

## Plasmid DNA MaxiPrep Kit (High Purity, Silica Precipitacion)

Catalog No.:  
D1061 (10 preps)

### Kit Contents

Component	D1061
	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
Silica Power Suspension	12 ml

### 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

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## Features

- Fast - procedure takes only 40 min
- High efficiency - 400-1000  $\mu\text{g}$  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  $-20^{\circ}\text{C}$ , other reagents at room temperature for up to 1 year.  
The Solution I with RNase A is stable for 6 months at  $4^{\circ}\text{C}$ . Any precipitate in the Solution II can be dissolved by incubating at  $37^{\circ}\text{C}$  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	D1061 (10 preps)
Wash Buffer PB	72 ml
Isopropyl Alcohol	48 ml
Total Volume	120 ml

Solution	D1061 (10 preps)
Wash Buffer W	40 ml $\times$ 3
Ethanol	60 ml $\times$ 3
Total Volume	100 ml $\times$ 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  $37^{\circ}\text{C}$  for 3-5 min to dissolve the precipitate, and cooling to  $25^{\circ}\text{C}$  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  $37^{\circ}\text{C}$  while shaking at 200-250 rpm. The bacterial culture should have a cell density of approximately  $10^9$  cells/ml or an absorbance of 1-1.5 at 600 nm ( $A_{600}$ ).

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## 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:** The neutralized bacterial lysate is cloudy and viscous.
5. Centrifuge at 8,000 rpm for 15 min.
6. Transfer the supernatant to a 50 ml centrifuge tube. Add Silica Powder Suspension at a ratio of 1.2 ml Suspension/25 ml supernatant. Mix thoroughly by inverting 6 times. Incubate at room temperature for 5 min.
7. Centrifuge at 8,000 rpm for 2 min. Discard the supernatant.  
**Note:** Do not add bleach to the flow-through.
8. Add 10 ml Wash Buffer PB, resuspend the precipitates by vortexing. Centrifuge at 8,000 rpm for 2 min and discard the supernatant.  
**Note:** Wash Buffer PB must be diluted with isopropyl alcohol previously.
9. Add 10 ml Wash Buffer W, resuspend the precipitates by vortexing. Centrifuge at 8,000 rpm for 2 min and discard the supernatant.  
**Note:** Wash Buffer W must be diluted with ethanol (96-100%) previously.
10. Repeat step 9 again.
11. Pipetting away any residual Wash Buffer W, open the cap, air dry the tube for 30 minutes or place the tube in a super clean bench to dry for 15 min.
12. Add 1.5-2 ml Elution Buffer. 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. Transfer the supernatant to a new tube. Store the purified plasmid DNA at -20°C.

## DNA Quantitation

Perform DNA quantitation using UV absorbance at 260 nm.

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