Plasmid DNA MaxiPrep Kit

(High Purity, Silica - membrane Spin Column)

Catalog No.:

D1051 (10 preps)

Kit Contents

Component	D1051
	10 preps
RNase A (10 mg/ml)	600 µl x 2
Solution I	100 ml
Solution II	100 ml
Solution III	120 ml
Wash Buffer PB	72 ml
Wash Buffer W	40 ml x 3
Elution Buffer (10 mM Tris-HCl, pH 8.5)	20 ml
Spin Columns	10 each

Description

Plasmid Maxiprep Kit is designed for rapid and cost-effective large-scale preparation of high quality plasmid DNA from transformed E.coli cultures. The kit utilizes an exclusive silica technology in the form of silica suspension which can recover 400-1,000 µg plasmid DNA from 100 ml overnight bacterial culture. The kit can be successfully used for efficient purification of any size plasmids and cosmids. The actual plasmid yield and optimal culture volume depend on the plasmid copy number and medium used for cultivation.

The purification theory: silica particles bound DNA selectively at high salt concentration and low pH, while protein and other impurities are removed. The pure DNA is eluted with low salt buffer or water. This method requires few manipulations, and is both faster and easier to perform than other organic-based extraction methods.

Downstream Applications

Purified DNA is free of impurities and enzyme inhibitors, and have an $A_{260/280}$=1.7-1.9, is suitable for applications such as:

PCR/qPCR/Restriction enzyme digestion/Sequencing/In vitro transcription

Features

·Fast - procedure takes only 40 min

·High efficiency - 400-1000 μg of plasmid DNA from one prep

·High purity - purified DNA without enzyme inhibitors, RNA or proteins, ready for downstream applications

Storage

Store RNase A at -20°C, other reagents at room temperature for up to 1 year.

The Solution I with RNase A is stable for 6 months at 4°C. Any precipitate in the Solution II can be dissolved by incubating at 37°C before use.

Important Notes

·Add all provided RNase A solution to the Solution I and mix well.

·Prior to the initial use of the kit, dilute the Wash Buffer PB, Wash Buffer W with isopropyl alcohol or ethanol (96-100%):

Solution	D1051 (10 preps)
Wash Buffer PB	72 ml
Isopropyl Alcohol	48 ml
Total Volume	120 ml

Solution	D1051 (10 preps)
Wash Buffer W	40 ml × 3
Ethanol	60 ml × 3
Total Volume	100 ml × 3

Mix well, mark the labels on the bottle that isopropyl alcohol or ethanol is added.

·Ensure that no DNases are introduced into the sterile solutions of the kit.

·Make sure there is no precipitates in Solution II. If any precipitate is visible, warming the solution at 37°C for 3-5 min to dissolve the precipitate, and cooling to 25°C before use.

·Wear disposable gloves when handling Solution II, Solution III and Wash Buffer PB as they contain guanidine hydrochloride or other denaturing agent.

·All purification steps should be carried out at room temperature.

Preparation Work - Growth of Bacterial Cultures

Pick a single colony from a fresh selective plate to inoculate in LB medium with appropriate antibiotic. Incubate for 12-16 hours at 37°C while shaking at 200-250 rpm. The bacterial culture should have a cell density of approximately 109 cells/ml or an absorbance of 1-1.5 at 600 nm (A_{600}).

Protocol

1. Harvest 100 ml (high-copy plasmid) or 250-500 ml (low-copy plasmid) overnight culture. Centrifuge at 8,000 rpm for 1 min. Remove the medium thoroughly.
   ***Note**: Do not overload the culture.*

2. Resuspend the cells in 7.5 ml Solution I. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain. Incubate the lysate at room temperature for 5-10 min.
   ***Note**: Ensure RNase A has been added to the Solution I.*

3. Add 7.5 ml Solution II and mix gently by inverting the tube ~12 times. Do not vortex. Incubate the lysate at room temperature for 3-5 min.
   ***Note**: Do not incubate for more than 5 min.*

4. Add 10 ml Solution III and mix immediately and thoroughly by inverting the tube ~12 times. Do not vortex.
   ***Note**: It is important to mix thoroughly and gently after the addition of the Solution III to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate is cloudy and viscous.*

5. Centrifuge at 8,000 rpm for 15 min.

6. Transfer the supernatant to the supplied spin column. Incubate at room temperature for 5 min.
   ***Note**: Do not over 15 ml one time. Avoid transferring the white precipitate.*

7. Centrifuge at 8,000 rpm for 2 min. Discard the flow-through and place the column back into the same collection tube.
   ***Note**: Do not add bleach to the flow-through.*

8. Add 10 ml Wash Buffer PB to the spin column. Centrifuge at 8,000 rpm for 2 min and discard the flow-through. Place the column back into the same collection tube.
   ***Note**: Wash Buffer PB must be diluted with isopropyl alcohol previously.*

9. Add 10 ml Wash Buffer W to the spin column. Centrifuge at 8,000 rpm for 2 min and discard the flow-through. Place the column back into the same collection tube.
   ***Note**: Wash Buffer W must be diluted with ethanol (96-100%) previously.*

10. Repeat step 9 again.

11. Centrifuge at 8,000 rpm for 4 min to remove residual wash solution. Discard the collection tube with flow-through. Dry the column in the air for 5 min.

12. Place the spin column into a clean 50 ml recovery tube, and pipet 1.5-2 ml Elution Buffer to the center of the column without touching the membrane. Incubate at room temperature for 5 min and centrifuge at 8,000 rpm for 2 min.
    ***Note**: Prewarm Elution Buffer to 65°C will increase the yield of DNA, especially the plasmids or cosmids > 20 kb.*
    ***Note**: A second elution step will recover residual DNA from the membrane and increase the overall yield by 10-20%.*

13. Discard the column. Store the purified plasmid DNA at -20°C.