Quantum Prep™ Plasmid Midiprep Kit

Instruction Manual

Catalog Number 732-6120

For Technical Service
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Table of Contents

Section 1
Introduction

1.1 Overview

The Quantum Prep plasmid midiprep kit has been optimized for the rapid purification of DNA from 40 to 50 ml of liquid culture. This kit uses the optimized alkaline lysis protocol and patented DNA binding matrix found in the Quantum Prep plasmid miniprep Kit.[1] This matrix, made up of the silicon dioxide exoskeleton of diatoms, results in DNA of the highest purity and yield with the minimum of processing steps. A single midiprep can be processed in approximately 45 minutes and requires no ethanol precipitation. The Quantum Prep kit was optimized using high-copy number plasmids grown in either Luria Broth or Terrific Broth.[2] It requires only 40 ml of culture and a single harvesting step. Multiple samples can be processed simultaneously with minimal increase in time. DNA purified using the Quantum Prep midiprep kit can be used directly for enzymatic modification, cell transfection and transformation, fluorescent sequencing and *in vitro* transcription. Each lot is qualified for automated fluorescent sequencing, yield, and enzymatic manipulation. Yields of high copy plasmid are in the range of 100 µg to over 300 µg from 40 ml of culture.

1.2 Contents

The Quantum Prep plasmid midiprep kit contains sufficient reagents for 20 plasmid midipreps. Additional Midiprep Wash Buffer will be required if following recommendations for EndA+ strains such as HB101 and MC1061.

Cell Resuspension Solution	110 ml
Cell Lysis Solution	110 ml
Neutralization Solution	110 ml
Quantum Prep matrix	20 ml
Midiprep Wash Buffer	125 ml
Spin columns and caps	20
2 ml microcentrifuge tubes	40

1.3 Storage and Stability

All components are guaranteed for 12 months from the date of purchase, when stored at room temperature and used as described in this manual.

Section 2
Protocol

Yields of plasmid DNA depend on factors such as vector copy number, insert DNA, growth conditions, and media. This midiprep protocol has been optimized for 40 ml of culture of high-copy number plasmids grown in Luria Broth or Terrific Broth. It is not recommended that larger volumes of culture be used for medium to high-copy

plasmids, as that will not usually result in higher yields using this protocol. For low-copy number plasmids (*e.g.* pBR322), up to 60 ml of culture can be used with the volumes in this protocol. Larger volumes may not result in higher yields due to the increase of other cellular contaminants.

It is highly recommended that the plasmid DNA be isolated from a host strain that contains a mutation in the endonuclease I gene (*endA*) of *E. coli*. Isolation of DNA from strains containing active endonuclease I gene product (such as HB101 and MC1061) may result in samples which contain trace amounts of nuclease. Additional precautions and wash steps are required with these strains (see Section 2.1 and 2.2). For the highest quality DNA, these host strains should be avoided.

2.1 Recommendations for Best Results

A precipitate may form in the lysis and neutralization solutions due to cold temperatures from ambient winter shipping conditions, cool laboratory temperatures, or factory storage conditions. This will not affect the performance of the product. If a precipitate is observed, warm the bottles to 37 °C until it is redissolved. Use and store at room temperature.

- Add 125 ml of 95% ethanol to the Midiprep Wash Buffer before first use.
- For increased plasmid yield a second elution may be performed. Eluting with water or TE heated to 70 °C may also result in an improved yield.

- To increase DNA concentration, elute with less than the recommended elution volume or concentrate the DNA by ethanol precipitation.
- If using EndA+ strains, grow in Luria Broth and purify DNA from only 20 ml of culture using recommended volumes and extra wash steps noted.

2.2 Protocol

Note: All steps are performed at room temperature unless otherwise indicated.

1. Inoculate plasmid-containing bacteria into 50 ml of liquid media in a 250 ml Erlenmeyer flask. Incubate overnight (15–18 hr) at 37 °C in a rotary shaker at 300 rpm.
2. Transfer 40 ml of the overnight culture to a 50 ml screw-cap centrifuge tube. Spin down cells by centrifugation for 5 minutes at 5,000 rpm (approximately 3,000 x g). Discard the supernatant.
3. Add 5 ml of Cell Resuspension Solution to the cell pellet. Vortex the cells to resuspend the pellet. Be sure that the cell pellet is completely resuspended.
4. Add 5 ml of Cell Lysis Solution. Mix by inverting the tube 6–8 times. Do not vortex, since this may cause shearing of the chromosomal DNA, resulting in contamination of the plasmid DNA. The solution should become viscous and somewhat clear.

5. Add 5 ml of Neutralization Solution. Cap the tube and mix by inverting the tube 6–8 times. The solution should become cloudy and develop a flocculant white precipitate.
6. Centrifuge for 10 minutes at 8,000 rpm (7,500 x g). Carefully pour the supernatant into a new tube. Try not to transfer any of the precipitate. However, a small amount of the debris will not affect plasmid purification.
7. Resuspend the Quantum Prep matrix by shaking vigorously. Add 1.0 ml of Quantum Prep matrix to the cleared lysate from step 6. Swirl gently for 15–30 seconds to mix. Centrifuge for 2 minutes at 8,000 rpm to pellet the matrix.
8. Carefully pour off the supernatant from the pelleted matrix.

   Add 125 ml of 95% ethanol to the wash buffer before first use.
9. Add 10 ml of wash buffer to the matrix. Resuspend the matrix in the wash buffer by shaking.
10. Centrifuge for 2 minutes at 8,000 rpm. Carefully pour the wash buffer from the pelleted matrix. Add 600 µl of wash buffer to the matrix and resuspend. **If isolating DNA from an EndA+ strain do an additional 10 ml wash step at this point.**

    Additional wash buffer can be ordered using Bio-Rad catalog number 732-6134. Alternatively the Midiprep Wash Buffer, composed of 200 mM NaCl, 40 mM Tris, 4 mM EDTA, pH 7.5 plus an equivalent amount of 95% ethanol, may be made.

11. Remove a spin column from the bag and snap off the tab at the tip. Place the spin column inside a 2 ml collection tube. Transfer the resuspended matrix from the previous step to the spin column. Puncture a hole in a spin column cap with a small, sharp object and cap the column with it. Spin 30 seconds in a microcentrifuge at 12–14,000 x g. Remove the spin column from its microcentrifuge tube, discard the wash buffer at the bottom of the tube and replace the filter in the same tube.
12. Add 500 µl of wash buffer and spin 30 seconds in a microcentrifuge at 12–14,000g. Remove spin column and discard Wash Buffer. **If isolating DNA from an EndA+ strain do two additional 500 µl wash steps.**
13. Replace the spin column in the tube and spin an additional 2 minutes at maximum speed to remove any residual wash buffer.
14. Transfer the spin column to a clean 2 ml microcentrifuge tube. Add 600 µl of water or TE to the matrix. Spin for 2 minutes in a microcentrifuge and discard the spin column containing the matrix. Store plasmid DNA at -20 °C.

Section 3
References

1. U.S. Patent 5,075,430 issued to Bio-Rad Laboratories.
2. Ausubel *et al*., Current Protocols in Molecular Biology, Wiley-Interscience, New York (1987).

Section 4
Product Information

Catalog Number	Product Description
732-6137	**Quantum Prep MaxiPrep Spin Baskets,** 10
732-6134	**Quantum Prep Maxi/Midiprep Wash Buffer,** 270 ml
732-6120	**Quantum Prep Plasmid Midiprep Kit,** 20 preps
732-6122	**Quantum Prep Neutralization Solution,** 110 ml
732-6116	**Quantum Prep Midi Spin Columns,** 20 with caps
732-6024	**Quantum Prep Wash Buffer,** 250 ml
732-6100	**Quantum Prep Plasmid Miniprep Kit,** 100 preps
732-6110	**Quantum Prep Matrix,** 20 ml