Plasmid Miniprep Kit

I. DESCRIPTION

The Kit is a reagent kit from ShineGene. It contains all the necessary reagents, mini-columns, and other supplies for quick, reliable, high-quality plasmid DNA or cosmid DNA preparation from bacteria such as *E. coli* cell culture. Plasmid and cosmid DNA molecules are selectively adsorbed onto the column, and other impurities such as proteins, salts and nucleotides are washed away. Eluted in a small volume low-salt buffer with complete removal of contaminants and inhibitors, the purified plasmid or cosmid DNA is immediately ready for many downstream applications such as PCR, transformation, restriction enzyme digestion, cloning, sequencing, *in vitro* translation, and transfection.

II. KIT CONTENTS

Kit contains enough supplies for 50/100 minipreps.

Components	ZN00301(50Preps)(EUR60.00)	ZN00302(100Preps)(EUR100.00)
Solution I	5ml	10ml
Solution II	10ml	20ml
Solution III	20ml	40ml
Wash Solutin	18ml	36ml
TE	5ml	10ml
Column	50	100
2ml Collection Column	50	100
Protocol	1	1

III. APPLICATIONS

The Kit enables the preparation of high-quality plasmid DNA ready for most downstream applications.

such as:

PCR and cloning
Restriction enzyme digestion
Transformation
Sequencing
In vitro translation
Transfection

IV. KEY FEATURES

- Easy to perform: Kit's simple and rapid procedure purifies DNA in five minutes.
- High capacity: Each column has a capacity of 20 μg plasmid DNA.
- High purity: The kit completele removes contaminants and inhibitors.
- Reproducible yields: Recovery is typically between 90% and 95% pure plasmid DNA, reproducible every time.

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V. STORAGE

This kit should be stored dry at room temperature. So stored, the kit is stable for 12 months. Solution I with RNase A added should be stored at 2-8°C and remains stable for 6 months (for long-term storage, store at -20°C).

VI. PLASMID MINIPREP PROTOCOL

The following steps may be performed ahead of time:

1. Solution I with RNase A should be stored at 4 °C for frequent use or at -20 °C for long-term storage.
2. Add **47 ml** of 96-100% of ethanol to 18 ml of **wash solution** and mix well.
3. Some precipitate may form in solution II after long periods of storage. Dissolve the precipitate by mixing gently. Otherwise, warm the container to 37 °C for a few minutes.

Procedure:

1. Transfer 1.5 ml of the overnight culture to a 1.5 ml microcentrifuge tube and centrifuge at 12,000 rpm for 30 seconds. Remove and discard the supernatant. For low copy number plasmids, use 3-5 ml of the overnight culture (spin down cells 2-3 times in the same tube) and double the volume of solutions I, II and III.
2. Add 100 µl of solution I to the pellet, cap the tube and resuspend the cells. This can be done by vortexing, but the following method may be faster: hold the microcentrifuge tube from the top and run the narrow end quickly along the top of an empty microcentrifuge storage rack. Repeat four times or until the cells are resuspended.
3. Add 200 µl of solution II to the mixture. Mix gently by inverting the tube 4-6 times. To avoid contamination by genomic DNA, do not vortex.
4. Add 400 µl of solution III, and mix gently by inverting the tube 4-6 times.
5. Centrifuge at 12,000 rpm for five minutes. Transfer the supernatant to the column. Centrifuge at 12,000 rpm for 30 seconds.
6. Remove and discard the flow-through. Add 650 µl of wash solution to the column, and centrifuge at 12,000 rpm for 30 seconds. If necessary, repeat wash procedure once.
7. Remove and discard the flow-through. Centrifuge at 12,000 rpm for additional 60 seconds to remove residual wash solution.
8. Transfer the column to a clean 1.5-ml microcentrifuge tube. Add 30-50 µl of TE to **the center of the column membrane**. Let the column stand at room temperature for one minute. Centrifuge at 12,000 rpm for one minute.
9. Store the purified DNA at -20ºC.

Note: It is extremely important to add the TE into the center part of the column. Incubating the column with the TE at higher temperatures (37 to 50ºC) may slightly increase the yield especially of large (>10,000bp) DNA Plasmids. Prewarming the TE at 55ºC to 80ºC may also slightly increase elution efficiency.