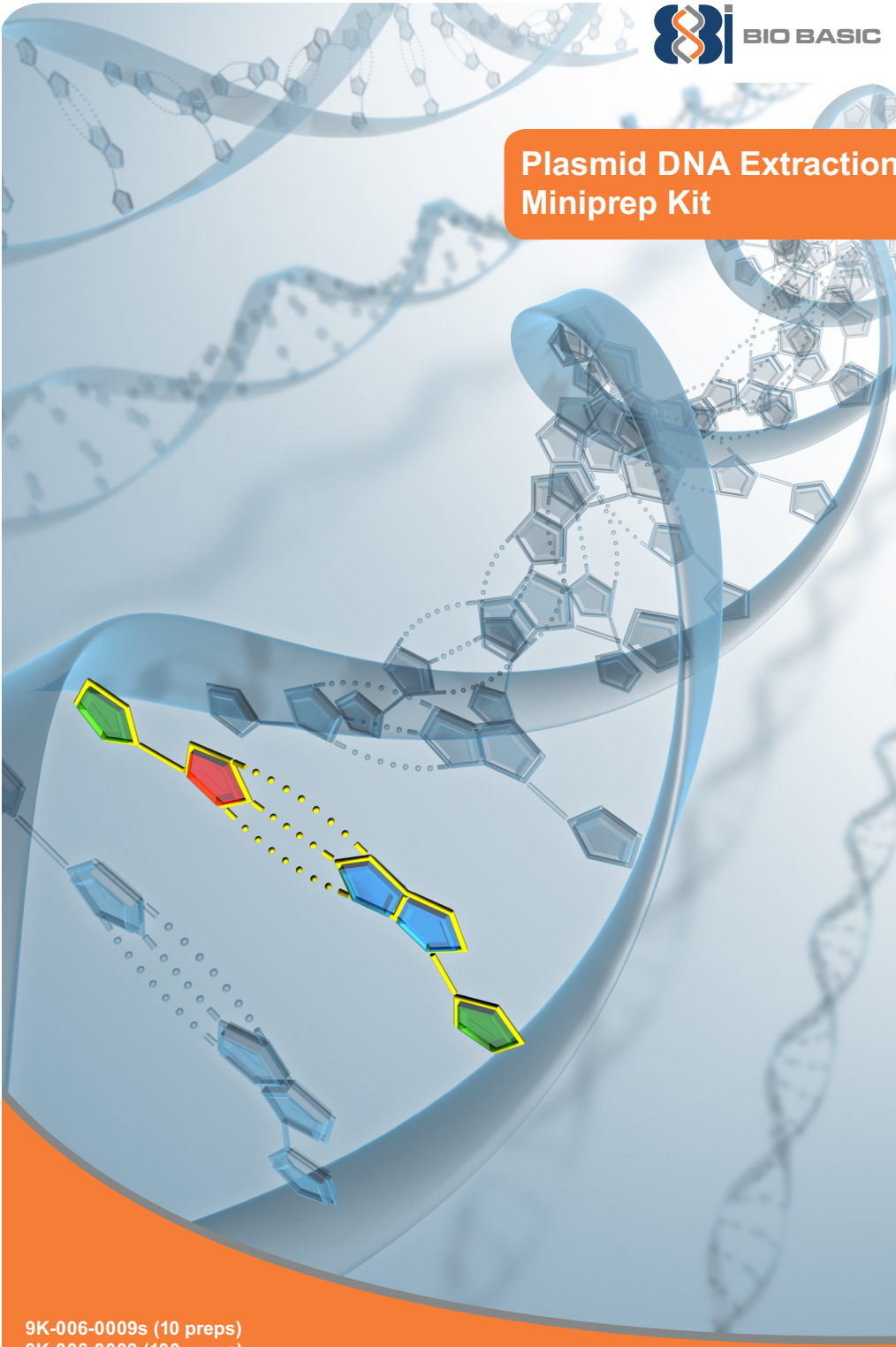

BIO BASIC

Plasmid DNA Extraction Miniprep Kit

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

For Research Use Only

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BIO BASIC INC.

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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 min. The high quality plasmid DNA can be used directly for any downstream applications.

Specification

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

Kit Contents

	9K-006-0009s (10 preps)	9K-006-00010 (100 preps)	9K-006-00010 (200 preps)
MINI 1 Resuspension Solution	3 mL	30 mL	60 mL
MINI 2 Cell Lysis Solution	3 mL	30 mL	60 mL
MINI 3 Neutralization Solution	4 mL	40 mL	80 mL
MINI 4 Washing 1 Solution (concentrated)*	3,5 mL	35 mL	70 mL
MINI 5 Washing 2 Solution (concentrated)**	2 mL	20 mL	40 mL
MINI 6 Elution Solution	1,5 mL	15 mL	30 mL
RNase A (50mg/ml)	6 µL	60 µL	120 µL
MINI Column	10 pcs	100 pcs	200 pcs
Collection Tube	10 pcs	100 pcs	200 pcs
User Manual	1	1	1

* Add 1,5 mL / 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.

Plasmid DNA Extraction Miniprep Kit

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 mins if any recipitation formed. Prevent vigorous shaking of the **MINI 2** *Cell Lysis Solution*.

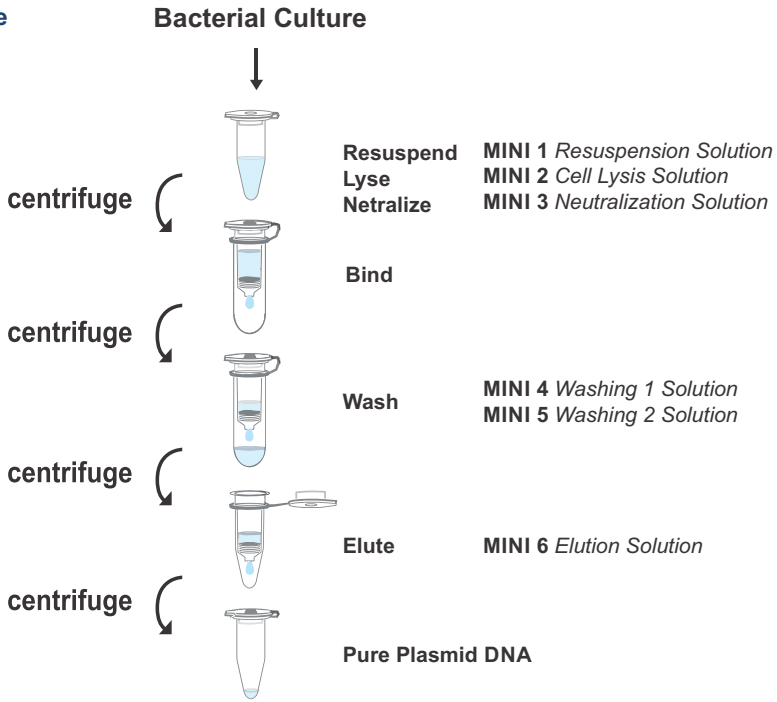
4. To avoid acidification of **MINI 2** *Cell Lysis Solution* from CO_2 in the air, close the bottle immediately after use.

5. For 9K-006-0009s, add 1,5 mL ethanol (96~100%) to **MINI 4** *Washing 1* Solution before first use.
   For 9K-006-0009, add 15 mL ethanol (96~100%) to **MINI 4** *Washing 1* Solution before first use.
   For 9K-006-0010, add 30 mL ethanol (96~100%) to **MINI 4** *Washing 1* Solution before first use.

6. For 9K-006-0009s, add 8 mL ethanol (96~100%) to **MINI 5** *Washing 2* Solution before first use.
   For 9K-006-0009, add 80 mL ethanol (96~100%) to **MINI 5** *Washing 2* Solution before first use.
   For 9K-006-0010, add 160 mL 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 Protocol

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 min 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 min.
   - 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 min.

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 min. During centrifuging, place a **MINI** Column in a Collection Tube.

7. Transfer the supernatant carefully to **MINI** Column. Centrifuge for 1min then discard the flow-through.
   - Do not transfer any white pellet into the column.

8. Add 400 µL of **MINI 4** Washing 1 Solutionto **MINI** Column. Centrifuge for 1min 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 1min 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 min 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 min.
    - Make sure that the **MINI 6** *Elution Solution* is dispensed on the center of the membrane and is absorbed completely.

13. Centrifuge for 1 min to elute plasmid DNA.

14. Store plasmid DNA at 4 °C or -20 °C.

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 10kb, 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 min or incubation at 60 °C for 3 min.

Genomic DNA Contamination

Lysate prepared improperly.

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

Troubleshooting

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 min 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 min.

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 min in **MINI 2** *Cell Lysis Solution.*

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