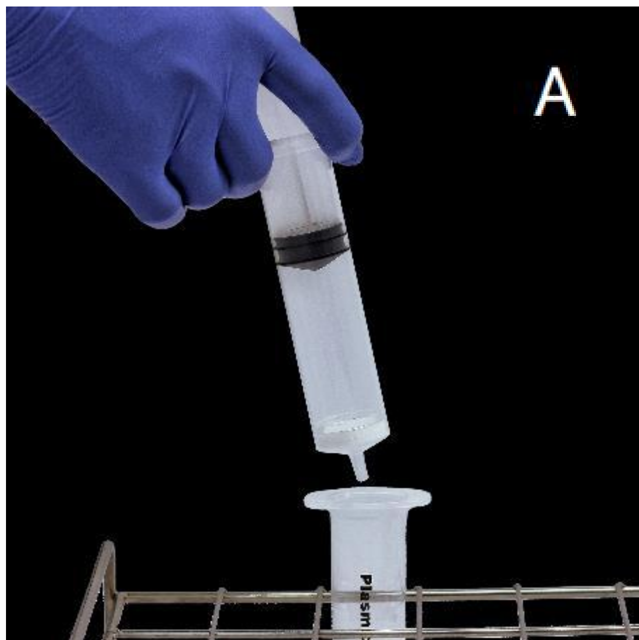
It is recommended to use a double-layer tube rack to separate the Plasmid DNA Midi Columns from the waste collection tubes to minimize contamination. If only a single-layer rack is available, ensure that the Plasmid DNA Midi Columns do not come into contact with the waste liquid. Typical flow rates are approximately 1 min/ml.

② Vacuum method:

Plasmid DNA Midi Columns are compatible with various vacuum manifolds; compatibility should be confirmed before use. Due to differences in vacuum system performance, filtration speeds may vary even at the same vacuum pressure. A flow rate of 1–3 s/ml is generally recommended.

③ Pressure-driven (plunger) method:

The plunger of the Column Filter can be used to push liquid through the Plasmid DNA Midi Columns. Do not insert the plunger too deeply, as pulling it out may introduce air bubbles into the anion-exchange resin and reduce binding capacity. Insert the plunger only slightly (e.g., until the black rubber tip just enters the barrel, as shown in Figure B), and apply repeated gentle pushes to achieve a controlled flow rate, typically 10–30 s/ml.



8) Prevention of endotoxin contamination

During operation, endotoxin contamination must be prevented. Please note the following:

① The working environment should be kept free from bacterial contamination.

② All centrifuge tubes (except waste collection tubes), pipettes, and pipette tips used during the procedure must be pyrogen-free and endotoxin-free. Glassware should be treated at 180 °C overnight before use.

9) Elution and long-term storage

Plasmid DNA may be eluted using Endo-free TE Buffer or Endo-free water (to be provided by the user). For long-term storage of purified plasmid DNA, it is recommended to store the DNA in Endo-free TE Buffer.

10) Storage of purified plasmid DNA

Purified plasmid DNA may be stored at 4 °C for short-term use. For long-term storage, keep the DNA at –20 °C.

7. Protocol

Column Equilibration

1) Add 20 ml of Buffer PEL to the Plasmid DNA Midi Columns, and allow the solution to flow through the column by gravity.

Bacterial Cell Harvesting

2) Collect 100–300 mL of overnight bacterial culture into 50 mL centrifuge tubes.
Centrifuge at 8,000 rpm for 10 min at room temperature, then discard the supernatant.

Note: ① If the culture volume exceeds 50 mL, collect the culture in multiple rounds into the same 50 mL centrifuge tube: add up to 50 mL culture, centrifuge, discard the supernatant, then add additional culture and repeat this step; alternatively, use centrifuge tubes with a larger capacity.
② For high-copy-number plasmids, it is recommended to collect 100–200 mL of overnight culture.
For low-copy-number plasmids, it is recommended to collect 100–300 mL of overnight culture.

Resuspension, Lysis, and Neutralization

3) Add 10 mL of Buffer PRS (containing RNase A) to the centrifuge tube containing the harvested bacterial pellet.
Fully resuspend the pellet by vortexing or repeated pipetting until no visible cell clumps remain.
Note: Ensure that an appropriate volume of RNase A has been added to Buffer PRS (RNase A : Buffer PRS = 1:100, v/v).

4) Add 10 mL of Buffer PLS to the resuspended cells. Gently invert the tube 6–8 times until the solution becomes clear and viscous.
Note: Mix gently. Do not vortex or mix vigorously, as harsh mixing may release genomic DNA and contaminate the plasmid DNA.

5) Add 10 mL of Buffer PBS to the lysate. A white precipitate will form. Gently invert the tube 6–8 times to mix thoroughly.
Note: ① The total duration of Steps 4 and 5 should not exceed 5 min.
② Mix gently. Do not mix vigorously, as harsh mixing may release genomic DNA and contaminate the plasmid DNA.

6) Incubate the lysate at room temperature for 2 min, then centrifuge at 12,000 rpm for 15 min at room temperature.

Optional Step

If further clarification of the alkaline lysate supernatant is required, this step may be performed.
If additional clarification is not necessary, this step can be skipped and the protocol may proceed directly to <Procedure – Binding, Washing, and Elution>.

7) Transfer the centrifuged supernatant to a Column Filter. Slowly push the plunger to filter the lysate, and collect the filtrate in a clean 50 mL centrifuge tube.
Note: ① When transferring the supernatant, handle gently and avoid disturbing the pellet.
② If a pressure-based method is used in the subsequent <Binding, Washing, and Elution> steps, the Column Filter plunger may be retained and used to push the solution through the Plasmid DNA Midi Columns.

Binding, Washing, and Elution

(Three methods available; choose one)

Method A: Gravity Flow

8) Transfer the clarified supernatant/filtrate to the equilibrated Plasmid DNA Midi Columns. Allow the solution to pass through the column by gravity flow, and discard the flow-through.
9) After the solution has completely passed through, add 30 mL Buffer PWB to the column. Allow the buffer to flow through by gravity, and discard the flow-through.
10) After the buffer has completely passed through, place the Plasmid DNA Midi Columns onto a new endotoxin-free, pyrogen-free 50 mL centrifuge tube. Add 10 mL Endo-free Elution Buffer to elute the plasmid DNA. Allow the eluate to pass through by gravity and collect the eluate.

Method B: Vacuum Manifold

8) Transfer the clarified supernatant/filtrate to the equilibrated Plasmid DNA Midi Columns.
Mount the columns properly onto a vacuum manifold, open the vacuum valve, and allow the solution to pass through under vacuum. Discard the flow-through.
Note: The recommended flow rate under vacuum is 1–3 s/mL.
9) After the solution has completely passed through, add 30 mL Buffer PWB to the column. Allow the buffer to pass through under vacuum, and discard the flow-through.
10) After the buffer has completely passed through, place the Plasmid DNA Midi Columns onto a new endotoxin-free, pyrogen-free 50 mL centrifuge tube. Add 10 mL Endo-free Elution Buffer to elute the plasmid DNA. Allow the eluate to pass through under vacuum and collect the eluate.

Method C: Pressure (Plunger) Method

8) Transfer the clarified supernatant/filtrate to the equilibrated Plasmid DNA Midi Columns. Use the plunger from the Column Filter to gently push the solution through the column. Discard the flow-through.
Note: The recommended flow rate for the pressure method is 10–30 s/mL.
9) After the solution has completely passed through, remove the plunger. Add 30 mL Buffer PWB to the column, reinsert the plunger, and push the buffer through the column. Discard the flow-through.
10) After the buffer has completely passed through, remove the plunger and place the Plasmid DNA Midi Columns onto a new endotoxin-free, pyrogen-free 50 mL centrifuge tube. Add 10 mL Endo-free Elution Buffer to elute the plasmid DNA. Push the solution through the column using the plunger and collect the eluate.

Precipitation, Washing, and Dissolution

11) Add 7 mL of isopropanol (pre-chilled on ice) to the 50 mL centrifuge tube containing the eluate. Mix thoroughly by gentle inversion, then centrifuge at 12,000 rpm for 20 min at 4°C. Carefully discard the supernatant.
Note: ① When removing the supernatant, handle gently and avoid disturbing the plasmid DNA pellet to prevent pellet loss and reduced DNA yield.
② If the plasmid DNA pellet is not compact after centrifugation, the centrifugation time may be extended as needed.
12) Add 5 mL of 70% ethanol to wash the plasmid DNA pellet. Centrifuge at 12,000 rpm for 5 min at 4°C, then carefully remove the supernatant.
Note: ① Prepare 70% ethanol by adding 42 mL of absolute ethanol to the Endo-free Water for 70% Ethanol provided in this kit.
② Add 70% ethanol gently to avoid dislodging the pellet.
③ When removing the supernatant, handle gently and avoid disturbing the plasmid DNA pellet to prevent pellet loss and reduced DNA yield.
④ If the plasmid DNA pellet is not compact after centrifugation, the centrifugation time may be extended as needed.
13) Air-dry the plasmid DNA pellet at room temperature until the surface ethanol has completely evaporated (approximately 10 min) to remove residual ethanol.
Note: ① To shorten the drying time, briefly centrifuge the tube and carefully remove residual ethanol with a pipette, then air-dry at room temperature for 2–5 min.
② Do not over-dry the pellet, as excessive drying may make the plasmid DNA difficult to dissolve.
③ Remove residual liquid as thoroughly as possible; residual droplets on the tube wall or rim may be carefully removed with a pipette.
14) Add 200–1000 μL of Endo-free TE Buffer or Endo-free Water to dissolve and resuspend the plasmid DNA. After the pellet has completely dissolved, transfer the solution to RNase-free tubes. Store the purified plasmid DNA at –20°C until use.
Note: ① For plasmid DNA intended for DNA sequencing, it is recommended to elute the DNA with Endo-free Water.
② The volume of Endo-free TE Buffer or Endo-free Water may be adjusted according to pellet size.
For smaller pellets, reducing the elution volume can help increase plasmid DNA concentration.

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