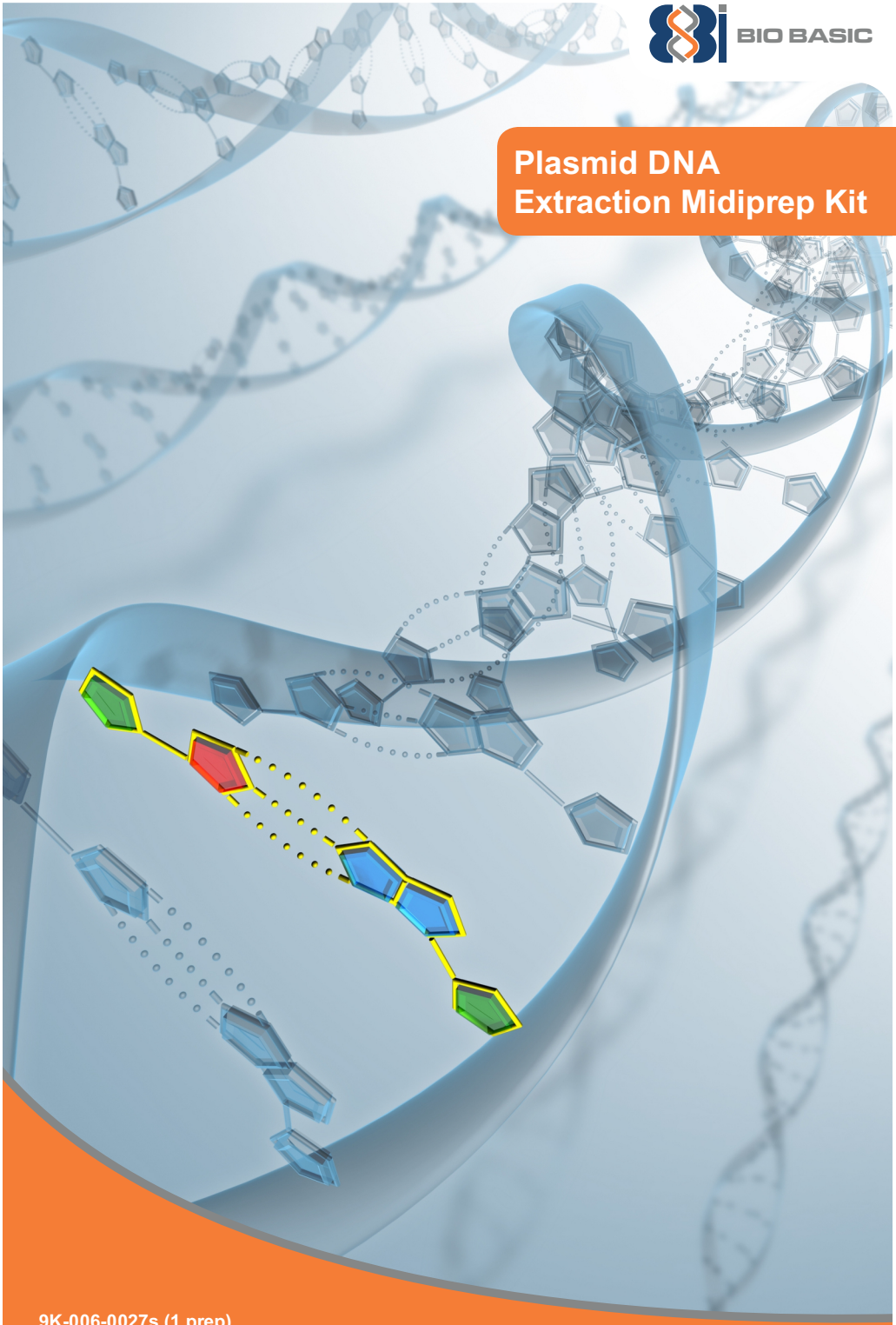
BIO BASIC

Plasmid DNA Extraction Midiprep Kit



9K-006-0027s (1 prep)

9K-006-0027 (25 preps)

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BIO BASIC INC.

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Introduction

Bio Basic Plasmid DNA Extraction Midiprep Kit is an excellent tool offering a rapid and economic method to purify plasmid DNA from bacteria cultures. This technology is based on alkaline lysis and purification by Anion-exchange chromatography. Compared with other harmful and time-consuming procedures such as phenol / chloroform extraction and ethanol precipitation, Bio Basic Plasmid DNA Extraction Kit shortens the handling time to about 2 hours. The high quality plasmid DNA can be used directly for any downstream applications.

Specification

Sample Size	Yield	Handling Time
25~50 mL of bacteria culture for high copy plasmids 100~200 mL of bacteria culture for low copy plamids	up to 100 µg for high-copy plasmids	About 2 hours

Kit Contents

	9K-006-0027s (1 prep)	9K-006-0027 (25 preps)
MIDI 1 *Resuspension Solution*	11 mL	110 mL
MIDI 2 *Cell Lysis Solution*	11 mL	110 mL
MIDI 3 *Neutralization Solution*	11 mL	110 mL
MIDI 4 *Equilibration Solution*	11 mL	110 mL
MIDI 5 *Washing Solution*	55mL	2x275 mL
MIDI 6 *Elution Solution*	13.5 mL	135 mL
RNase A (50 mg / mL)	22 µL	220 µL
MIDI Column	1 pcs	25 pcs
User Manual	1	1

Plasmid DNA Extraction Midiprep 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. Transfer tube content into **MIDI 1** *Resuspension Solution* bottle. Add 250µL of **MIDI 1** *Resuspension Solution* into RNase A tube, rinse tube inside and transfer back into **MIDI 1** *Resuspension Solution* bottle. Store at 4℃.

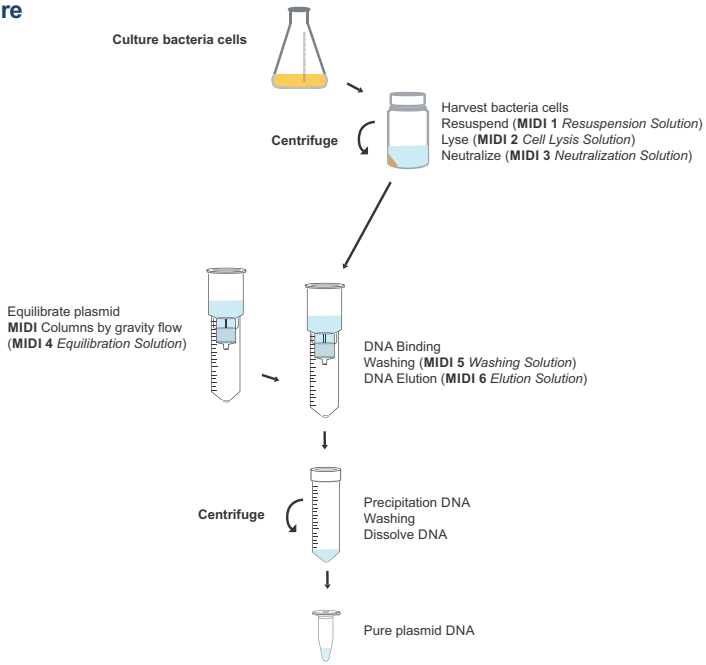
3. Check **MIDI 2** *Cell Lysis Solution* before use. Warm **MIDI 2** *Cell Lysis Solution* at 37°C if any precipitation formed. Prevent vigorous shaking of the **MIDI 2** Cell Lysis Solution.

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

Additional Material Required

1. 50 mL centrifuge tube.
2. Isopropanol
3. 70 % Ethanol

Brief Procedure



General Protocol

1. Harvest the bacterial culture by centrifugation at 6,000 x g for 15 minutes.

2. Add 4 ml of **MIDI 1** *Resuspension Solution* (RNase A added) and resuspend the cell pellet by vortexing or pipetting.

3. Add 4 ml of **MIDI 2** *Cell Lysis Solution* and mix gently by inverting the tube 10 times. Do not vortex to avoid shearing of genomic DNA.

4. Incubate for 3 minutes at room temperature until lysate clears.

5. Add 4 mL of **MIDI 3** *Neutralization Solution* and mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing of genomic DNA.

6. Centrifuge at 15,000 x g for 20 minutes at 4 °C.

7. Transfert supernatant from step 6 in a new tube. Centrifuge at 15,000 x g for 20 minutes at 4°C.

8. Place a **MIDI** Column into a 50 mL centrifuge tube, add 4 mL of **MIDI 4** *Equilibration Solution* to equilibrate the **MIDI** Column and allow the column to empty by gravity flow. Discard the filtrate.

9. Transfer the supernatant from step 7 to the equilibrated **MIDI** Column, and allow the column to empty by gravity flow. Discard the filtrate.

10. Add 10 ml of **MIDI 5** *Washing Solution* to wash the **MIDI** Column and allow the column to empty by gravity flow. Discard the filtrate.

11 Repeat step 10.

12. Place the **MIDI** Column into a clean 50 mL centrifuge tube (not provided) and add 4 mL of **MIDI 6** *Elution Solution* to elute DNA by gravity flow.

13. Precipitate DNA by adding 3 mL of isopropanol to the eluted DNA from Step 12.

14. Mix gently and centrifuge at 20,000 g for 30 minutes at 4 °C.

15. Carefully remove the supernatant and wash the DNA pellet with 2 mL of room temperature 70 % ethanol.

16. Centrifuge at 20,000 g for 10 minutes at 4°C.

17. Carefully remove the supernatant and air-dry the DNA pellet for 10 minutes.

18. Dissolve the DNA pellet in a suitable volume of 10mM tris pH 8.5 or ddH_2O.

Troubleshooting

Low yield

Bacterial cells were not lysed completely.

- Too many bacterial cells were used.
- After **MAXI 3** *Neutralization Solution* addition, break up the precipitate by inverting.
- DNA pellet was lost after precipitation.
- DNA pellet was insufficiently redissolved.

Purified DNA doesn't perform well in downstream application.

RNA contamination

- Make sure that RNase A was has been added in **MAXI 1** *Resuspension Solution* when first using. If RNase A added in **MAXI 1** *Resuspension Solution* has expired, add additional RNase A.
- Too many bacterial cells were used, reduce the sample volume.
- Elution buffer contains EDTA.

Genomic DNA contamination

- Do not use overgrown bacterial culture.
- During **MAXI 2** *Cell Lysis Solution* and **MAXI 3** *Neutralization Solution* addition, mix gently to prevent genomic DNA shearing.
- Lysis time was too long (over 5 minutes).

Too much salt residual in DNA pellet

- Wash the DNA pellet twice with 70% ethanol.

Notes:

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Worldwide

For more information on pricing, complete product line or to locate a Point of Sales near you, please visit our web site or contact one of our Customer Service Representatives.

Email	order@biobasic.com
Phone	1 (905) 474-4493
Toll Free	1 (800) 313-7224
Fax	1 (905) 474-5794

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