# BIO BASIC
# Worldwide

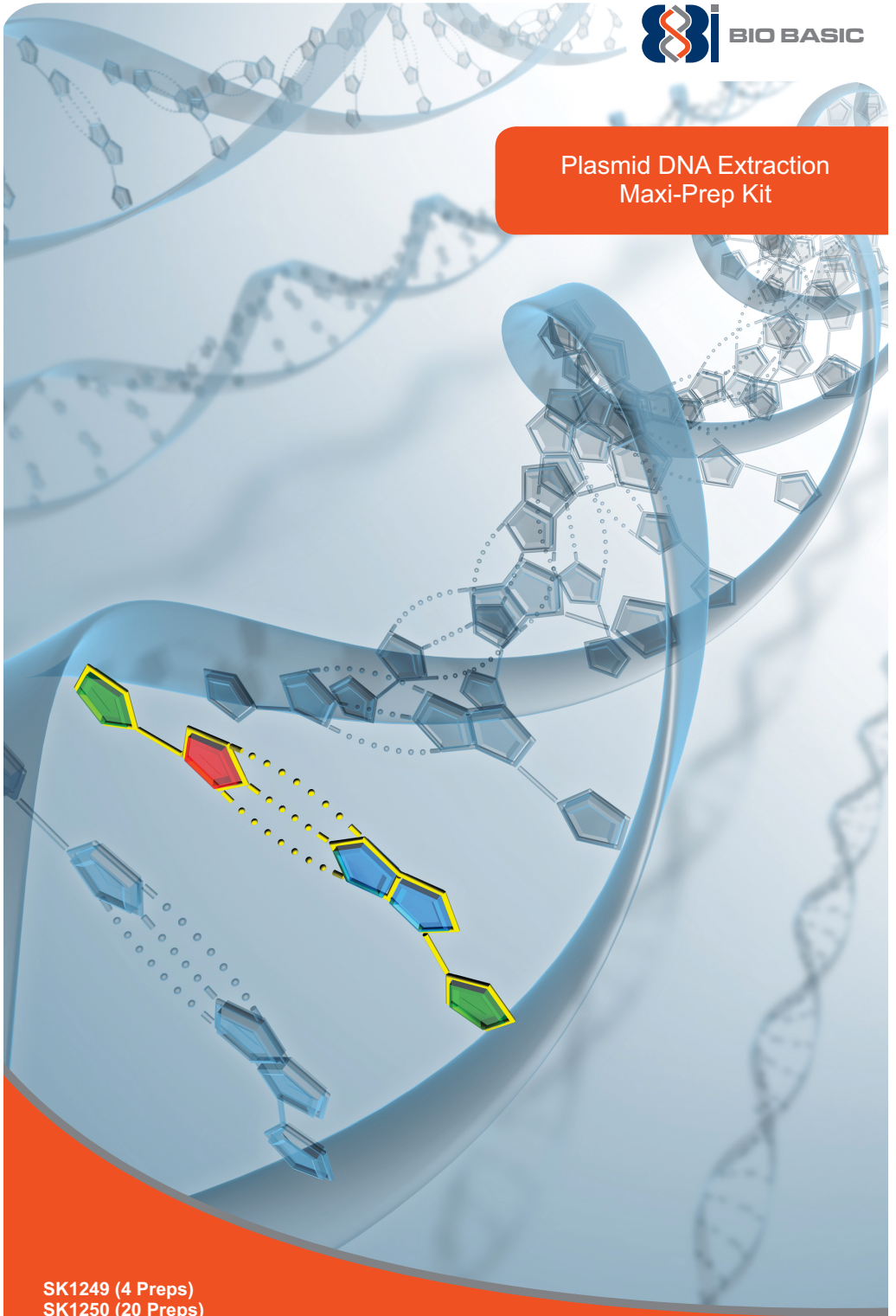
For more information on pricing, complete product line or to locate a Point of Sales near you, please visit our website or contact one of our Customer Service Representatives.


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


BIO BASIC INC.

BIO BASIC

# Plasmid DNA Extraction Maxi-Prep Kit



**SK1249 (4 Preps)**
**SK1250 (20 Preps)**
*QF 24 TV4*
*CV1 2020*

***For Research Use Only***

## *Plasmid DNA Extraction Maxi-Prep Kit*
## *Code: SK1249 (4 Preps)*
## *SK1250 (20 Preps)*



### Description

Bio Basic's Plasmid DNA Extraction Maxi-Prep 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's 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 |
|---|---|---|
| 100-250 ml 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 | | |



**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.

15. Centrifuge at ⩾8000 x g (⩾10,000 rpm) for 5 minutes at room temperature. (The solution is divided into two layers). If the separation of two layers is not clear, you can still continue and proceed step 16.
16. Transfer the supernatant to a new endotoxin-free tube.
17. Repeat step 13 to step 15 1-2 times.
18. Precipitate DNA by adding and 600 µl of 3M Sodium acetate (pH=5.2) and 4.5 ml of isopropanol to the eluted DNA from Step 12.
19. Mix gently and centrifuge at 20,000 x g for 20 to 30 minutes at 4°C.
20. Carefully remove the supernatant and wash the DNA pellet with 5 ml of room temperature 70% ethanol.
21. Centrifuge at 20,000 x g for 10 minutes at 4°C.
22. Carefully remove the supernatant and air-dry the DNA pellet for 10 minutes.
23. Dissolve the DNA pellet in suitable volume of 10mM tris pH8.5 (endotoxin free) or $ddH_2O$ or MAXI 7 Buffer.

## 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.



## Kit Contents

| Components | SK1249 (4 Preps) | SK1250 (20 Preps) |
|---|---|---|
| MAXI 1 Resuspension Solution | 55 ml | 2 x 110 ml |
| MAXI 2 Cell Lysis Solution | 55 ml | 2 x 110 ml |
| MAXI 3 Neutralization Solution | 55 ml | 2 x 110 ml |
| MAXI 4 Equilibration Solution | 54 ml | 2 x 135 ml |
| MAXI 5 Washing Solution | 4 x 55 ml | 4 x 275 ml |
| MAXI 7 Elution Solution | 30 ml | 150 ml |
| Liquid Endotoxin Eliminator | 5 ml | 25 ml |
| 3M Sodium Acetate (pH5.2) | 5 ml | 25 ml |
| Rnase A (50 mg/mL) | 88 µL | 2 x 220 µL |
| MAXI Column | 4 pcs | 20 pcs |
| User Manual | 1 | 1 |

**Additional Material Required:**

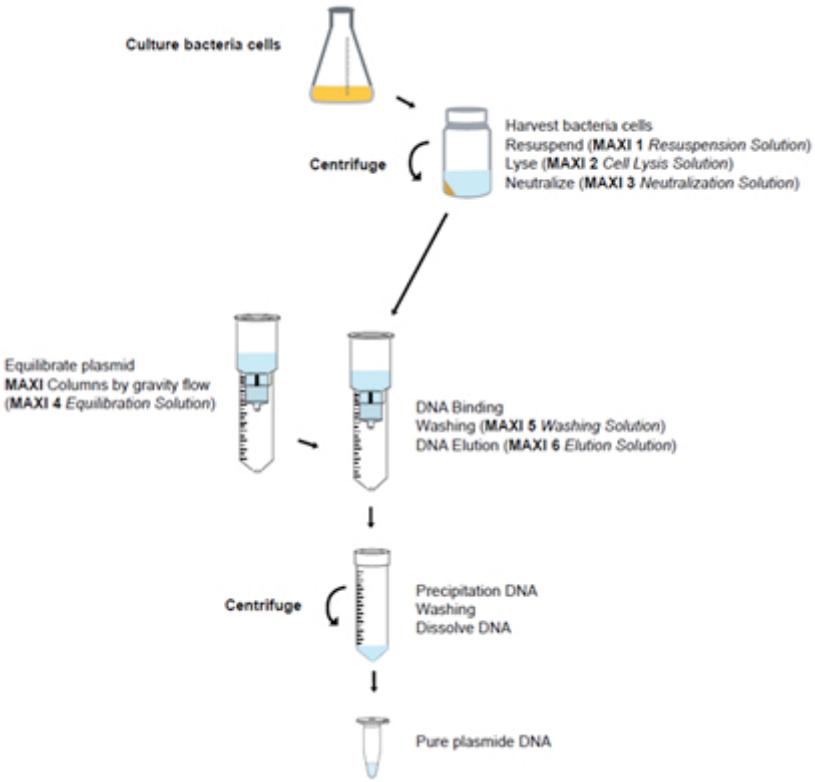
- 50 ml centrifuge tube
- Isopropanol
- 70% Ethanol

## 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.
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 6 ml of MAXI 7 Elution Solution to elute DNA by gravity flow.
13. Add 600 µl of pre-cold Liquid Endotoxin Eliminator to 6 ml of plasmid DNA solution. Incubate on Ice for 10 minutes.
14. Incubate at 65ºC for 1-5 minutes (Solution may show some level of turbidity).