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Plasmid DNA Extraction Miniprep Kit

9K-006-0009s (10 prep)
9K-006-0009 (100 prep)
9K-006-0010 (200 prep)

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For Research Use Only

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Plasmid DNA Extraction Maxiprep Kit

Introduction

Bio Basic Plasmid DNA Extraction Miniprep Kit is an excellent tool offering a speed and economic method to purify plasmid DNA from bacteria cultures. This technology is based on binding DNA to silica-based membranes in chaotropic salts and washing DNA with specially formulated solutions. Compared with other harmful and time-consuming procedures, such as phenol/chloroform extraction and ethanol precipitation, Bio Basic Plasmid DNA Extraction Miniprep Kit shortens the handling time to about 25 minutes. The high quality plasmid DNA can be used directly for any downstream applications.

Specifications

Sampling	Yield	Handling Time
1~5 mL overnight culture	up to 20 µg for high-copy plasmids	about 25 minutes

RNA Contaminates Plasmid DNA

Insufficiency of RNase A activity in MINI 1 Resuspension Solution because of long-term storage

- Prior to use **MINI 1** Resuspension Solution, ensure that RNase A was added. If RNase A added to **MINI 1** Resuspension Solution Buffer is out of date, add additional RNase A into **MINI 1** Resuspension Solution to a concentration of 50 µg/ml then store at 4°C.
- Too many bacterial cells were used, reduce sample volume.

Smearing or degradation of Plasmid DNA

Nuclease contamination

- If used host cells have high nuclease activity (e.g., endA+ strains), perform this optional wash step to remove residual nuclease.
- After DNA Binding Step, add 400µl of **MINI 4** Washing 1 Solution into **MINI** Column and incubate for 2 minutes at room temperature.
- Centrifuge at full speed (14,000 rpm or 10,000 xg) for 30 seconds.
- Proceed to standard **MINI 5** Washing 2 Solution Step.

Plasmid DNA is not adequate for enzymatic digestions

Eluted plasmid DNA contains residual ethanol

- Discard the flow-through after washing with **MINI 5** Washing 2 Solution and centrifuge for an additional 3 minutes.

Denatured Plasmid DNA migrate faster than supercoiled form during electrophoresis

Incubation in **MINI 2** Cell Lysis Solution too long

- Do not incubate longer than 5 minutes in **MINI 2** Cell Lysis Solution.

Troubleshooting

Low Yield

Bacterial cells were not lysed completely

- Too many bacterial cells were used. If using bacterial culture with an OD600>10, separate it into three tubes.
- After **MINI 3** Neutralization Solution addition, break up the precipitate by inverting to ensure higher yield.

Overgrown of bacterial cells

- Incubation time should not exceed 16 hours.

Bacterial cells were insufficient

- Ensure that bacterial cells have grown to an expected amount (OD600>1) after incubation under suitable shaking conditions and temperature.

Incorrect DNA **MINI 6** Elution Solution Step

- Ensure that **MINI 6** Elution Solution was added and absorbed to the center of **MINI** Column Matrix.

Incomplete DNA elution

- If size of DNA plasmids is larger than 10 kb, use preheated **MINI 6** Elution Solution (60~70°C) on Step 12 to improve the elution efficiency.

Incorrect **MINI 5** Washing 2 Solution

- Ensure that ethanol was added to **MINI 5** Washing 2 Solution prior to use.

Eluted DNA does not perform well

Residual ethanol contaminants

- After **MINI 5** Washing 2 Solution Step, dry **MINI** Column with additional centrifugation at top speed for 3 minutes or incubation at 60°C for 3 minutes.

Genomic DNA Contamination

Lysate prepared improperly

- Gently invert the tube after adding **MINI 2** Cell Lysis Solution. The incubation time should not exceed 5 minutes.
- Do not use overgrown bacterial culture.

Kit Contents

Component	9K-006-0009s, 10 Prep	9K-006-0009, 100 Prep	9K-006-0010 200 Prep
MINI 1 Resuspension Solution	3ml	30ml	60ml
MINI 2 Cell Lysis Solution	3ml	30ml	60ml
MINI 3 Neutralization Solution	4ml	40ml	80ml
MINI 4 Washing 1 Solution*	3.5ml	35ml	70ml
MINI 5 Washing 2 Solution**	2ml	20ml	40ml
MINI 6 Elution Solution	1.5ml	15ml	30ml
RNase A (50mg/ml)	6µl	60µl	120µl
MINI Column	10 pcs	100 pcs	200 pcs
Collection Tube	10 pcs	100 pcs	200 pcs
Protocol	1	1	1

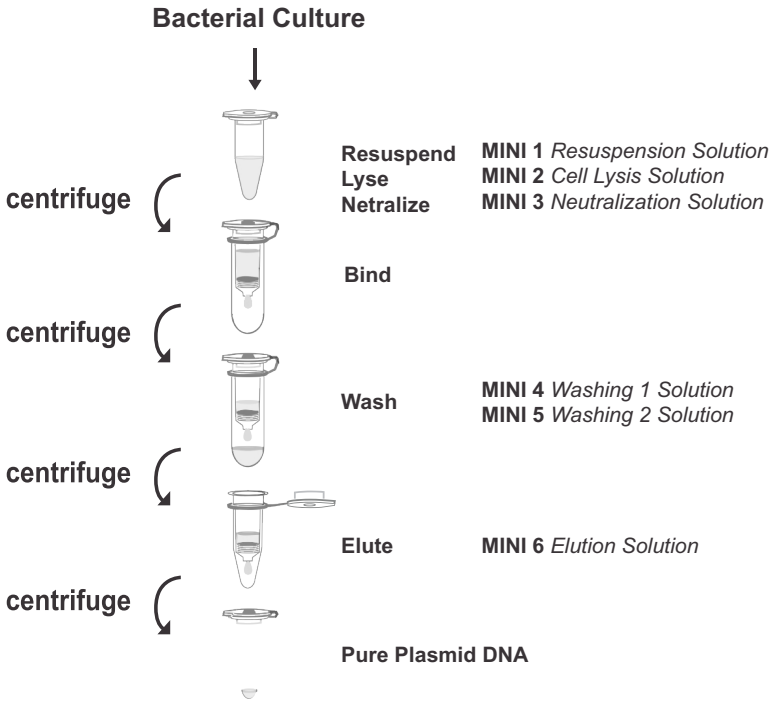
*: Add 1.5ml/15 ml/30 ml ethanol (96 ~ 100%) to **MINI 4** Washing 1 Solution before first use.

: Add 8 ml/80 ml/160 ml ethanol (96 ~ 100%) to **MINI 5 Washing 2 Solution before first use.

Important Notes

1. Solutions provided in this kit contain irritants, wear gloves and lab coat when handling.
2. Briefly spin RNase A tube to remove drops from the inside of the lid. Add 0.1 mL of **MINI 1** Resuspension Solution into RNase A tube and mix well. Transfer the mixture into **MINI 1** Resuspension Solution bottle and store at 4°C.
3. Check **MINI 2** Cell Lysis Solution before use. Warm **MINI 2** Cell Lysis Solution at 55°C for 10 minutes if any precipitation formed. Prevent vigorous shaking of the **MINI 2** Cell Lysis Solution.
4. To avoid acidification of **MINI 2** Cell Lysis Solution from CO_2 in the air, close the bottle immediately after use.
5. 9K-006-0009s: add 1.5ml ethanol (96~100%) to **MINI 4** Washing 1 Solution before first use.
   9K-006-0009: add 15ml ethanol (96~100%) to **MINI 4** Washing 1 Solution before first use.
   9K-006-0010: add 30 ml ethanol (96~100%) to **MINI 4** Washing 1 Solution before first use.
6. 9K-006-0009s: add 8ml ethanol (96~100%) to **MINI 5** Washing 2 Solution before first use.
   9K-006-0009: add 80ml ethanol (96~100%) to **MINI 5** Washing 2 Solution before first use.
   9K-006-0010: add 160ml ethanol (96~100%) to **MINI 5** Washing 2 Solution before first use.
7. All centrifuge steps are performed at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.

Brief Procedure



General Procedure

1. Transfer 1-5ml of well-grown bacteria culture to a microcentrifuge tube (not provided).
2. Spin down the bacteria by centrifuging for 1-2 minutes and discard the supernatant completely.
3. Add 250 µL of **MINI 1** Resuspension Solution to the pellet and resuspend the cells completely by pipetting or vortexing.
   - Make sure that RNase A has been added into **MINI 1** Resuspension Solution.
   - No cell pellet should be visible after resuspension of the cells.
4. Add 250 µL of **MINI 2** Cell Lysis Solution, invert the tube 5 times to lyse the cells and incubate at room temperature for 2 minutes.
   - Do not vortex at this step, as it may shear genomic DNA.
   - Continue inverting the tube until the lysate becomes clear.
   - Do not let the mixture incubate for more than 5 minutes.
5. Add 350 µL of **MINI 3** Neutralization Solution and invert the tube 5 times immediately but gently.
   - Invert immediately after adding **MINI 3** Neutralization Solution - will avoid localised precipitation.
   - Do not vortex at this step, as it may shear genomic DNA.
6. Centrifuge for 10 minute. During centrifuging, place a **MINI** Column in a Collection Tube.
7. Transfer the supernatant carefully to **MINI** Column. Centrifuge for 1 minute then discard the flow-through.
   - Do not transfer any white pellet into the column.
8. Add 400 µL of **MINI 4** Washing 1 Solution to MINI Column. Centrifuge for 1 minute then discard the flow-through.
   - Make sure that ethanol (96-100 %) has been added into **MINI 4** Washing 1 Solution.
9. Add 750 µL of **MINI 5** Washing 2 Solution to **MINI** Column. Centrifuge for 1 minute then discard the flow-through.
   - Make sure that ethanol (96-100 %) has been added into **MINI 5** Washing 2 Solution.
10. Centrifuge for an additional 3 minute to dry the column.
    - **Important:** This step will remove any residual liquid that could inhibit subsequent enzymatic reaction.
11. Place **MINI** Column to a new 1.5 mL microcentrifuge tube (not provided).
12. Add 50 µL~100 µL of **MINI 6** Elution Solution or ddH2O (PH:7.0-8.5) to the center of **MINI** Column membrane. Stand the column for 1 minute.
    - Make sure that the **MINI 6** Elution Solution is dispensed on the center of the membrane and is absorbed completely.
13. Centrifuge for 1 minute to elute plasmid DNA.
14. Store plasmid DNA at 4°C or -20°C.