



## ***IsoPure*<sup>™</sup> Maxi II Prep DNA Purification Kit**

### **Product Information and Kit Protocol**

#### TABLE OF CONTENTS

I.	Introduction	2
II.	Components List	3
III.	Additional Materials and Equipment Required	4
IV.	Plasmid DNA Purification Procedures	5
V.	Frequently Asked Questions	7
V.	Appendix	8

**Caution: Buffer C contains a chaotropic agent. Please read this manual carefully before using this kit.**

**Always wear gloves and follow proper lab safety procedures when handling chemicals and reagents.**

## **I. Introduction**

IsoPure Plasmid Maxi II Prep Kit is designed for purification of up to 1.2 mg of high purity plasmid DNA from 200 - 300 ml of bacterial culture. The principle of the kit is based on the modified alkaline lysis technique for plasmid DNA purification.

Here are a few reasons why IsoPure Plasmid Maxi II Prep Kit is superior to its competitors:

- The procedure is simple and can be completed in about an hour;
- Column washing is carried out with 70% ethanol. Other kits use wash buffers containing salts which could inhibit downstream enzymatic applications;
- Purified plasmid DNA is directly eluted into TE buffer or water at the final step. Unlike other plasmid purification kits, the IsoPure Plasmid Maxi II Prep Kit does not require alcohol precipitation and DNA pellet resuspension steps, which often leads excess salts that can downstream enzymatic applications.

## **I. Components List**

### 1) Buffer A (150 ml)

Dissolve RNase with 1 ml of buffer A. Add the dissolved RNase to buffer A and mix well before use. Store the solution at 4 °C after the addition of RNase A.

### 2) Buffer B (150 ml)

Store the solution at room temperature. If the solution is not clear due to precipitation, warm the solution at 37 °C for 10 min. and mix the contents before use.

### 3) Buffer C1 (180 ml)

Store the solution at room temperature. If the solution is not clear due to precipitation, warm the solution at 37 °C for 10 min. and mix the contents before use.

**Caution: Buffer C1 contains a chaotropic agent. Handle with care.**

### 4) RNase (one vial)

Dissolve RNase with 1 ml of buffer A. Add to Buffer A and mix well before use.

### 5) DNA binding column unit (10 units)

### 6) TE buffer (30 ml)

Store at room temperature.

### 7) 2 ml centrifuge tubes (20 tubes)

For plasmid DNA storage.

### 8) 50 ml centrifuge tubes (10 tubes) for plasmid DNA elution.

### 9) Instruction manual.

## **III. Additional Materials and Equipment Required.**

### 1) High speed centrifuge that is able to reach 13,000 rpm

(14,000 - 18,000 x g) with appropriate rotor to hold standard 50 ml high-speed centrifuge tubes.

### 2) Low speed centrifuge with swing-bucket rotor (preferred) or fixed-angle rotor that can hold standard 50 ml centrifuge tubes, and 450 ml centrifuge bottles.

### 3) 50 ml centrifuge tubes for high speed centrifugation.

### 4) 450 ml centrifuge bottles for pelleting bacteria from culture.

### 5) Heat block or water bath for heating TE or sterile water to 65 - 70°C.

### 6) High quality 70% ethanol.

### 7) Sterile water for plasmid DNA elution.

### 8) Spectrophotometer for DNA quantitation.

#### IV. Plasmid DNA Purification Procedure

1. Inoculate 200-300 ml LB containing appropriate antibiotic in a 1 liter flask with 1.5 ml of an overnight culture of *E. Coli* containing the desired plasmid. Grow the culture at 37 °C for 12 - 16 hours with vigorous shaking (200-300 rpm).
2. Transfer the culture to a 450 ml centrifuge bottle. Pellet down the bacteria by centrifugation for 10 min. at 5,000 x g at room temperature.
3. Resuspend the bacterial pellet in **10 ml** of buffer A by pipetting (Complete resuspension of bacteria is critical for high yield). **Be sure to add RNase into buffer A before use.**
4. Transfer the suspension to a 50 ml centrifuge tube. Add **10 ml** of Buffer B. Close the cap and mix the tube gently and thoroughly by inverting the tube 10 times. Let it stand at room temperature for 5 min. The mixture should become clear and viscous. **Do Not Vortex! Vortexing shears genomic DNA and leads to genomic DNA contamination.**
5. Add **15 ml** of buffer C1 to the tube. Gently mix the solution by inverting the tube 10 times. White precipitation should appear after mixing. **Caution: Buffer C1 contains chaotropic agent. Handle with care. Do Not Vortex! Vortexing shears genomic DNA and leads to genomic DNA contamination.**
6. Pour the mixture directly to a high speed centrifuge tube. Centrifuge the mixture for 10 min. at 13,000 rpm (14,000 - 18,000 x g) at room temperature or 4 °C\*. Alternatively if a high speed centrifuge is not available the sample can be spun longer at lower g force, e.g. 20 min. at 9,000 x g.
7. Carefully transfer the supernatant to a 50 ml centrifuge tube. Keep the tube at room temperature or 4 °C (on ice)\*.
8. Transfer half of the supernatant to the DNA binding column unit. Centrifuge the column for 5 min. at 5,000 x g at room temperature or 4 °C\*. Carefully remove the DNA binding column from the unit and discard the pass through from the collection tube. Reassemble the DNA binding column unit.
9. Repeat step 8 for the remaining supernatant using the same column.

10. Add **20 ml** of 70% ethanol to the DNA binding column unit. Centrifuge the unit at for 5 min. 5,000 x g at room temperature. Carefully remove the DNA binding column from the unit and discard the pass through from the collection tube. Reassemble the DNA binding column unit.

11. Repeat step 10.

12. Centrifuge the unit for an additional 10 min. at 5,000 x g at room temperature. Open the cap of the unit and let the unit stand at room temperature for 10 min. to dry any leftover ethanol.

**Optional step for automatic DNA sequencing application: Centrifuge the unit for 20 min instead of 10 min. at 5,000 x g at room temperature, let the open column stand for 10 min to ensure complete removal of wash buffer.**

13. Transfer the DNA binding column to a new 50 ml centrifuge tube. Add **1 ml** of preheated (65 - 70 °C) TE or sterile water (not included) to the center of the DNA binding column and let it stand at room temperature for 1 min. Elute the plasmid DNA by centrifuging the unit for 5 min. at 5000 x g at room temperature.

**For DNA sequencing or any other salt sensitive applications (such as difficult ligation or transformation), elute the plasmid DNA with sterile water instead TE. Plasmid DNA suspended in water needs to be stored frozen (-20°C) to prevent hydrolyzation.**

14. For maximum efficiency repeat step 13.

**60 – 75% of plasmid DNA is eluted from the column with the first elution. If high concentration of plasmid DNA is desired do not combine the first elution with the second elution. Use the first elution Plasmid for applications requiring high DNA concentration.**

15. Transfer the eluted plasmid DNA from the collecting tube to a 2 ml tube (included) and store the DNA at 4 °C if it is eluted with TE, or - 20 °C if it is eluted with water.

**\* Note 1: Steps 6 - 9 can be performed at room temperature. If steps 6 - 9 are carried out at 4 °C, the yield of supercoiled plasmid DNA will significantly increase.**

## **V. Frequently Asked Questions**

Q. Low yield

- A. 1) Too many cells and not enough buffer to lyse cells completely. After resuspension of cell pellet in buffer A (step 3), the final total volume should not exceed 12 ml. If the volume exceeds 12 ml, double the volume of buffer A, B and C1 in the purification process to ensure complete lysis.
- 2) Low copy plasmid.

Harvest twice amount of cell culture. Growing cells in rich medium such as Terrific Broth can increase the density of cells. Remember to use twice the volume of buffer A, B and C1 in the purification process to ensure complete lysis.

- 3) Make sure the cells are resuspended in buffer A (step 3) completely. Incomplete resuspension of cells decreases the efficiency of lysis.
- 4) Make sure there is no precipitation in buffers B and C1. Precipitation in these buffers decreases the efficiency of lysis. Warm the solutions at 37 °C for 10 min. and Vortex or shake well to redissolve the precipitants if necessary.

Q. Contamination of high molecular weight chromosomal DNA

A. During step 4 and 5, samples should not be vortexed or shaken vigorously. Also step 4 (lysis step) should not exceed more than 10 min. Both can cause shearing of the genomic DNA and lead to high molecular weight chromosomal DNA contamination.

Q. Sample contains RNA

- A. 1) Too many cells were harvested. Use twice amount buffer A, B and C1 in the purification process.
- 2) RNase activity is weakened. Buffer A with RNase A should be stored at 4°C to maintain its full activity. Add more RNase (50 ug/ml) to buffer A if the activity is lost.

Q. Sample floats upon loading in agarose gel

A. Sample contains ethanol from the washing step. Make sure to follow step 12 closely. If problem persists, spin the unit for 20 min. instead of 10 min. as recommended. Let the column air dry after spinning.

Q. O.D. ratio between 260 nm and 280 nm above 2.2

The kit normally produces plasmid DNA with O.D. ratio between 1.9 to 2.2. If the ratio is above 2.2,

- 1) Washing (steps 10-11) was not completed. Repeat step 11 one more time.
- 2) Sample contains ethanol from the washing step. Make sure step 12 is followed closely. If problem persists, spin the unit for 20 min. instead of 10 min. as recommended.

## **VI. Appendix.**

### **A. Culture Media recipes**

#### **LB broth**

*10 g of Tryptone; 5 g of yeast extract; 5 g of NaCl*

Adjust the pH of the medium to 7.4 with NaOH. Add water to the medium to a final volume of 1000 ml. Sterilize the medium by autoclaving. Preheat or cool down the media to the incubation temperature before inoculating.