



**96-well  
Plasmid Miniprep  
Manual for  
Non-Kit Users**

**Whatman®**

# Contents

<b>Introduction</b> .....	<b>.3</b>
Microplate Components Required .....	.3
Buffer Formulations .....	.4
Equipment Required .....	.4
Precautions .....	.5
<b>Plasmid Miniprep Procedure</b> .....	<b>.6</b>
Overview of Procedure .....	.6
Cell Growth .....	.7
Harvesting the Bacterial Cells .....	.7
Cell Lysis .....	.7
Vacuum Filtration Protocol .....	.8
Centrifugation Protocol .....	.11
Plasmid Purification Automation .....	.13
<b>Product Performance</b> .....	<b>.14</b>
<b>Troubleshooting</b> .....	<b>.15</b>
<b>Additional Accessories</b> .....	<b>.18</b>

# Introduction

The 96-well Plasmid Miniprep System is designed and optimized for the high-throughput laboratory. This system includes the microplate components and buffer formulations needed to rapidly isolate and purify plasmid DNA. The design of the protocol allows for easy adaptation to different equipment in the laboratory.

The 96-well Plasmid Miniprep procedure is based on alkaline lysis of bacterial cells followed by the clearing of lysate through a Lysate Clarification Plate. The plasmid DNA is then captured by a DNA Binding Plate and eluted with isotonic buffer. The eluted DNA is ready for use and does not require precipitation or other extraction techniques. The plasmid DNA is of high quality and suitable for downstream applications such as fluorescent DNA sequencing, cloning, bacterial transformation, and PCR.

## Microplate Components Required

Catalog No.	Description	Qty/Case
7701-5205	Irradiated Growth Plate/Lid Unit	25
7720-2830	Lysate Clarification Plate	25
7700-2810	DNA Binding Plate	25
7701-5250	250 $\mu$ L V-bottom Collection Plate	50
7701-5200	2 mL Waste Collection Plate	25
7704-0001	96-well Adhesive Plate Seals	100

## Buffer Formulations

Buffer	Qty needed (per plate)	Formulation
P1 Buffer*	20 mL	100 mM Tris, 20 mM EDTA, pH 7.8. Prior to use add RNase A to a final concentration of 0.1 mg/mL
P2 Buffer	20 mL	0.2N NaOH, 1% (w/v) SDS
P3 Buffer	20 mL	4M Guanidine-HCl, 0.75 M Potassium Acetate, pH 4.5
Wash Buffer 1	40 mL	4M Guanidine-HCl, 0.75 M Potassium Acetate, pH 4.6
Wash Buffer 2	40 mL	40% (v/v) 100 mM Tris, 20 mM EDTA, 0.4M NaCl, pH 7.5. Requires addition of ethanol prior to use. Wash Buffer to yield a final concentration of 60% (v/v) ethanol.
Elution Buffer	10 mL	10 mM Tris, 0.1 mM EDTA, pH 7.5

\*P1 buffer containing RNase(A) is stable for up to 6 months from the date of RNase addition when stored at 2-8C. All buffers listed in the table above are stable for 1 year at room temperature.

## Equipment Required

### Vacuum Filtration Protocol:

- Appropriate pipettes and pipette tips.
- Centrifuge capable of 1800 x g with a rotor for microplates.
- Device capable of providing variable plate agitation, such as a plate shaker or vortex.
- Whatman® Vacuum Manifold System (Cat. No. 7705-0107 contains manifold with gauge, regulator, two-way control valve and Teflon®/silicone vacuum assist) or equivalent system compatible with Whatman plates
- 300 µL Small Spacer Plate (Cat. No. 7701-1350 or equivalent)
- 750 µL Large Spacer Plate (Cat. No. 7701-5750 or equivalent)
- Sufficient tubing and moisture trap
- Vacuum source capable of at least -15 inches Hg

### *Pressure Conversion Chart*

Conversions from inches Hg to:	Multiply by:
Millibars (mbar)	33.86
Millimeters of mercury (torr)	25.4
Kilopascals (kPa)	3.386
Pounds per square inch (psi)	0.4912

### Centrifugation Protocol:

- Appropriate pipettes and pipette tips.
- Centrifuge capable of 1800 x g with a rotor for microplates.
- Device capable of providing variable plate agitation, such as a plate shaker or vortex.
- Microplate carrier capable of accommodating 7.5 cm stack height
- Whatman 750 µL Collection Plate (Cat. No. 7701-5750)

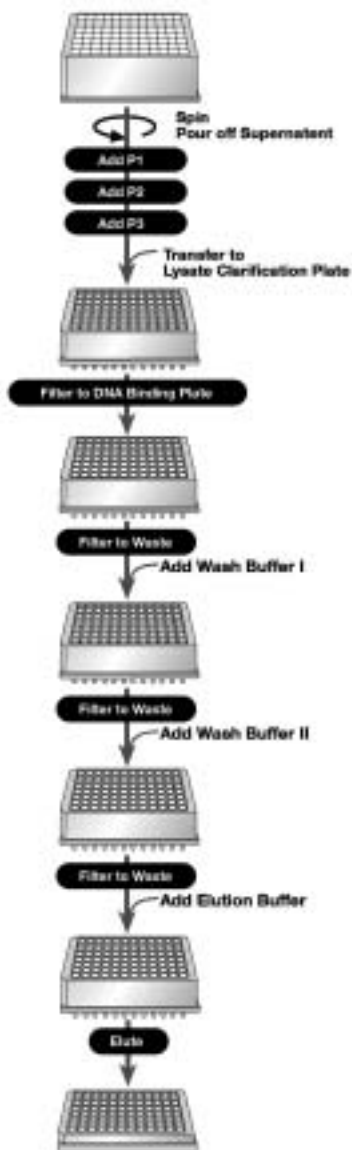
## Precautions

- Several buffers are potential eye/skin irritants. Proper laboratory practices should be followed.
- The Vacuum Assist is recommended for processing runs less than 96 wells. Alternatively, unused wells can be sealed with the 96-well Adhesive Plate Seals provided.
- This procedure is designed for research use only; not for use in diagnostic procedures.
- MSDS sheets are available upon request.
- Whatman plates are designed to be disposable, single-use-only products.

# 96-Well Plasmid Miniprep Procedure

This section includes all of the information necessary to isolate and purify plasmid DNA from bacterial cells using vacuum filtration and centrifugation systems. The protocol can also be adapted for use on liquid handling systems.

## Overview of Procedure



## Cell Growth

The 96-well Plasmid Miniprep procedure has been optimized for use with bacterial cultures grown in Luria-Bertani (LB) media. When using rich growth media such as Terrific Broth or 2X LB, lysate clarification time may be increased by 5 minutes to ensure all DNA passes through the Lysate Clarification Plate.

Fill the appropriate wells of the Growth Plate with 1.5 mL of media containing the appropriate antibiotic. Use a single colony from a freshly streaked plate to inoculate and grow overnight (16-24 hours). The Growth Plate should be incubated with shaking (325 rpm) at 37°C. The cultures should be grown to a level of OD A<sub>600nm</sub> 1.8-2.0.

## Harvesting the Bacterial Cells

Harvest the bacterial cells by centrifuging the Growth Plate for 5 minutes at 1800 x g in a centrifuge with a rotor for microplates. Discard the supernatant by inverting the Growth Plate over a waste container. Tap the inverted plate gently on a paper towel to fully remove any remaining medium.

## Cell Lysis

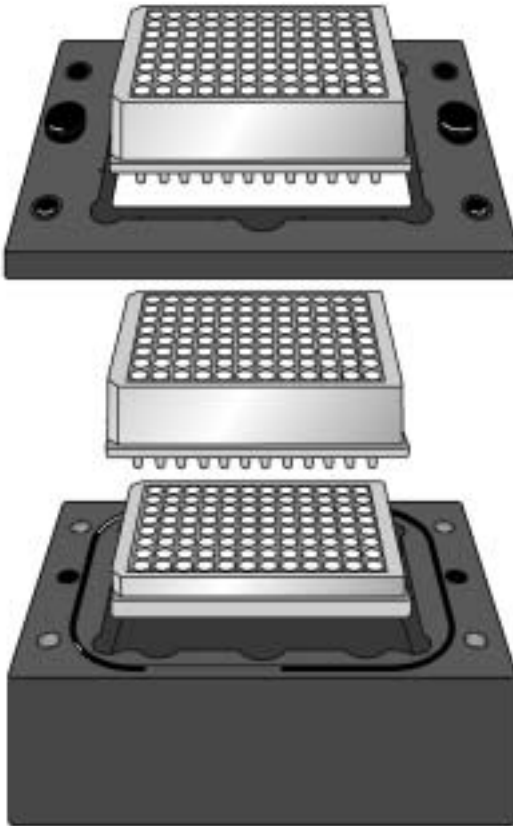
**Note:** Ensure that RNase A has been added to the P1 Buffer. If RNase A has not been added to the P1 Buffer, refer to “Buffer Formulations”.

- 1. Add 200 µL P1 Buffer to the cell pellet. Resuspend completely with a pipette or brief vortexing.** The pelleted cells should be resuspended completely. Incomplete resuspension of the bacterial pellet will result in low yield.
- 2. Add 200 µL P2 Buffer. Mix the Growth Plate on a plate shaker at low speed for 2 minutes.** Avoid vigorous vortexing, as this will cause shearing of the bacterial genomic DNA. Add the P2 Buffer to the center of the well with adequate force. Do not let P2 Buffer roll down the side of the wells. A repeat pipettor is best suited for this delivery. Do not allow the bacterial cells to incubate with P2 Buffer for more than 5 minutes.
- 3. Add 200µL P3 Buffer. Mix the growth Plate on a plate shaker at low speed for 2 minutes.** Avoid vigorous vortexing, as this will cause shearing of the bacterial genomic DNA. Add the P3 Buffer to the center of the well with adequate force. Do not let the P3 Buffer roll down the side of the well. A repeat pipettor is best suited for this delivery.

## Vacuum Filtration Protocol

### 1. Prepare the vacuum manifold:

As illustrated below, place the 300  $\mu$ L Small Spacer Plate in the manifold base. Place the DNA Binding Plate on top of the Small Spacer Plate. Attach the manifold collar. Place the Lysate Clarification Plate on top of the vacuum manifold collar. Ensure that the two filter plates are aligned alphanumerically (A1 to A1).



**2. Transfer the lysate from step 3 of Cell Lysis into the Lysate Clarification Plate.**

Ensure that all of the lysate from step 3 is transferred to the Lysate Clarification Plate. Avoid excessive foaming of the lysate at this point. A multichannel pipette with a 1 mL fill volume is recommended for this step.

**Note:** When transferring lysate, be sure to track the samples carefully.

**3. Apply vacuum between -15 to -20 inches Hg for 10 minutes. *The Vacuum Assist must be used to ensure proper vacuum.***

After 10 minutes, release the vacuum using the manifold switch and check to see that all liquid has been evacuated from each well. If the wells and/or the drip directors have any remaining liquid, apply vacuum until all the liquid has been transferred to the DNA Binding Plate.

**4. Reconfigure vacuum manifold.**

Remove the Lysate Clarification Plate, the DNA Binding Plate and the 300  $\mu$ L Small Spacer Plate from the vacuum manifold. Place 2 mL Waste Collection Plate in the vacuum manifold and reassemble the manifold. Place the DNA Binding Plate on the top of the vacuum manifold. Ensure that the two microplates are aligned alphanumerically (A1 to A1).

**5. Apply vacuum between -15 to -20 inches Hg until all of the material has passed through the DNA Binding Plate.**

Plasmid DNA is now bound to the filter media in the DNA Binding Plate. Release the vacuum using the manifold switch and check to see that all liquid has been evacuated from each well. If fluid remains in any of the wells, repeat this step until all liquid has cleared.

**6. Add 400  $\mu$ L of Wash Buffer 1 to each well. Apply vacuum between -15 to -20 inches Hg until all Wash Buffer has passed through the DNA Binding Plate.**

Release the vacuum using the manifold switch and check to see that all liquid has been evacuated from each well. If fluid remains in any of the wells, repeat this step until all liquid has cleared.

**Note:** Ensure that ethanol has been added to Wash Buffer 2. If ethanol has not been added to Wash Buffer 2, then refer to “Buffer Formulations”.

- 7. Add 400  $\mu\text{L}$  of Wash Buffer 2 to each well. Apply vacuum between -15 to -20 inches Hg until all Wash Buffer has passed through the DNA Binding Plate.**

Release the vacuum using the manifold switch and check to see that all liquid has been evacuated from each well. If fluid remains in any of the wells, repeat this step until all liquid has cleared.

- 8. Apply maximum vacuum for an additional 2 minutes to dry the DNA Binding Plate.**

This step removes residual Wash Buffer 2 from the DNA Binding Plate. Seal any unused wells of the DNA Binding Plate before drying. Ensure that the wells in use are exposed to ambient airflow for proper ethanol drying. Release the vacuum using the manifold switch. Using a clean absorbent paper towel, blot the bottom of the DNA Binding Plate to remove any residual Wash Buffer 2 from the drip directors. The removal of ethanol present in the Wash Buffer 2 is important for downstream applications.

- 9. Prepare the vacuum manifold for DNA collection.**

Remove the 2 mL Waste Collection Plate and place the 750  $\mu\text{L}$  Large Spacer Plate in the bottom of the vacuum manifold. Place the 250  $\mu\text{L}$  Collection Plate (v-bottom) on top of the Spacer Plate and reassemble the manifold collar. Place the DNA Binding Plate on top of the manifold. Ensure that the two microplates are aligned alphanumerically (A1 to A1).

- 10. Add 50  $\mu\text{L}$  of Elution Buffer to the center of each well.**

Incubate the plate at room temperature for 1 minute. Apply vacuum between -15 to -20 inches Hg for 1 minute until all Elution Buffer has passed through the DNA Binding Plate.

- 11. Repeat step 10.**

This step can be omitted if a more concentrated plasmid DNA is desired. As elution volume is decreased, some loss in volume might be observed due to liquid retention in the filter media.

- 12. Remove 250  $\mu\text{L}$  Collection Plate from the vacuum manifold.**

For storage, cover the Collection Plate with the 96-well Adhesive Plate Seal. The DNA is ready for use in downstream applications such as fluorescent DNA sequencing, cloning, bacterial transformation, and PCR.

## Centrifugation Protocol

When using the centrifugation protocol outlined below, ensure that the centrifuge meets the criteria noted in the “Equipment Required” section. Refer to the manufacturer’s manual for operating instructions.

### 1. Prepare the plates for lysate clarification.

Place the Lysate Clarification Plate on top of a 750  $\mu$ L Collection Plate (not included, Cat. No. 7701-5750). Ensure that the two filter plates are aligned alphanumerically (A1 to A1).

### 2. Transfer the lysate from step 3 of Cell Lysis into the Lysate Clarification Plate.

Ensure that all of the lysate from step 3 is transferred to the Lysate Clarification Plate. Avoid excessive foaming of the lysate at this point. A multichannel pipette with a 1 mL fill volume is recommended for this step.

**Note:** When transferring lysate, be sure to track the samples correctly.

### 3. Centrifuge the Lysate Clarification Plate for 10 minutes at 1800 x g.

After 10 minutes, the lysate should be completely transferred to the Collection Plate. Remove the stack from the centrifuge.

### 4. Prepare the plates for DNA binding.

Place the DNA Binding Plate on top of a 2 mL Waste Collection Plate. Ensure that the two plates are aligned alphanumerically (A1 to A1).

### 5. Transfer the clarified lysate to the DNA Binding Plate.

Ensure that all of the clarified lysate is transferred to the DNA Binding Plate. Failure to transfer all of the clarified lysate will result in lower yields. Ensure that the two plates are aligned alphanumerically (A1 to A1).

### 6. Centrifuge the DNA Binding Plate for 2.5 minutes at 1800 x g.

Plasmid DNA is now bound to the filter media in the DNA Binding Plate.

### 7. Add 400 $\mu$ L Wash Buffer 1 to each well of the DNA Binding Plate.

Centrifuge the DNA Binding Plate stack for 2.5 minutes at 1800 x g.

**Note:** Ensure that ethanol has been added to Wash Buffer 2.  
If ethanol has not been added, refer to “Buffer Formulations”.

**8. Add 400  $\mu$ L of Wash Buffer 2 to each well of the DNA Binding Plate.**

Centrifuge the DNA Binding Plate stack for 4.5 minutes at 1800 x g.

**9. Remove the stacked plates from the centrifuge and blot the bottom of the DNA Binding Plate with a paper towel.**

The full removal of ethanol from the plate is required for downstream applications. Ensure complete removal before elution.

**10. Place the DNA Binding Plate on top of a 250  $\mu$ L Collection Plate.**

Ensure that the two plates are aligned alphanumerically (A1 to A1).

**11. Add 50  $\mu$ L of Elution Buffer to the center of each well.**

Incubate the plate at room temperature for one minute. Place the stacked plates into the centrifuge and spin for 2.5 minutes at 1800 x g.

**12. Repeat step 11.**

This step can be omitted if a more concentrated plasmid DNA is desired. As elution volume is decreased, some loss in the collection volume might be observed due to liquid retention in the filter media.

**13. Remove 250  $\mu$ L Collection Plate from the Centrifuge.**

For storage, cover the Collection Plate with the 96-well Adhesive Plate Seal. The DNA is ready for use in downstream applications such as fluorescent DNA sequencing, cloning, bacterial transformation, and PCR.

## **Plasmid Purification Automation**

The Whatman 96-well Plasmid Miniprep system is compatible with high-throughput platforms. Automated protocols for specific high throughput systems can be obtained through Whatman technical service, or by download at [www.whatman.com](http://www.whatman.com).

## Product Performance

By following the protocols in this User's Manual and adhering to general laboratory practices, each 1.5 mL bacterial culture containing high copy number plasmid yields 5-8 µg of high quality DNA. The DNA is ready for use in downstream applications such as fluorescent DNA sequencing, cloning, bacterial transformation, and PCR.

The 96-well Plasmid DNA Miniprep Kit can be used to isolate and purify plasmid DNA from EndA<sup>-</sup> and EndA<sup>+</sup> bacterial strains.

Plasmids	Host Bacterial Strains	
	EndA <sup>-</sup>	EndA <sup>+</sup>
pGEM <sup>®</sup>	DH5α <sup>™</sup>	HB101
pSport	JM109	
pBR322	XL 1-Blue	
	TOP	

**Table 1.** Plasmids and bacterial strains used with the 96-well Plasmid Miniprep Kit.



**Figure 1.** 0.8% Agarose gel showing plasmid DNA purified using the 96-well Plasmid Miniprep Kit.

Plasmid	Copy Number	Average Yield
pGEM <sup>®</sup>	300-400	5-8 µg
pBR322	15-20	1-3 µg

**Table 2.** Plasmids with different copy number purified using the 96-well Plasmid Miniprep Kit.

# Troubleshooting

## Problem

## Comments/Suggestions

Low Yield

**Growth of cells.** Adhere to the growth conditions described in the protocol.

Ensure that the cells grown in the Growth Plate have reached an acceptable level. (OD  $A_{600nm}$  1.8-2.0)

Bacterial cultures for plasmid preparation should always be grown from a single colony picked from a freshly streaked plate containing appropriate antibiotic.

**Cell Resuspension.** Resuspend the bacterial cells completely. An additional gentle vortex or mix with a pipette can be done to ensure bacterial cells are completely resuspended. View cells from the bottom of the Growth Plate to see if cell clumps are remaining.

**Lysis.** The addition of P2 Buffer and P3 Buffer should be to the center of each well in the Growth Plate. Adding buffers to the sides of the wells may result in incomplete mixing.

**Incomplete mixing of the lysate after the addition of P2 and P3 Buffers.**

The shaking step needs to be quick, but the cells should not be vigorously vortexed.

Low Yield

**Incorrect Plate Configuration**

Correct plate usage in each step is critical of optimal yields.

**Elution.** Ensure the Elution Buffer is added to the center of the wells. Low yield may result if the Elution Buffer is added to the sides of the well rather than to the filter media.

## Problem

RNA in the Eluate

## Comments/Suggestions

**RNase A was not added to the P1 Buffer.**

**RNase A solution has expired.**

After the addition of RNase A the P1 Buffer has a shelf life of 6 months when stored at 2-8°C.

**Genomic DNA in Eluate**

**Improper Mixing.** The lysate must be handled gently after the addition of P2 and P3. The shaking step needs to be quick, but the cells should not be vigorously vortexed.

**Slow/Clogged Filtration**

**Insufficient Vacuum Pressure.**

Make sure you are obtaining the correct vacuum pressure as described in the protocol.

**Vacuum Hardware Incompatibility.**

For optimal results, the Whatman Vacuum Manifold System should be used according to the protocol.

**Incorrect Plate Configuration.**

Correct plate usage in each step is critical for optimal filtration.

**Poor DNA Performance in Downstream Applications**

**Sequencing Failures**

**Ethanol in eluate.** Ensure that the Wash Buffer drying process is followed carefully and completely.

**Restriction Digest**

**Incomplete removal of culture media.**

Completely decant culture media after pelleting the bacterial cells.

**Ethanol in eluate.** Ensure that the Wash Buffer drying process is followed carefully and completely.

# Additional Accessories

Catalog No.	Description	Qty/Case
7705-0107	Vacuum Manifold System (contains: Manifold with gauge, regulator, two-way control valve, and Teflon®/silicone vacuum assist)	1 per/box
7705-0205	Teflon/Silicone Vacuum Assist (replacement for vacuum assist contained in Vacuum Manifold System)	6 per/pack
7701-5200	2 mL Waste Collection Plate	25 per/case
7701-5750	750 µL Collection Plate (for centrifuge processing)	25 per/ case
7725-0118	96-well Filtrate Directors (for Biomek® 2000 processing)	25 per/case
7705-0120	Small Whatman Vacuum Adapter Collar for Biomek 2000	1 per/pack
7705-0121	Medium Whatman Vacuum Adapter Collar for Biomek 2000	1 per/pack

## Notice

The information in this document is given in good faith, and is for guidance only. It is not intended to override or void any of Whatman's Standard Conditions of Sale. Those Conditions remain in full and complete effect, and govern all relations between Whatman and the purchaser of the goods which relate to this document.

DH5α is a trademark of Life Technologies Inc.

pGEM is a registered trademark of Promega Corp.

PCR is a patented process covered by U.S. Patents 4,683,195 and 4,683,202

and foreign equivalents owned by Hoffman-La Roche AG

Biomek® is a registered trademark of Beckman Coulter, Inc.

Whatman® is a registered trademark of Whatman Inc.

©Copyright, Whatman Inc., 2002 Printed in USA

# Whatman

[www.whatman.com](http://www.whatman.com)

For pricing and availability, please contact:

## USA

Whatman Inc.  
9 Bridewell Place  
Clifton, NJ 07014  
Toll Free: 800-WHATMAN  
Fax: 973-773-0168  
E-mail: [info@whatman.com](mailto:info@whatman.com)

## Europe/Middle East/Africa

Whatman International Ltd.  
Whatman House  
St. Leonard's Road  
20/20 Maidstone  
Kent, England  
ME 16 OLS  
Tel: +44 (0) 1622 676670  
Fax: +44 (0) 1622 677011  
E-mail: [info@whatman.com](mailto:info@whatman.com)

## China

Whatman Shanghai Liaison Office  
No. 8 Dong An Room 909  
Pine City Hotel  
Shanghai 200032  
P.R.China  
Tel: 86 21 6443 7176  
Fax: 86 21 6443 2568  
E-mail: [wmansha@online.sh.cn](mailto:wmansha@online.sh.cn)

## Japan

Whatman Japan Ltd.  
Daiwa Ueno Building 1F 6-10  
Ueno 5-chrome, Taito-ku  
Tokyo 110-0005 Japan  
Tel: +81 3 3832 6707  
Fax: +81 3 3832 6457  
E-mail: [japaninfo@whatman.co.jp](mailto:japaninfo@whatman.co.jp)

## Asia Pacific

Whatman Asia Pacific Pte Ltd.  
171 Chin Swee Road  
#08-01 San Centre  
Singapore 169877  
Tel: + 65 6534 0138  
Fax: + 65 6534 2166  
E-mail: [wap@whatman.com.sg](mailto:wap@whatman.com.sg)

## India

Whatman India Liaison Office  
503 Swastik Chambers U.B.Chowk  
V.N.Purav Marg, Chembur  
Mumbai 400071  
India  
Tel: 91 22 529 7035  
Fax: 91 22 529 7036  
E-mail: [whatmanb@bom3.vsnl.net.in](mailto:whatmanb@bom3.vsnl.net.in)

# Whatman®

*The Advantages Filter Through<sup>SM</sup>*

9 Bridewell Place, Clifton, NJ 07014  
[www.whatman.com](http://www.whatman.com)