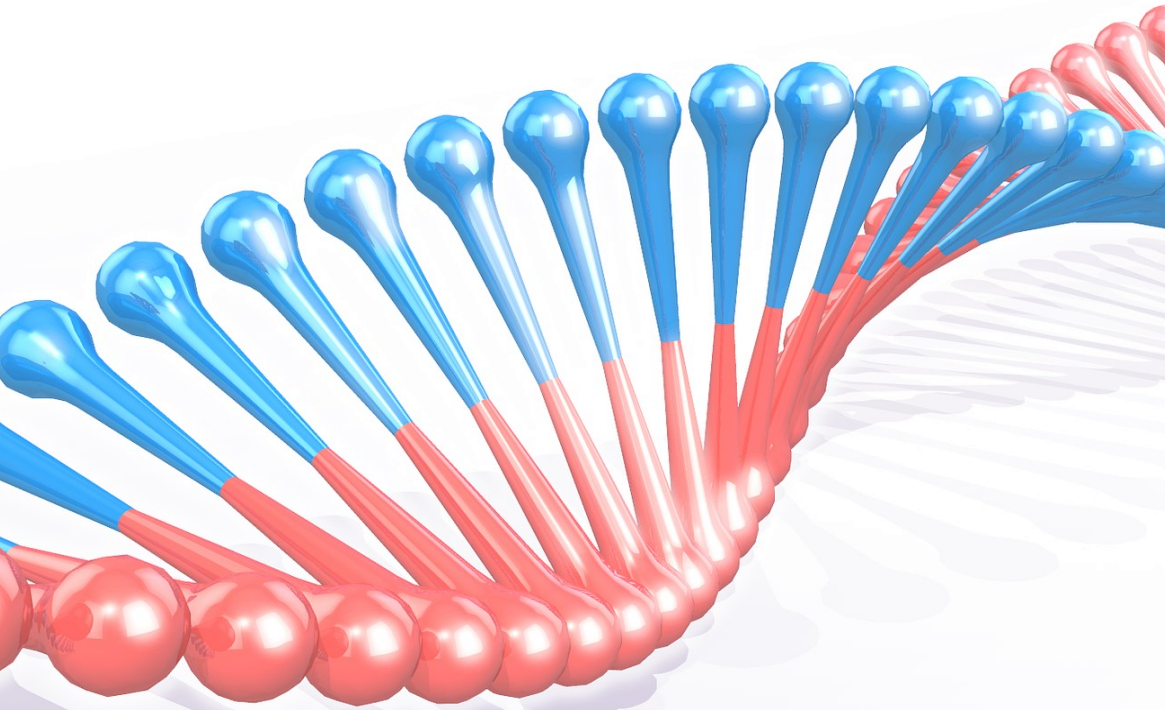
PRO TECH
波仕特生物科技

Gene-Spin™ MiniPrep

Plasmid Purification Kit - V^3

(For purification of high - quality plasmid DNA)
Ver. 6 Dec 2021

Innovative Tools for Nucleic Acid Purification



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KIT CONTENTS :

Gene-Spin MiniPrep kit Cat No.	50 reactions MP530-V3	250 reactions MP530XL-V3
Solution I	12 ml *2	60 ml *2
Solution II	12 ml *3	60 ml *3
Solution III	18 ml	90 ml
Washing Solution	16 ml *1	40 ml *2*1
Elution Solution	10 ml	50 ml
Gene-Spin spin Column	50 pcs	250 pcs
Collection tubes	50 pcs	250 pcs

REMARKS BEFORE YOU START :

1. Before Using Washing Buffer, add 64 ml (for 50 reactions) or 160 ml (for 250 reactions) 95~100 % ethanol and mix well.
2. Please store Solution I at 4 °C for longer storage.
3. The SDS in Solution II will precipitate at temperature below 20 °C. If this is the case, store the Solution II at 30 to 40 °C and mix well.

ATTENTION :

Solution III contain guanidine hydrochloride! Wear gloves and goggles when using this kit!

Description :

The Gene-Spin™ Plasmid MiniPreparation Kit is designed for the rapid, small-scale preparation of high-purity plasmid DNA. The entire minipreps procedure can be completed in 45 minutes or less, depending on the number of samples processed.

The purified plasmid DNA can be used directly for automated fluorescent DNA Sequencing. It also be suitable for restriction enzyme digestion and *in vitro* transcription.

The Gene-Spin Basic Principle

Gene-Spin MiniPrep procedure is based on alkaline lysis of bacterial cells and then creates appropriate conditions for binding of plasmid DNA to the membrane in the Gene -Spin MiniPrep Column.

The procedure consists of three basic steps:

- Preparation and clearing of the bacterial lysate
- Binding of plasmid DNA onto the Gene-Spin Mini-Prep Column
- Washing and elution of plasmid DNA

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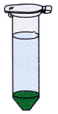
GeneSpin™ Plasmid MiniPrep Flow Chart

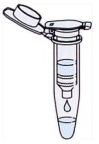
1~5 ml E. *coli* LB Culture

12,000 - 14, 000 g , 1 min



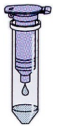
+ 200 ul Solution I
+ 200 ul Solution II
+ 300 ul Solution III

12,000 - 14, 000 g , 5 min



Load Supernatant ~ 700 ul

12,000 - 14, 000 g 1 min

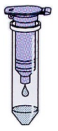


+ 700 ul Washing

12,000 - 14, 000 g , 1 min

12,000 - 14, 000 g , 3 min

60°C oven for 5 - 10 min to evaporate all the ethanol

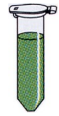


+ 50 ul Elution Buffer / ddH_2O

12,000 - 14, 000 g , 1 min

General Protocol :

1. Harvesting



Transfer an overnight 1 - 5 ml culture to a microcentrifuge tube. Spin down the cells for 30 s - 1 min at top speed (12,000 - 14,000 x g). Discard the supernatant.

12,000 x g , 30 s-1 min

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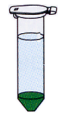
2. Cell lysis

Add **200 µl Solution I** and pipette up and down (or vortex) until the cells are completely resuspended.

+ 200 ul S I Resuspend

Add **200 µl Solution II** and mix by gently inverting the capped tube 5 - 6 times. ***Do not vortex.*** Incubate at room temperature for a maximum of 5 min.



+ 200 ul S II Mix RT, 5min

Add **300 µl Solution III** and mix by gently inverting the capped tube 5-6 times. ***Do not vortex.*** Incubate at room temperature for 1 - 3 min.

+ 300 ul S III Mix

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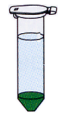
3. Clarification of lysate



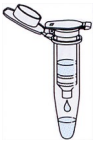
Pellet the precipitate for 5 mins at top speed (12,000 - 14,000 x g). A compact white pellet will form along the

12,000 x g , 5 min

side or at the bottom of the tube.

4. DNA binding

Insert the **spin column** into a **collection tube**, load the supernatant from step 3 directly to **spin column**, spin for 30 s - 1 min.

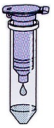


Load Supernatant

12,000 x g, 30 s-1 min

5. Wash

Remove the **spin column** from the **collection tube**, discard the flow-through and add **700 µl Washing Solution** and spin for 1 min. For fluorescent sequencing repeat this step for one more time is recommended.



+ 700 ul Washing Solution

12,000 x g, 1 min

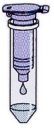
6. Dry

Discard the filtrate then centrifuge for 3 min at top speed to remove any residual trace of ethanol (see Note 2). Spin column incubate at 60°C oven for 5 - 10 min.

12,000 x g, 3 min

7. Elution

Remove the **spin column** and place the column in a new microcentrifuge tube. Add 50 - 100 µl H_2O or TE into the column. For plasmid larger than



+ 50 ul Elution Buffer / ddH_2O

7 kb, use preheated 60 - 70^{0}C H_2O or TE to elute.

12,000 x g , 1 min

Elute DNA by centrifugation for 1 min and store the eluted DNA at -20^{0}C.

Repeat step 7 may give 10-15% more DNA, but DNA concentration will be diluted.

Note:

1. Use an endA$^-$ *E.coli* strain for plasmid purification. The instability of plasmids isolated from endA$^+$ strains has been reported.
2. To remove residual trace of ethanol at step 8 is very important, if some trace ethanol still remains in binding resin, the eluted DNA may interfere with further application. If necessary, after 3 min centrifugation at step 8, incubate the spin column at 60^{0}C oven for 5-10 min to evaporate all the ethanol before elute the DNA.
3. Depending on the *E. coli* strain, plasmid copy numbers and medium, the yield of plasmid DNA is around 2-15 ug per ml *E.coli* culture at a purity of 1.8 (260/280).

FUNCTIONAL TEST DATA:

Plasmid DNA Yield Based on DNA Size

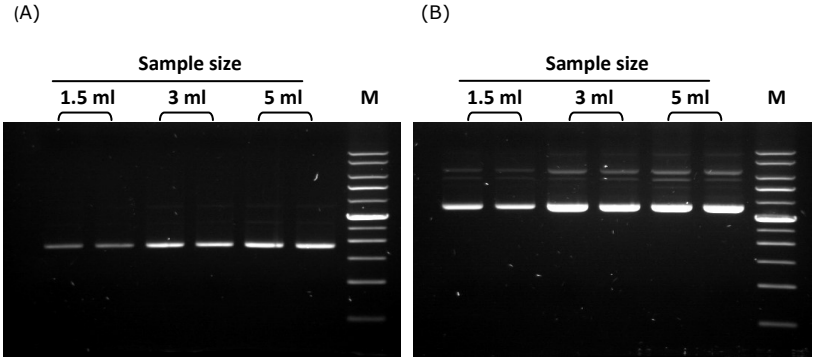


Figure. (A) 2.5 kb plasmid and (B) 5 kb Plasmid DNA was extracted using Gene-Spin™ MiniPrep purification Kit. 0.25 µl aliquots of a 100 µl eluate of purified supercoiled plasmid DNA from a 1.5, 3 and 5 ml overnight *E. coli* culture were loaded, and analyzed by electrophoresis on a 1% agarose gel. M: Protech 1 kb DNA Ladder.

Plasmid length (kb)	Sample size (ml)	A260/280	Total yield (µg)	Plasmid length (kb)	Sample size (ml)	A260/280	Total yield (µg)
	1.5	1.872	8.8		1.5	1.844	22.5
	1.5	1.889	8.5		1.5	1.825	20.8
2.5	3	1.827	17.9	5	3	1.812	49.1
	3	1.821	17.3		3	1.812	42.4
	5	1.81	21		5	1.855	47.5
	5	1.812	21.2		5	1.851	48.3

Table. Yield and Purity analysis of 2.5 and 5 kb plasmid DNA (100 µl eluate) from 1.5, 3 and 5 ml of DH5α cultured bacterial cells.

TROUBLESHOOTING

Comments and suggestions

Incomplete lysis of bacterial cells	⇨ ***SDS in Solution II precipitated*** - SDS in Solution II may precipitate upon storage. If this happens, incubate Solution II at 30-40ºC for 5 min and mix well. ⇨ ***Too many bacterial cells used*** - We recommend LB as the optimal growth medium. When using very rich media like TB, the cell density of the cultures may become to high.
Poor Plasmid Yield	⇨ ***In complete lysis of bacterial cells*** - See "Possible cause and suggestions" above. ⇨ ***Suboptimal precipitation of SDS and cell debris*** - Precipitation of SDS and cell debris will be slightly more effective when centrifuging at 4ºC instead of room temperature (step 5).

	Comments and suggestions
Poor Plasmid Yield	⇨ ***Suboptimal elution conditions*** - If possible, using a slightly alkaline elution buffer like elution solution (10mM Tris-HCl, pH8.5). If using nuclease-free water, check the pH of the water.
No Plasmid Yield	⇨ ***Reagent not applied properly*** - Add indicated volume of 96-100% ethanol to buffer concentrate washing solution and mix well. ⇨ ***Nuclease-rich host strains used*** - If using nuclease-rich strains like *E.coli* HB101 or strains of the JM series, be sure to perform the optimal washing solution step.
Poor Plasmid Quality	⇨ ***Genomic DNA contamination*** - Cell lysate was vortexed or mixed too vigorously after addition of solution II. Genomic DNA was sheared and thus liberated.

	Comments and suggestions
Poor Plasmid Quality	⇨ ***Smeared plasmid bands on agarose gel*** - If using nuclease-rich strains like *E.coli* HB101 or strains of the JM series, be sure to perform the optimal washing solution step. When working with nuclease-rich strains, keep plasmid preparations on ice or frozen in order to avoid plasmid DNA degradation.

APPENDIX: VACUUM PROTOCOL

1. Harvesting

Transfer an overnight 1-5 ml culture to a microcentrifuge tube. Spin down the cells for 30 s - 1 min at top speed (12,000-14,000 x g). Discard the supernatant.

2. Cell lysis

Add **200 ml Solution I** and pipette up and down (or vortex) until the cells are completely re-suspended.

Add **200 ml Solution II** and mix by gently inverting the capped tube 5-6 times. ***Do not vortex.*** Incubate at room temperature for a maximum of 5 min.

Add **300 ml Solution III** and mix by gently inverting the capped tube 5-6 times. ***Do not vortex.*** Incubate at room temperature for 1 - 3 min.

3. Clarification of lysate

Pellet the precipitate for 5 min at top speed (12,000-14,000 x g). A compact white pellet will form along the side or at the bottom of the tube.

4. DNA binding

Attach the vacuum maniford to the vacuum source, e.g. Axygen AxyVac Vacuum Maniford (AP-VM). Insert the column into the fitting on the vacuum maniford. Transfer the clarified supernatant from Step 3 to the column.

Switch on the vacuum source and adjust the regulator to achieve a negative pressure of -760 mmHg (equivalent to approximately -850-1,000 mbar and -12-15 psi). Allow the vacuum to continue until no liquid remains in the column (s).

5. Wash

Pipette 700 ml of Washing Solution along the wall of the column(s) to remove residual salt. Turn the vacuum on and adjust the regulator to -760mmHg (equivalent to approximately -850-1,000 mbar and -12-15 psi). Continue to apply vacuum until no fluid remains in the column(s).

Repeat this wash step with second 700ml of Washing Solution.

6. Dry

Discard the filtrate then centrifuge for 3 min at top speed to remove any residual trace of ethanol. Incubate spin column(s) at 60^{o}C oven for 5-10 min.

7. Elution

Remove the column and place the column in a new microcentrifuge tube. Add 50 – 100 ml H2O or Elution Buffer into the column. For plasmid larger than 7kb, use preheated 60-70 ^{o}C H2O or TE to elute.

Elute the DNA by centrifugation for 1min and store the eluted DNA at -20^{o}C. Repeat step 7 may give 10-15% more DNA, but DNA concentration will be diluted.

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