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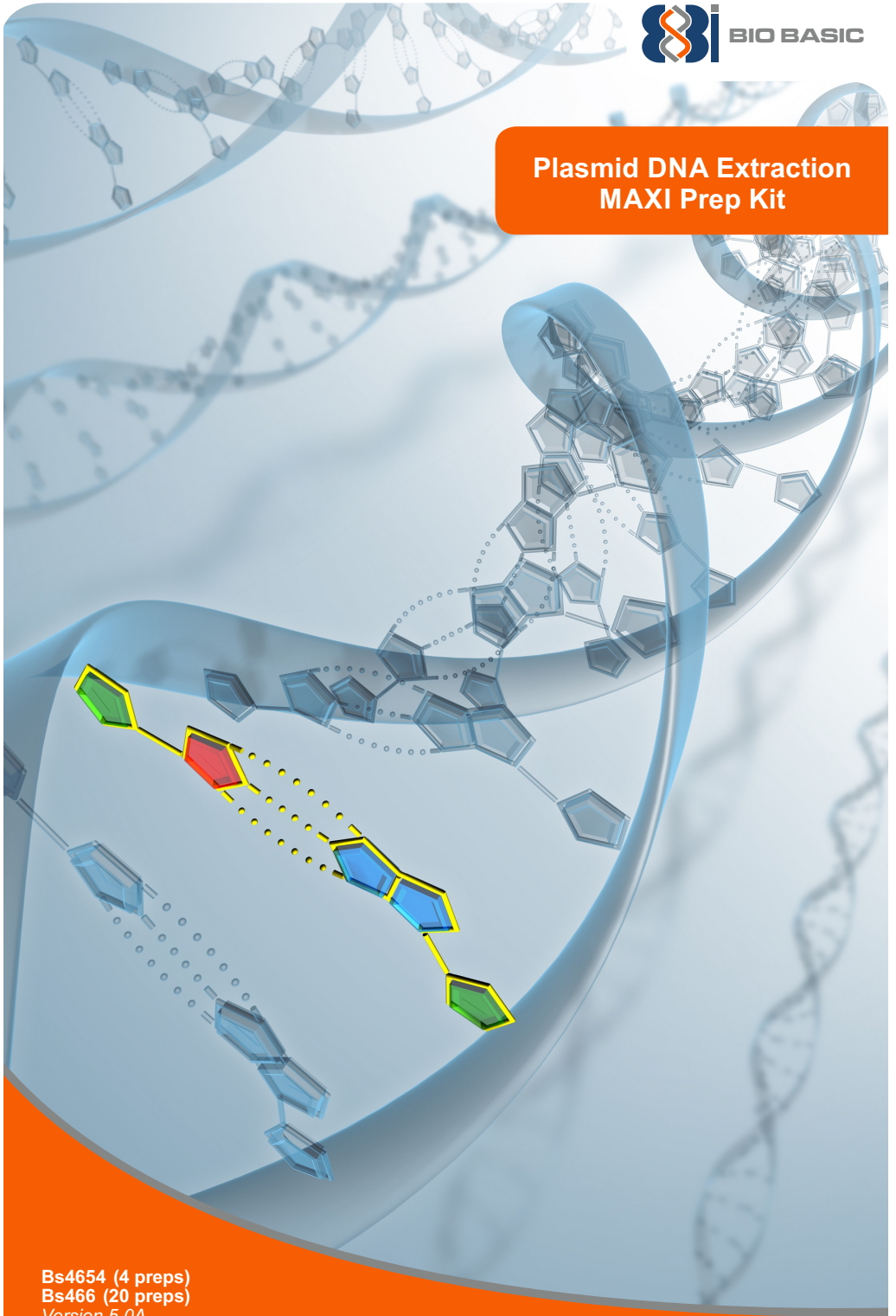
Plasmid DNA Extraction MAXI Prep Kit



Bs4654 (4 preps)
Bs466 (20 preps)
Version 5.0A
Rev 31.8.2015

For Research Use Only

Introduction

Bio Basic Plasmid DNA Extraction Maxiprep Kit is an excellent tool offering a rapid and economic method to purify plasmid DNA from bacterial 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 application.

Specification

Sample Size	Yield	Handling Time
100-250mL of bacterial culture for high copy plasmids	Up to 500 µg for high copy-plasmids	About 2 hours
200-400 mL of bacterial culture for low copy plasmids		

16. Centrifuge at 20,000 x 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 pH8.5 or ddH_2O.

Troubleshooting

Low yield

Bacterial cells were not lysed completely.

- Too many bacterial cells were used.
- After **MAXI 3** *Neutralization Solution*, break up the precipitate by inverting the tube several times.
- 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 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 bacteria 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.

5. Add 10 mL of **MAXI 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. Transfer supernatant from step 6 in a new tube. Centrifuge at 15,000 x g for 20 minutes at 4°C.

8. Place a **MAXI** Column into a 50 mL centrifuge tube, add 10 mL of **MAXI 4** *Equilibration Solution* to equilibrate the **MAXI** Column and allow the column to empty by gravity flow. Discard the filtrate

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

10. Add 25 mL of **MAXI 5** *Washing Solution* to wash the **MAXI** Column and allow the column to empty by gravity flow. Discard the filtrate.

11. Repeat Step 10.

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

13. Precipitate DNA by adding 9mL of isopropanol to the eluted DNA from Step 12.

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

15. Carefully remove the supernatant and wash the DNA pellet with 5mL of room temperature 70% ethanol.

Content

Component	BS4654 (4 preps)	BS466 (20 preps)
MAXI 1 *Resuspension Solution*	55mL	220 mL
MAXI 2 *Cell Lysis Solution*	55 mL	220 mL
MAXI 3 *Neutralization Solution*	55 mL	220 mL
MAXI 4 *Equilibration Solution*	54 mL	270 mL
MAXI 5 *Washing Solution*	4X55 mL	4x275 mL
MAXI 6 *Elution Solution*	54 mL	270 mL
RNase A (50mg/mL)	88 µL	440 µL
MAXI Column	4 pcs	20 pcs
User Manual	1	1

Additional Materials Required

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

Important Notes

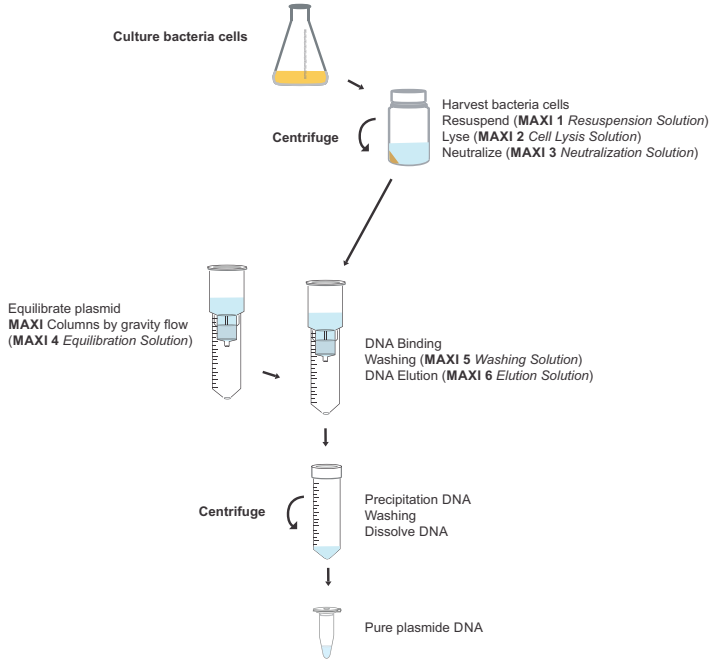
1. Solutions provided in this kit contain irritants, wear gloves and lab coat when handling.

2. Briefly spin RNase A tube to remove drops from the inside of the lid. Transfer tube contents into **MAXI 1** *Resuspension Solution* bottle. Add 250 µL of **MAXI 1** *Resuspension Solution* into RNase A tube, rinse tube inside and transfer back into **MAXI 1** *Resuspension Solution* bottle. Store at 4°C.

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

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

Brief Procedure



General Protocol

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

2. Add 10 mL of **MAXI 1** *Resuspension Solution* (RNase A added) and resuspend the cell pellet by vortexing or pippetting.

3. Add 10 mL of **MAXI 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.