

QuickClean 96-Well Plasmid Miniprep Kit

L00237


Technical Manual No. TM 0231

Version 0712007

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I. DESCRIPTION

The QuickClean 96-Well Plasmid Miniprep Kit is designed to purify up to 20 µg/well high-purity plasmid using 96-well plates in less than 60 minutes by centrifugation. Cells are lysed, filtered and the plasmid DNA is bound to a silica membrane plate. Then the plasmid DNA is washed and recovered in Tris buffer or water. The purified plasmid can be used for sequencing and other molecular cloning applications, including restriction enzyme digestion, transformation, PCR, *in vitro* translation and transfection.

II. KIT CONTENTS

The components included in the QuickClean 96-Well Plasmid Miniprep Kit are listed below. The kit includes sufficient reagents for 384 (4x96) minipreps.

| L00237 Components | Amount |
|---------------------------------------|---------------|
| 96-Well Growth Plate (2.2 ml) | 4 |
| 96-Deep-Well Plate (1.6 ml) | 4 |
| 96-Well Collection Plate | 4 |
| 96-Well Filter Plate | 4 |
| 96-Well Binding Plate | 4 |
| Solution I: Resuspension Solution | 110 ml |
| Solution II: Lysis Solution | 110 ml |
| Solution III: Neutralization Solution | 110 ml |
| Wash Solution (Concentrated) | 2x50 ml |
| Elution Solution | 40 ml |
| RNase A (11 mg/ml) | 1 ml |
| Adhesive Tape | 12 |

| | |
|------------------------|---|
| 96-Deep-Well Plate Pad | 8 |
| Manual | 1 |

III. APPLICATIONS

Overview of the method

The QuickClean 96-Well Plasmid Miniprep Kit enables the preparation of high-quality plasmid DNA ready for most downstream applications such as:

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

IV. KEY FEATURES

- ❖ Time-saving protocols: Our simplified procedures enable the purification of 192 samples (2×96) in less than 60 minutes
- ❖ High capacity: Each column has a capacity of up to 20 µg of plasmid DNA.
- ❖ High purity: The kit completely removes contaminants and inhibitors.
- ❖ Reproducible yields: The rate of pure plasmid DNA is typically between 90% and 95%.

V. STORAGE

This kit should be stored at room temperature and stable for 12 months while solution I with RNase A should be stored at 2-8°C and remain stable for 6 months. For long-term storage, store at -20°C.

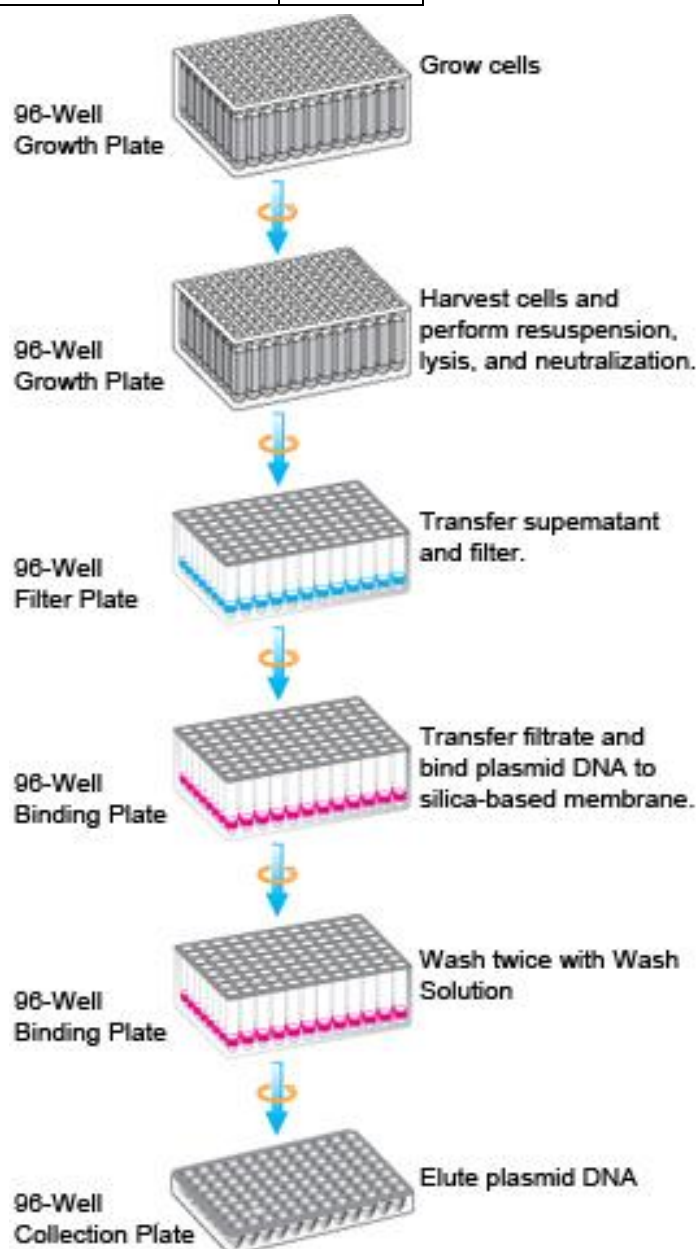
VI. Plasmid Miniprep Protocol

Materials needed:

- ❖ 96-100% ethanol.
- ❖ Water bath (37°C), optional.
- ❖ Culture medium containing antibiotic: High-nutrient media such as 2×YT are recommended for high-copy-number plasmid purification. For low-copy-number plasmid purification, high-nutrient media must be used.
- ❖ Sterile toothpick (optional).

Instruments needed:

- ❖ Centrifuge capable of 3,000×g, fitted with 96-well plate adapters.



- ❖ Multichannel pipettors (volume range: 50-1,000 μ l)
- ❖ Vortexer.
- ❖ Incubator.

Composition of liquid medium:

1. LB liquid medium: Dissolve 10g of Trptone, 5g of yeast extract and 10g of NaCl in 800 ml of distilled water. Adjust pH to 7.0 with 5N NaOH. Add distilled water to 1L. Autoclave the medium and cool to 37°C before adding antibiotic.
2. 2×YT liquid medium: Dissolve 16g of Trptone, 10g of Yeast extract and 5g of NaCl in 800 ml of distilled water. Adjust pH to 7.0 with 5N NaOH. Add distilled water to 1L. Autoclave the medium and cool to 37°C before adding antibiotic.
- 3.

Before use, prepare the following:

1. Add 1 ml of RNase A to Resuspension Solution, Mix well and store at 2 - 8°C.
2. Add 200 ml of 100% ethanol to 50 ml of Wash Solution, Mix well and store at room temperature.
3. Check reagents and plan the purification:
 - Before use, check the Neutralization Solution and Lysis Solution. If white precipitate is present in either, place the solution in a 37°C water bath until the salts re-dissolve and the solution becomes clear . Do not shake the Lysis Solution, since this can cause too much foam.
 - This protocol is optimized for processing 192 samples (2×96 well preps). If you plan to process less than this, divide your samples between two plates evenly so that you always have a balance. During the procedure, remember to mark the used wells, and the unused well can be used at a later date .

Procedure:**1. Growing cells**

- 1.1 Add an appropriate concentration of antibiotic to the medium and mix well.
- 1.2 Transfer 1.3 ml medium containing antibiotic into each well of 96-Well Growth Plate (2.2 ml, supplied in the kit).
- 1.3 Pick a single colony from an overnight plate and add to each well with sterile toothpick.
- 1.4 Cover the 96-Well Growth Plate with adhesive tape (supplied in the kit) and seal tightly.
- 1.5 Pierce 2-3 holes in the adhesive tape with a needle.
- 1.6 Incubate in a 37°C incubator on a shaker at 250-280 rpm for 18 - 22 hours. An OD₆₀₀ of 1.0-4.0 for the culture of the high-copy-number plasmid bacteria is appropriate for plasmid DNA extraction.

2. Harvesting cells and preparing lysates

- 2.1. Harvest cells by centrifuging the 96-Well Growth Plate at 2,500×g for five minutes.
- 2.2. Remove and discard the adhesive tape. Place the 96-Well Growth Plate inverted on a paper towel to remove the medium. Alternatively, tap them gently on the paper towel.
- 2.3. Transfer 250 μ l of Resuspension Solution with RNase A (as described above) to each well. Seal the well securely with fresh adhesive tape. Vortex the plate vigorously on a vortexer until the cell pellet is completely resuspended.
- 2.4. Carefully remove and discard the adhesive tape. Transfer 250 μ l Lysis Solution to each well and seal the top of the 96-Well Growth Plate with a piece of 96-Deep-Well Plate Pad. Mix gently but thoroughly by inverting the growth plate for 4-6 times. This step should be completed within five minutes.

- 2.5. Remove the 96-Deep-Well Plate Pad and discard. Add 250 μ l of Neutralization Solution to each well and seal the 96-Well Growth Plate with a new 96-Deep-Well Plate Pad. Mix gently but thoroughly by inverting the plate 6-8 times or until a uniform precipitate is formed within each well. Incubate at room temperature for five minutes.
- 2.6. Centrifuge the plate at 2,500 \times g for five minutes.

3. Filtering the lysate and binding plasmid

- 3.1. Place a 96-Well Filter Plate (supplied in the kit) on top of a 1.6 ml 96-Deep-Well Plate. and transfer the supernatant (clear lysate from Step 2.6) to each well of the 96-Well Filter Plate.
Note: Do not discard the cover for 1.6 ml 96 Deep Well Plate. It will be used in Step 3.3.
- 3.2. Centrifuge the stacked plates (96-Well Filter Plate on top of the 1.6 ml 96-Well Deep Plate) for 10 minutes at 3,000 \times g. Discard the 96-Well Filter Plate.
- 3.3. Place a 96 Well Binding Plate on top of the inverted cover for 1.6 ml 96-Deep-Well Plate. Transfer filtered lysates in the 96-Deep-Well Plate from Step 3.2 to the 96 Well Binding Plate. **Note:** Do not discard the 96-Deep Well Plate, this plate will be used to collect waste during the following centrifugation steps.
- 3.4. Place the 96-Well Binding Plate on top of the used 1.6 ml 96-Deep-Well Plate and centrifuge at 2,500 \times g for 5 minutes to bind the plasmid DNA on the membrane.

4. Washing

- 4.1. Discard the flow-through in the 1.6 ml 96-Deep-Well Plate.
- 4.2. Place the 96-Well Binding Plate on top of the used 1.6 ml 96-Deep-Well Plate. Add 500 μ l of Wash Solution with ethanol (as described above) to the 96-Well Binding Plate.
- 4.3. Centrifuge the stacked plates at 2,500 \times g for five minutes. Discard the flow-through.
- 4.4. Repeat the wash step one more time.
- 4.5. Place the 96-Well Binding Plate on top of the used 1.6 ml 96-Deep-Well Plate and centrifuge the stacked plates at 2,500 \times g for another five minutes to remove residual Wash Solution. Discard the 1.6 ml 96-Deep-Well Plate.

5. Eluting Plasmid DNA

- 5.1. Place the 96-Well Binding Plate on top of a 96-Well Collection Plate. Transfer 50 μ l of Elution Solution or sterile distilled water to the wells of the 96-Well-Binding Plate
- 5.2. Incubate the plate at room temperature for 1-2 minutes.
- 5.3. Centrifuge the stacked plates for five minutes at 2,500 \times g.
- 5.4. Transfer another 50 μ l of Elution Solution or sterile distilled water (pH>7.0) to the 96-Well Binding Plate and centrifuge the stacked plates for five minutes at 2,500 \times g.
- 5.5. Now the plasmid is ready for use. If the plasmid is not intended for immediate use, cover the 96-Well Collection Plate with the appropriate cap supplied in the kit and store the plasmid at 4°C for short periods (1-2 days) or at -20°C for longer periods.

VII. EXAMPLES

A: Consistent yields obtained with the QuickClean 96-Well Plasmid Miniprep Kit

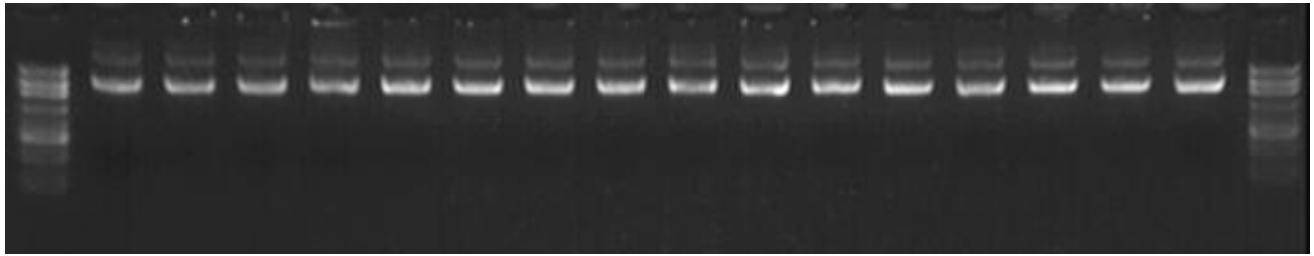


Figure 1: Isolation of pUC18 plasmid DNA (1.2% TAE agarose gel).

B: Sequencing result of a plasmid obtained with the QuickClean 96 Well Plasmid Miniprep Kit

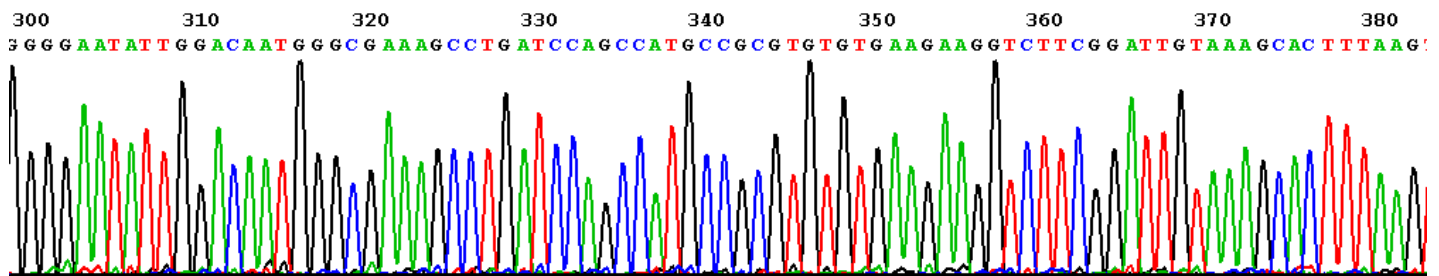


Figure 2: Part of plasmid DNA sequencing analysis

VIII. TROUBLESHOOTING

Use the table below to solve and avoid common problems.

| Problem | Causes and Comments |
|------------------------|--|
| Low plasmid DNA yield. | Poor cell lysis due to too many cells in culture medium. |
| | Poor resuspension of bacterial cell pellet. After addition of the Resuspension Solution, the cell pellet should be resuspended thoroughly. |
| | Low-copy-number plasmid. We recommend using high nutrient media such as 2X YT for low-copy-number plasmid miniprep. |
| | Cell growth. Poor cell growth (low OD) will result in low yields. |
| | Wrong reagents. Make sure that ethanol is added to Wash Solution and the RNase A is added to Resuspension Solution. |
| | Wrong elution solution. DNA elution is performed using a low-salt buffer or sterile distilled water with pH>7.0 for efficient plasmid elution. |
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|--------------------------------|--|
| Poor plasmid DNA quality. | Ensure that the 96-Well Binding Plate is free from any residual Wash Solution prior to elution. Traces of ethanol from the Wash Buffer can inhibit downstream enzymatic reactions. |
| Small size nucleic acid band. | RNA has co-purified with plasmid DNA. Check the storage time and conditions for reagents. Solution I with RNase A will remain stable for six months at 2 - 8 °C. |
| High-molecular-weight DNA band | Genomic DNA contamination. After the addition of Solution II, mix gently by inverting the tube 4 - 6 times. Do not vortex. |

X. ORDERING INFORMATION

QuickClean 96-Well Plasmid Miniprep Kit Catalog Number: L00237

For Research Use Only.

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References:

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