Protech Technology

Gene-Spin™ MiniPrep Plasmid Purification Kit - V²

(For purification of high-quality plasmid DNA)
Ver. 4.2 Sep 2009

Innovative Tools for Nucleic Acid Purification

This Kit is for research purposes only.
Not for use in diagnostic procedures.
For in vitro use only

Please do not hesitate to contact us if you have any questions.
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KIT CONTENTS :

Gene-Spin kit	50 reactions	250 reactions
Cat No.	MP530	MP530XL
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 X 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 20 minipreps procedure can be completed in 45 minutes or less, depending on the number of samples processed. The systems allows to purify up to 40 ug plasmid from 1-5 ml of a standard *E. coli* culture.

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 silica 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 MiniPrep Column
- Washing and elution of plasmid DNA

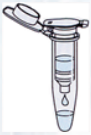
GeneSpin™ Plasmid MiniPrep Flow Chart

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 , 2 min

GENERAL PROTOCOL :

1. Transfer an overnight 1-5 ml culture to a micro-centrifuge tube. Spin down the cells for 30s-1min at top speed (12,000-14,000 x g). Discard the supernatant.
2. Add **200 µl Solution I** and pipette up and down (or vortex) until the cells are completely resuspended.
3. 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.
4. Add **300 µl Solution III** and mix by gently inverting the capped tube 5-6 times. ***Do not vortex.***
5. Pellet the precipitate for 5 mins 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.
6. Insert the **spin column** into a **collection tube**, load the supernatant from step 5 directly to **spin**

column, spin for 30 s.

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

8. 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.

9. Remove the **spin column** and place the column in a new microcentrifuge tube. Add 50-100 µl H_2O or Elution Buffer into the column. For plasmid larger than 7kb, use preheated 60-70 ^{0}C H_2O or TE to elute.

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

    Repeat step 9-10 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).

TROUBLESHOOTING

Q: 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.

Q: 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).

⇨ *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.

Q: 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.

Q: 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.

- ***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.

Manufactured for and distributed by
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