GeneMark

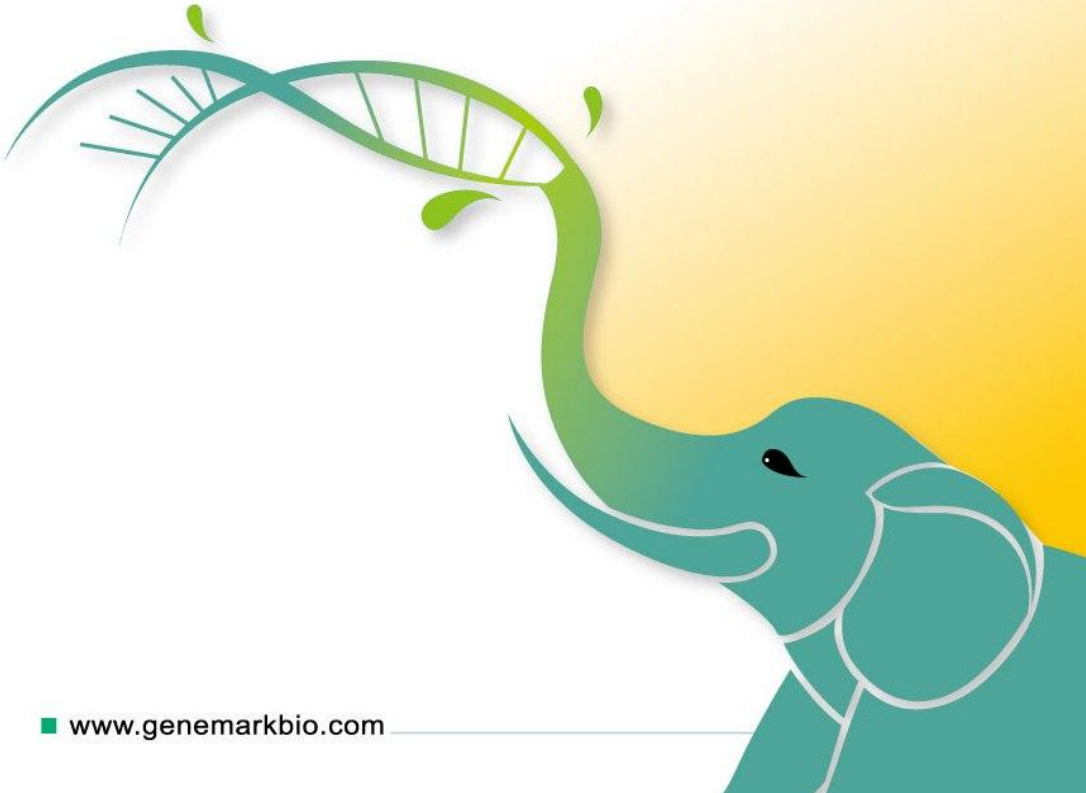
Plasmid Miniprep Purification Kit

Cat. # : **DP01 / DP01-300**

Size : **50/ 300** Reactions

Store at RT

For research use only



Description:

The **Plasmid Miniprep Purification Kit** is based on a very simple and rapid procedure using spin column format to purify plasmid DNA from 1~10 ml liquid culture. The yield of up to 60 µg plasmid DNA is quantitative and reproducible, and the plasmid DNA quality is sufficient for direct use in any applications such as PCR, restriction digestion, cloning, automated DNA sequencing, southern blot, and *in vitro* transcription assay.

Components of the Kit:

	DP01	DP01-300
1. Solution I	10 ml	60 ml
2. Solution II	10 ml	60 ml
3. Solution III	10 ml	60 ml
4. Wash solution	**16 ml (add 64 ml Ethanol before use)**	**96 ml (add 384 ml Ethanol before use)**
5. Elution solution	10 ml	60 ml
6. Spin column	50 pcs	150 pcs x 2
7. Collection tube	50 pcs	150 pcs x 2

*** Store the Solution I (contains RNase A) at 4°C, store all other kit components at RT.**

*** If precipitates have formed in Solution II, warm the buffer at 35°C in a water bath until fully dissolved.**

General Procedure:

1. Pellet 1~10 ml of bacteria culture by centrifugation for 1 min at top speed (12~14,000x *g*) in a microcentrifuge. Discard the supernatant and remove any excess media.

Note: For liquid culture > 5 ml, increase Solution I, Solution II and Solution III volume to prevent product loss.

2. Resuspend the cell pellet completely in 200 µl of **Solution I** by pipetting or vortexing.
3. Add 200 µl of **Solution II** and mix by inverting the tube 5 times; the cell suspension should turn clear immediately.
4. Add 200 µl of **Solution III** and mix by inverting the tube 5 times.
5. Centrifuge the lysate at top speed in a microcentrifuge for 5 min. 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**, carefully transfer all of the clear lysate from **step 5** to spin column, centrifuge at top speed for 1 min.
7. Discard the filtrate from the collection tube and add 700 µl of **Wash Solution** to the **spin column** and centrifuge at top speed for 1 min. Repeat this step once more.
8. Discard the filtrate and centrifuge at top speed for additional 3~5 min to remove residual trace of ethanol.
   ** If centrifugation speed is lower than 10,000x g or residual ethanol must be removed completely, incubate the spin column in a heat oven (45~60°C) for 5 min to evaporate all of the ethanol.*
9. Transfer the **spin column** into a new microcentrifuge tube and add 50~100 µl of **Elution Solution** or **H_2O (pH 7.0~8.5)** into the column and wait for 1~2 min.
   (For plasmid DNA larger than 7 kb, use preheated (**60~70**°C) Elution solution to elute.)
10. Centrifuge at top speed for 1 min to elute the DNA. Store the eluted plasmid DNA at -20°C.
    *The yield of plasmid DNA is 6~60 µg for 1~10 ml E. coli culture at purity of 1.8~2.0 (A260/A280).

Troubleshooting Guide

	Comments and Suggestion
Low or no yield	
a) Plasmid did not propagate	Inoculate the liquid medium containing recommended antibiotic with single colony of bacterial cells from a freshly streaked plate and grow the culture under appropriate conditions.
b) Overgrown bacteria	Overgrowth of culture leads to lysis of cells and degradation of DNA. Do not grow cultures longer than 12~16 hours.
c) Not enough bacterial cells	Ensure optimum growth of culture (OD_{600} > 1) by incubating the culture overnight at recommended temperature with vigorous shaking
d) Low-copy-number plasmid	When using low-copy-number plasmids, use larger culture volumes. Even with larger culture volumes, yields of low-copy-number plasmids will be lower than those of high-copy-number plasmids.
e) Poor cell lysis	1) Excessive growth of bacteria. The culture medium should contain recommended antibiotic. 2) Poor cell suspension. Ensure complete resuspension of bacterial pellet by votexing or pipetting before adding Solution II.
f) Incomplete DNA Elution	1) If plasmid is larger than 7 Kb, use preheated Elution solution (60~70°C) in the **Step 9**. 2) DNA should be eluted only with a low-salt buffer [e.g., Elution solution (10 mM Tris·Cl, pH 8.5) or water]. Elution efficiency is

	dependent on pH. The maximum efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH value is within this range. 3) Ensure that Elution solution is added onto the center of the membrane and is completely absorbed.
Genomic DNA contamination a) Overgrown bacteria	Overgrowth of culture leads to lysis of cells and degradation of DNA. Do not grow cultures longer than 12~16 hours.
b) Lysate prepared incorrectly	1) The lysate must be handled gently after addition of Solution II to prevent genomic DNA shearing. 2) Lysis in **step 3** must not exceed 5 minutes.
c) Solution III added incorrectly	Upon addition of Solution III, mix immediately, but gently.
RNA contamination RNase A digestion insufficient	1) If Solution I containing RNase A is stored beyond a year, add additional RNase A to Solution I. 2) Reduce the culture volume, if necessary.

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