QuickClean 5M Miniprep Kit **Cat. No. L00193**

Technical Manual No. 0181 **Update Date 03252008**

I. DESCRIPTION

The QuickClean 5M Miniprep Kit is a reagent kit from GenScript. It contains all the necessary reagents, mini-columns, and other supplies for quick, reliable, high-quality plasmid DNA or cosmid DNA preparation from bacteria such as *E. coli* cell culture. Plasmid and cosmid DNA molecules are selectively adsorbed onto the QuickClean column, and other impurities such as proteins, salts and nucleotides are washed away. Eluted in a small volume low-salt buffer with complete removal of contaminants and inhibitors, the purified plasmid or cosmid DNA is immediately ready for many downstream applications such as PCR, transformation, restriction enzyme digestion, cloning, sequencing, *in vitro* translation, and transfection.

II. KIT CONTENTS

QuickClean 5M Miniprep Kit contains enough supplies for 250 minipreps.

L00193 Components	250 Preps
RNase A	1 ml (3 mg/ml)
Solution I	30 ml
Solution II	60 ml
Solution III	100 ml
Wash solution	55 ml
Elution buffer	25 ml
QuickClean columns	250
2 ml Collection tubes	250
Protocol	1

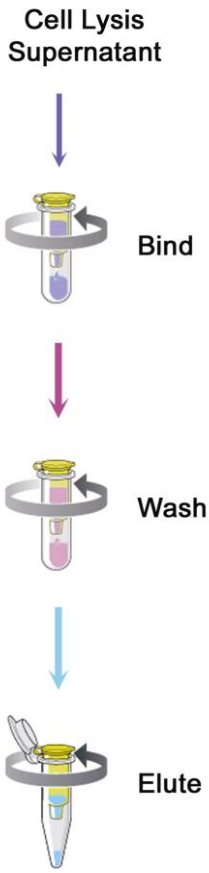
III. APPLICATIONS

The QuickClean 5M Miniprep Kit enables the preparation of

Overview Procedure

high-quality plasmid DNA ready for most downstream applications such as:

- PCR and cloning
- Restriction enzyme digestion
- Transformation
- Sequencing
- *In vitro* translation



IV. KEY FEATURES

- Easy to perform: QuickClean's simple and rapid procedure purifies DNA in five minutes.
- High capacity: Each column has a capacity of 20 µg plasmid DNA.
- High purity: The kit completele removes contaminants and inhibitors.
- Recovery is typically between 50% and 90%.

V. STORAGE

This kit should be stored dry at room temperature. So stored, the kit is stable for 12 months. Solution I with RNase A added should be stored at 2-8°C and remains stable for 6 months (for long-term storage, store at -20°C).

VI. PLASMID MINIPREP PROTOCOL

The following steps may be performed ahead of time:

1. Transfer 1 ml of RNase A solution to solution I and mix well. Solution I with RNase A should be stored at 4 °C for frequent use or at -20 °C for long-term storage.
2. Add 220 ml of 96-100% of ethanol to 55 ml of wash solution and mix well.
3. Some precipitate may form in solution II after long periods of storage. Dissolve the precipitate by mixing gently. Otherwise, warm the container to 37 °C for a few minutes.

Procedure:

1. Transfer 1.5 ml of the overnight culture to a 1.5 ml microcentrifuge tube and centrifuge at 12,000 rpm for 30 seconds. Remove and discard the supernatant. For low copy number plasmids, use 3-5 ml of the overnight culture (spin down cells 2-3 times in the same tube) and double the volume of solutions I, II and III.
2. Add 100 µl of solution I to the pellet, cap the tube and resuspend the cells. This can be done by vortexing, but the following method may be faster: hold the microcentrifuge tube from the top and run the narrow end quickly along the top of an empty microcentrifuge storage rack. Repeat four times or until the cells are resuspended.
3. Add 200 µl of solution II to the mixture. Mix gently by inverting the tube 4-6 times. To avoid contamination by genomic DNA, do not vortex.
4. Add 300 µl of solution III, and mix gently by inverting the tube 4-6 times.

5. Centrifuge at 12,000 rpm for five minutes. Transfer the supernatant to the column. Centrifuge at 12,000 rpm for 30 seconds.
6. Remove and discard the flow-through. Add 500 µl of wash solution to the column, and centrifuge at 12,000 rpm for 30 seconds. If necessary, repeat wash procedure once.

7. Remove and discard the flow-through. Centrifuge at 12,000 rpm for additional 60 seconds to remove residual wash solution.
8. Transfer the column to a clean 1.5-ml microcentrifuge tube. Add 50 µl of elution buffer to **the center of the column membrane**. Let the column stand at room temperature for one minute. Centrifuge at 12,000 rpm for one minute.

Note: If residual ethanol is present in the elution solution, it may complicate the loading into gels for analysis. The loading solution may rise or "blow up", instead of sinking smoothly into the wells of gel. The addition of more loading buffer can prevent this phenomenon. For example, use 5X loading buffer as 3X loading buffer or double the volume of loading buffer.

VII. EXAMPLES

A. Plasmid DNA preparation from *E. coli* bearing high copy number plasmid.

The QuickClean 5M Miniprep Kit is here compared to a commercially available kit (Competitor A) for rapid plasmid purification from *E. coli* bearing high copy number plasmid. Both kits are used in compliance with the protocols provided by the manufacturers. The results are shown in Figure 1.

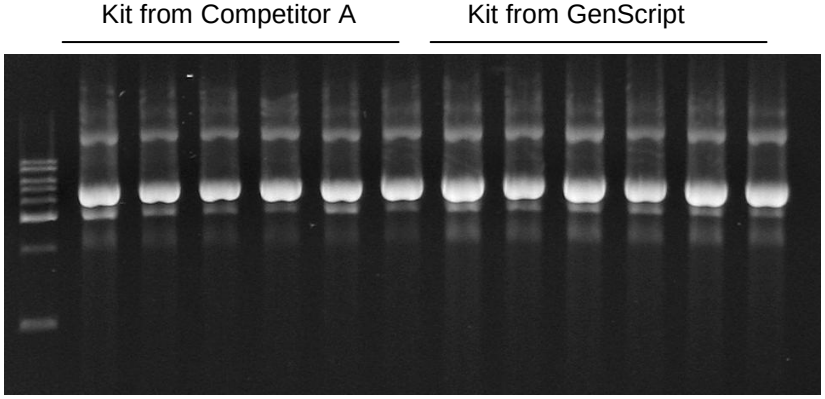


Figure 1. The QuickClean 5M Miniprep Kit and a kit from Competitor A were used for rapid plasmid purification from *E. coli* bearing high copy number plasmid. Both kits were used in compliance with the protocols provided by the manufacturers. Six minipreps were performed using each kit. The same volume of cell culture was used in each miniprep.

Figure 1 shows that both kits enabled the rapid purification of the plasmid. However, the GenScript QuickClean 5M Miniprep Kit provided a high and reproducible yield each time.

B. Plasmid DNA preparation from *E. coli* bearing low copy number plasmid.

The Quickclean 5M Miniprep Kit is also compared with the kit from Competitor A for rapid plasmid purification from *E. coli* bearing low copy number plasmid. Both kits have been used in compliance with the protocols provided by the manufacturers. The results are shown in Figure 2.

Kit from Competitor A Kit from GenScript

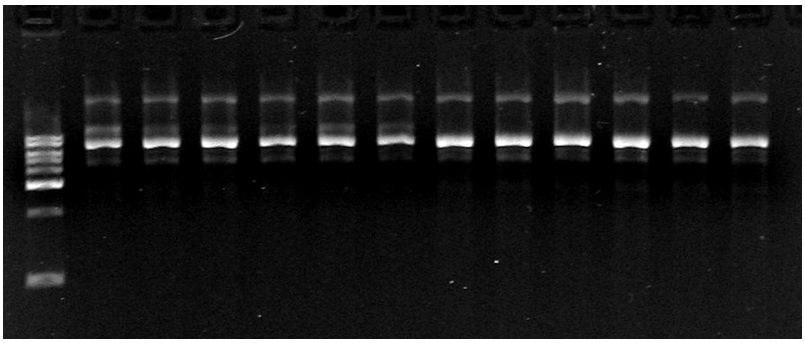


Figure 2. The QuickClean 5M Miniprep Kit and a kit from Competitor A were used for rapid plasmid purification from *E. coli* bearing low copy number plasmid. Both kits were used in compliance with the protocols provided by the manufacturers. Six minipreps were performed using each kit. The same volume of cell culture was used in each miniprep.

Figure 2 shows that both kits enabled the rapid purification of plasmid from *E. coli* bearing low copy number plasmid. The GenScript QuickClean 5M Miniprep Kit, however, purified the plasmid more reliably and with a higher yield from each miniprep.

VIII. Troubleshooting

Use the table below to solve and avoid common problems.

Problem	Probable Cause	Solution
Low plasmid DNA yield	Low copy number plasmid	Use more bacterial culture. Try 3-5 ml instead of 1.5 ml.
	The cell pellet is not resuspended well (1).	When more culture (3-5 ml) is used, double the volume of solution I, II and III.
	The cell pellet is not resuspended well (2).	The cell pellet can be resuspended by vortexing or by the following simple method: Hold the microcentrifuge tube from the top and run the narrow end quickly along the top of an empty microcentrifuge storage rack. Repeat four times or until the cells are resuspended.
	Another elution solution has been used.	Elution buffer is 2.0 mM Tris-HCl pH 8.5. TE buffer (pH 8.0) or water can also be used, but the yield will be slightly lower. Eluting plasmid DNA with 25 µl of elution buffer two times will yield more DNA. More elutions are also possible.
Small nucleic acid band	The RNA is co-purified with plasmid DNA.	Check the storage time and condition of the reagents. Solution I with RNase A added will be stable for six months at 4-8°C.
High molecular	Genomic DNA contamination.	After the addition of solution II, mix gently by inverting the

weight DNA band		tube 4-6 times. Do not vortex.

IX. ORDERING INFORMATION

QuickClean 5M Miniprep Kit Cat. No. L00193

X. REFERENCE

Kui Shin Voo and Britta M. Jacobsen. 1998. Rapid Resuspension of Pelleted Bacterial Cells for Miniprep Plasmid DNA Isolation. *BioTechniques* 24:240-243.

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