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Life Science Group

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Quantum Prep® Plasmid Miniprep Kit

Instruction Manual

Catalog Number 732-6100

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Section 1
Introduction

1.1 Overview

The original alkaline lysis method for purifying plasmid DNA from bacterial cultures requires organic reagents and time-consuming steps to obtain high quality DNA. The Quantum Prep plasmid miniprep kit has been optimized for the rapid purification of high-quality, high-yield plasmid DNA from 1–2 ml liquid cultures. This kit uses the silicon dioxide exoskeleton of diatoms as the DNA binding matrix.[1] The advantages of this porous substrate include ease of resuspension, high affinity for DNA, simple and efficient processing, elution in deionized water, and an inherently large surface-to-volume ratio. All of these properties contribute to the highest purity and yields of DNA. Plasmid DNA purified with the Quantum Prep kit can be used directly for fluorescent sequencing, cell transfection, electroporation, and enzymatic restriction and modification.

Plasmid yield depends on a number of factors, such as the vector copy number, culture volume and aeration, bacterial strain, and growth media. The Quantum Prep kit was optimized using high copy number plasmids grown in rich media, such as Terrific Broth.[2] The Quantum Prep kit permits yields as high as 25 µg from a 1.5 ml culture of DH5αF'[pTZ18U] grown in Terrific Broth. Yields as high as 40 µg have been obtained with other plasmids using the Quantum Prep miniprep kit.

1.2 Contents

The Quantum Prep kit contains reagents sufficient for 100 plasmid minipreps.

20 ml	Cell Resuspension Solution
25 ml	Cell Lysis Solution
25 ml	Neutralization Solution
20 ml	Quantum Prep matrix
63 ml	Wash Buffer
100	Spin Filters
100	Wash Tubes
100	Collection Tubes

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

2.1 Recommendations for Best Results

- The lysis and neutralization solutions may exhibit salt precipitation due to cold temperatures from ambient winter shipping conditions or cool lab temperatures. The product will not perform optimally if the salt precipitates out of the solution. If precipitation is observed upon receipt, warm the bottles to 37 °C with occasional mixing until redissolved. Store at room temperature.
- The 1.5 ml microcentrifuge collection tubes supplied with this kit will accommodate the Spin Filters for elution of purified plasmid. The Spin Filters will also fit in most (but not all) commonly available 1.5 ml microcentrifuge tubes.

- To improve the total yield of DNA, elute with twice the recommended volume, *i.e.*, 200 ml, of deionized H_2O or TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). Eluting with H_2O yields more DNA than eluting with TE. Eluting with H_2O or TE pre-heated to 70 °C will also improve the yield. These high temperatures may partially denature large plasmids.
- To increase the concentration of DNA eluted, use one half the recommended volume of H_2O or TE, *i.e.*, 50 ml. Alternatively, the purified DNA may be ethanol precipitated and resuspended in 10–20 ml of water or TE to achieve a higher concentration. See Figure 1 for a detailed comparison of DNA yield and concentration versus elution volume.

2.2 Protocol

All centrifugation steps are performed at maximum speed (12–14,000 x g).

1. Transfer an overnight culture (1–2 ml) of plasmid-containing cells to a microcentrifuge tube. Pellet the cells by centrifugation for 30 seconds. Remove all of the supernatant by aspirating or pipetting.

2. Add 200 µl of the Cell Resuspension Solution and vortex or pipet up and down until the cell pellet is completely resuspended.
3. Add 250 µl of the Cell Lysis Solution and mix by gently inverting the capped tube about 10 times (do not vortex). The solution should become viscous and slightly clear if cell lysis has occurred.
4. Add 250 µl of the Neutralization Solution and mix by gently inverting the capped tube about 10 times (do not vortex). A visible precipitate should form.
5. Pellet the cell debris for 5 minutes in a microcentrifuge. A compact white debris pellet will form along the side or at the bottom of the tube. The supernatant (cleared lysate) at this step contains the plasmid DNA.
6. While waiting for the centrifugation step at step 5, insert a Spin Filter into one of the 2 ml wash tubes supplied with the kit. Mix the Quantum Prep matrix by repeated shaking and inversion of the bottle to insure that it is completely suspended. (No tubes are supplied with the sample size kit. However, most 2.0 and 1.5 ml tubes will accommodate the Spin Filters.)
7. Transfer the cleared lysate (supernatant) from step 5 to a Spin Filter, add 200 µl of thoroughly suspended matrix, then pipet up and down to

mix. If you have multiple samples, transfer the lysates first, then add matrix and mix. When matrix has been added to all samples and mixed, centrifuge for 30 seconds.

The correct final formulation of the Wash Buffer is 50% ethanol, added by the user. To achieve this formulation , add one volume, 63 ml, of 95% or 100% ethanol to the Wash Buffer before first use of the Quantum Prep kit. (If using a sample size kit, please observe the smaller volumes involved.)

8. Remove the Spin Filter from the 2 ml tube, discard the filtrate at the bottom of the tube, and replace the filter in the same tube. Add 500 µl of Wash Buffer and wash the matrix by centrifugation for 30 seconds.
9. Remove the Spin Filter from the 2 ml tube, discard the filtrate at the bottom of the tube and replace the filter in the same tube. Add 500 µl of Wash Buffer and wash the matrix by centrifugation for a full **2 minutes** to remove residual traces of ethanol.
10. Remove the Spin Filter and discard the microcentrifuge tube. Place the filter in one of the 1.5 ml collection tubes supplied with the kit or any other standard 1.5 ml microcentrifuge tube which will

accommodate the Spin Filter. Add 100 µl of deionized H_2O or TE. Elute the DNA by centrifugation for 1 minute at top speed.

11. Discard the Spin Filter and store the eluted DNA at -20 °C.

2.3 Helpful Hints

1. It is recommended that the cells do not let sit longer than 5 minutes at step 3 before proceeding to step 4. Additionally, it is recommended that the cells do not let sit longer than 10 minutes at step 4 before proceeding to step 5.
2. Due to the fixed angle for most microcentrifuges, the matrix may pellet against the side of the Spin Filter. For optimal elution of DNA from the matrix in Step 10 of the protocol, the elution liquid (water or TE) should be applied at the highest portion of the matrix, *i.e*, near the side of the Spin Filter.
3. The use of strains deficient in the endonuclease I gene product (*end* A1 genotype), such as DH5αF', is recommended for improving the quality of plasmid DNA prepared from minipreps.

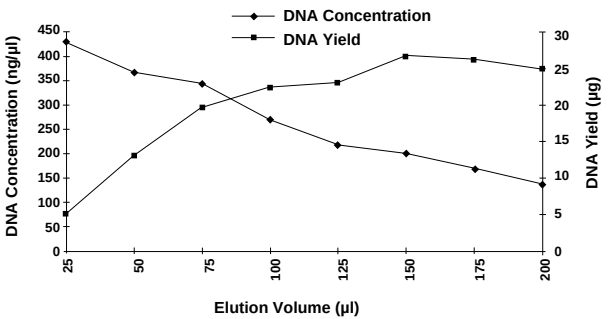

Fig. 1. Effect of elution volume on DNA concentration and yield from a 1.5 ml culture of DH5αF'[pTZ18U] grown in terrific broth and purified using the Quantum Prep plasmid miniprep kit.

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
Plasmid Preps	
732-6100	**Quantum Prep Plasmid Miniprep Kit**
732-6110	**Quantum Prep Matrix,** 20 ml
732-6027	**Quantum Prep Mini Spin Filters,** 100
732-6115	**Quantum Prep Neutralization Solution,** 25 ml
732-6122	**Quantum Prep Neutralization Solution,** 125 ml
732-6024	**Quantum Prep Wash Buffer,** 250 ml
732-6120	**Quantum Prep Plasmid Midiprep kit,** 20 preps
732-6130	**Quantum Prep Plasmid Maxiprep kit,** 10 preps

Catalog Number	Product Description
	Purification From Agarose
732-6160	**Quantum Prep Gel Slice Kit**
732-6165	**Quantum Prep Freeze 'N Squeeze Spin Columns,** 25
732-6166	**Quantum Prep Freeze 'N Squeeze Spin Columns,** 100
	Purification of PCR Reactions
732-6300	**Quantum Prep PCR Kleen Spin Columns,** 25
732-6301	**Quantum Prep PCR Kleen Spin Columns,** 100
	Purification of Labeling Reactions
732-6223	**Micro Bio-Spin P-30 Tris,** 25
732-6224	**Micro Bio-Spin P-30 Tris,** 100
732-6250	**Micro Bio-Spin P-30 Tris, RNase-free,** 25
732-6251	**Micro Bio-Spin P-30 Tris, RNase-free,** 100

Contact your local Bio-Rad Laboratories representative for more information on the following related products.

DNA Purification Kits

Nucleic Acid Electrophoresis Cells and Power Supplies

Nucleic Acid Blotting Equipment and Membranes

Electrophoresis Buffers and Gel Reagents