Genorise

Cat.NO. GERC-101079-500

Store the kit at 15 to 25℃

Genorise

Genorise Pure Plasmid Mini Kit 500

For purification of molecular biology grade DNA

Plasmid

Large plasmids (>10 kb)

Low-copy plasmids and cosmids

For Research Use only

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Plasmid DNA Purification （*e.g.*, PUC-19）

Prepare sample	Centrifuge 0.5-4 ml *E. coli* culture
1 Resuspend pellet	 250 µL Buffer G1 + RNase A
2 Lyse sample	 250 µL Buffer G2
3 Neutralization	 350µL Buffer G3 10,000 X *G* 10 Min
4 Pre-equilibrate theColumn 5 Bind DNA	 250µL Buffer GL 【*Optional steps*】 10,000 X *G*  1 Min
6 Wash silica membrane 7 Wash silica membrane 8 Wash silica membrane	 1 wash 500ul GW1 2 Wash 500ul GW2 700ul GW2  10,000 X *G*，1 Min
9 Dry silica membrane	 10,000 X *G* 2 Min
10 Elute highly pure DNA	 60 µL GE(70°C) RT 1 Min 10,000 X *G* 1 Min

Kit Contents

Genorise Pure Plasmid Mini Kit	500
Cat No.	GERC-101079-500
DNA binding columns	500
Colletion Tubes	500
Buffer GL 【*Optional steps*】	125ml
Buffer G1	125ml
Buffer G2	125ml
Buffer G3	100ml×2
Buffer GW1	（80ml +50ml ethanol）×2
Buffer GW2	（38ml +92ml ethanol）×5
Buffer GE	50ml
RNase A(50mg/ml)	300µL

7.Supplementary Information

A. Selection and Preparation of Plasmids and E. coli Strains

Plasmid DNA can be purified from overnight cultures of *E. coli* with the Genorise Minipreps DNA Purification System. The yield of plasmid will vary depending on a number of factors, including the plasmid copy number, cell density of bacterial culture, type of culture medium and the bacterial strain used.

Plasmid copy number is an important factor affecting plasmid DNA yield. Copy number is determined primarily by the region of DNA surrounding and including the origin of replication. This region, known as the replicon, controls replication of plasmid DNA by bacterial enzyme complexes. Some DNA sequences, when inserted into a particular plasmid, can lower the copy number of the plasmid by interfering with replication.

Choose a single, well-isolated colony from a fresh Luria-Bertani (LB) agar plate (containing antibiotic) and use the colony to inoculate 1–10ml of LB media (also containing antibiotic). The inoculated medium should be incubated overnight (12–16 hours) at 37°C. An A_{600} of 2.0–4.0 for high copy plasmids ensures that bacteria have reached the proper growth density for harvesting and plasmid DNA isolation.

B. Choosing a Bacterial Strain

Endonuclease I is a 12kDa periplasmic protein that degrades double-stranded DNA. This protein is encoded by the gene endA. The E. coli genotype endA1 refers to a mutation in the wildtype endA gene, which produces an inactive form of the nuclease. E. coli strains with this mutation are referred to as EndA–.

Table 1. EndA⁻ and EndA⁺ Strains of E. coli.

EndA⁻	EndA⁺
BJ5183	BL21(DE3)
DH1	CJ236
DH20	HB101
DH21	JM83
DH5α™	JM101
JM103	JM110
JM105	LE392
JM106	MC1061
JM107	NM522 (all NM series strains are EndA⁺)
JM108	NM554
JM109	G2392
MM294	PR700 (all PR series strains are EndA⁺)
SK1590	Q358
SK1592	RR1
SK2267	TB1
SRB	TG1
XL1-Blue	Y1088 (all Y10 series strains are EndA⁺) XLO
	BMH71–18
	ES1301

The absence of endA1 (or endA) in an E. coli genotype denotes the presence of the wildtype gene, which expresses an active endonuclease I. The wildtype is indicated as EndA+. Using the *Genorise* Plasmid Minipreps DNA Purification System, high-quality DNA is easily obtained from both EndA+ and EndA– strains. However, some EndA+ strains can be problematic for a number of applications.

In general, we recommend the use of EndA– strains whenever possible, particularly for applications such as automated fluorescent sequencing.

For applications such as fluorescent DNA sequencing, special considerations should be given to the selection of plasmid and E. coli strains to optimize yield and plasmid quality. Optimal automated fluorescent sequencing results are obtained by using high copy plasmids and EndA– strains of E. coli for plasmid propagation.

6. Troubleshooting

Low nucleic acid

①**Buffers or other reagents were exposed to conditions that reduced their effective**
- Store all buffers at +15 to +25℃.
- After reconstitution of RNase with Suspension Buffer store aliquots at +2 to +8℃.
- Close all reagent bottles tightly after each use to preserve pH, stability, and freedom from contamination.

②**Ethanol not added to Wash ?Add absolute ethanol to all Wash Buffers Buffer before using.**
- After adding ethanol, mix the Wash Buffer well and store at +15 to +25℃
- Always mark Wash Buffer vial to indicate whether ethanol has been added or not.

③**Reagents and samples not mixed**
- Always mix the sample tube well after add completely. tion of each reagent.

Absorbance (A_{260}) reading of product too high Sample "pops"out of wells in agarose gels

Eluate containing the purified DNA product is contaminated with ethanol from the Wash Buffer.
- After the last wash step, make certain flowthrough solution containing Wash Buffer does not touch the bottom of the High Pure Filter Tube.
- If this has occurred, empty the Collection Tube, reinsert the contaminated filter, and recentrifuge for 30 seconds.

Low plasmid yield

①**Too few cells in starting**
Grow *E. coli* to an absorbance (A_{600}) of 1.0-1.9 material. before harvest.

②**Incomplete cell lysis**
- Be sure the E. coli pellet is completely resuspended in Suspension Buffer
- Make sure the lysate is clear and viscous after the lysis step (incubation with **Buffer G2**).
- Make sure a cloudy white precipitate forms when **Buffer G3** is added to the lysate. The precipitate should pellet completely during centrifugation.

③**Lysate did not bind completely to DNA Binding Column**
- Pre-equilibrate the silica fleece in the DNA Binding Column by adding 250 µL Buffer GL to the Column before applying sample. (If you want to increase your yield in the standard protocol, always perform this extra pre-equilibration step.), Centrifuge for 1 min at 10,000 x g and discard the flowthrough .

Plasmid is degraded or no plasmid is obtained.

High levels of nuclease activity
- Use optional Buffer GW1 (step⑥of protocol) to eliminate nuclease activity in *E. coli* strains with high levels of nuclease (for example, HB101).

RNA present in final product.

①**RNase A not completely dissolved.**
- Follow the instructions given under “Add RNase A to Buffer G1”

▲ Reconstituted mixture is stable for 6 months when stored properly.

②**Too many cells in starting material.**
- Do not use more than 4 ml of an overnight *E.coli* culture as starting material.

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1. Introduction

Genorise Pure Plasmid Mini Kit provides a fast, simple, and cost-effective plasmid miniprep method for routine molecular biology laboratory applications.
- The Kit use silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. ●Plasmid DNA purified is immediately ready for use.
- Phenol extraction and ethanol precipitation are not required, and high-quality plasmid DNA is eluted in a small volume of Tris buffer (included in each kit) or water.
- Isolation of up to 20 µg purified plasmid DNA from bacterial cultures, which may be used directly in downstream applications such as restriction enzyme digestion, PCR, cloning, sequencing, in vitro transcription, or labeling reactions.
- The Elution Buffer is now 10 mM Tris, pH 8.5 which is the optimum sample buffer for subsequent applications.
- The procedure can be adapted to purify larger quantities of plasmid DNA.

2.Storage

- Kits should be stored dry at room temperature (15-25°C). Kits can be stored for up to 12 months without showing any reduction in performance and quality.
- For longer storage these kits can be kept at 2-8°C.
- If any precipitate forms in the buffe after storage at 2-8°C. it should be redissolved by warming the buffers to 37°C before use.
- After addition of RNase A, Buffer G1 is stable for 6 months when stored at 2-8°C. RNase A can be stored at -20°C .

3. Equipment and Reagent to Be Supplied by User

- Absolute ethanol（95-100%）
- Microcentrifuge tubes, 1.5 ml or 2.0 ml, sterile
- Heating block or water bath (70°C).

4. DNA Purification Protocols

4.1 Sample Material

- 0.5- 4.0 ml E. coli cultures
- 4.0-10 ml E. coli cultures(Low-copy plasmids and cosmids).

▲ Bacterial cultures should be grown for 12 to 16 hours, in fluid medium (e.g.,LB) containing a selective antibiotic, to a density of 1.5 to 5.0 A_{600} units/ml .

▲ Do not use more highly concentrated samples, since these will overload the High Pure filter tube and produce unsatisfactory yields.

4.2 Protocols

Prepare the starting material:

- Pellet the bacterial cells from 0.5 - 4.0 ml of E. coli culture. The cells should have a density of 1.5 - 5.0 A_{600} units per ml.
- Discard the supernatant.
- Add 250 µL **Buffer G1** + RNase A to the centrifuge tube containing the bacterial pellet.
- Resuspend the bacterial pellet and mix well.

250 µL **Buffer G1**

Treat the resuspended bacterial pellet as follows:

- Add 250 µL **Buffer G2**.
- Mix gently by inverting the tube 3 to 6 times.

▲To avoid shearing genomic DNA, do not vortex!

- Incubate for 5 min at any temperature between +15 and +25°C.

▲Do not incubate for more than 5 min!

250 µL **Buffer G2**

Treat the lysed solution as follows:

- Add 350 µL **Buffer G3**.
- Mix gently by inverting the tube 3 to 6 times.
- Incubate on ice for 5 min. The solution should become cloudy and a flocculant precipitate should form.
- Centrifuge for 10 min at approx. 10,000 x g in a standard tabletop microcentrifuge.

350 µL **Buffer G3**

【*Optional steps*】 *Prepare the DNA Binding Column:*

- Insert one DNA Binding Column into one Collection Tube.
- Pre-equilibrate the silica fleece in the DNA Binding Column by adding 250 µL **Buffer GL** to the Column before applying sample. Centrifuge for 1 min at 10,000 x g and discard the flowthrough .

250 µL **Buffer GL**

⑤After centrifugation:

- Insert the DNA Binding Column into the Collection Tube.
- Transfer entire supernatant from Step③ into upper buffer reservoir of the Column.
- Insert the DNA Binding Column assembly into a standard tabletop microcentrifuge.
- Centrifuge for at 10,000 x g.

10,000 x g 1 min

⑥After centrifugation:

- Remove the DNA Binding Column from the Collection Tube, discard the flowthrough liquid, and re-insert the Column in the same Collection Tube.
- If the E. coli strain in Step① has a high nuclease content (e.g., HB101 or JM strains), perform the optional wash step below before going to Step ⑦.
- If the E. coli strain in Step ① does not have a high nuclease content (e.g., XL1 blue or DH5 strains), skip the optional wash step and perform Step⑦.

Optional wash step: To eliminate high nuclease activity from the preparation:

- Add 500 µL of **Buffer GW1** to the upper reservoir of the Column.
- Centrifuge for 1 min at 10,000 x g and discard the flowthrough.

500 µL **Buffer GW1**
10,000 x g 1 min

⑦To wash the preparation:

- Add 500µL of **Buffer GW2** to the upper reservoir of the Column.
- Centrifuge for 30 - 60 s at 10,000 x g and discard the flowthrough.

500µL **Buffer GW2**
10,000 x g 1 min

To wash the preparation:

- Combine the DNA binding column with the Collection Tube.
- Add 700µL of **Buffer GW2** to the upper reservoir of the Column.
- Centrifuge 1 min at 10,000×g and discard the flowthrough.

700µL **Buffer GW2**
10,000 x g 1 min

⑨After discarding the flowthrough liquid:

- Centrifuge the DNA binding column for additional 2 min at full speed.
- Discard the Collection Tube.

▲ The extra centrifugation time ensures removal of residual Wash Buffer.

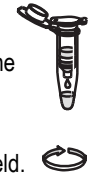
full speed
2 min

⑩To elute the DNA:

- Insert the DNA binding column into a clean, sterile 1.5 ml Microcentrifuge tube.
- Add 50-100 µL prewarmed Elution Buffer to the upper reservoir of the DNA binding column.
- Centrifuge the tube assembly for 1 min at 10,000×g 1min.

▲ Elution with 50µL (instead of 100µL) increases the final DNA concentration in the eluate , but also decreases the overall DNA yield.

▲ Warm up the Elution Buffer to 70℃ increases the final DNA concentration in the eluate.



10,000 x g 1 min

5.Results

①**Purity** Plasmid DNA is free of all other bacterial components, including RNA.

②**Yield**

Yield is variable and depends both on the particular E. coli strain used and the cell density of the bacterial culture.

Dependence of Plasmid Yield (pUC19) from E. coli strain used				
Culture	Volume			
E.coli hosstrain/densitiy	0.5 mL	1.0 mL	2.0 mL	4.0 mL
XL1 blue (3.6 A_{600} units/ mL)	4.9 µg	8.6µg	11.8µg	14.6µg
DH5 alpha(1.5 A_{600} units/ml)	0.9µg	1.7µg	3.3µg	6.2µg
HB101 (4.7A_{600} units/ml)	1.8µg	3.5µg	5.9µg	8.2µg

Dependence of Plasmid Yield (pUC19) from Cell Densitiy (2 ml *E. coli* HB101 suspension)					
Culture	Volume				
Cell densitiy(A_{600} unitsml)	0.4	0.8	1.4	3.4	5.6
Plasmid yield	0.3µg	0.5µg	0.8µg	4.0µg	6.9µg