3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Automated Protocol for GenElute™ HP 96 Well Plasmid Miniprep Kit

Using the Biomek® FX Workstation (Beckman Coulter)

Catalog Number **NA9604**

Automation Guide

I. Description

The GenElute HP 96 Well Plasmid Miniprep Kit has been developed for use as a high-throughput method for the purification of plasmid DNA from bacterial cultures. This rapid and cost-effective system allows for the purification of up to 10 μg of high copy plasmid DNA. Purified DNA is suitable for various molecular biology applications including restriction enzyme digestion, sequencing, and transfection. Designed to complement Sigma's MISSION® shRNA product line, the GenElute HP 96-Well Plasmid Miniprep Kit is optimized for the most demanding downstream transfection protocols. The result is purified plasmid DNA that can generate best-in-class transfection grade plasmid that delivers the highest viral titers of any 96 well plasmid kit on the market.

The GenElute HP 96 Well Plasmid Miniprep Kit is compatible with liquid handling instrumentation. An automated method has been developed for the Biomek FX from Beckman Coulter, and is available for download at www.sigma.com/automation. The protocol is simple and rapid, 96 samples can be processed in under 50 minutes.

II. Product Components

Reagents Provided	Product Code	4 × 96 Preps
Column Preparation Solution	C2112	1 × 375 ml
RNase A Solution	R6148	1 × 1.5 ml
Resuspension Solution	R1149	1 × 240 ml
Lysis Solution	L1912	1 × 240 ml
Neutralization Solution	N1285	1 × 240 ml
Binding Solution	B4683	1 × 280 ml
Wash Solution 1	W0623	1 × 375 ml
Wash Solution 2	W4629	1 × 75 ml
Elution Solution	E7777	2 × 115 ml
Filter Plate	F3555	4
Binding Plate	B6686	4
Elution Plate	CLS3790	4
Foil Plate Seals	A2350	12

III. Storage

Store the kit at room temperature (18–25 °C). Once the RNase A Solution is added to the Resuspension Solution, store at 2–8 °C.

IV. Materials to Be Supplied by the User

1. Ethanol, 95-100% (Sigma-Aldrich, E7148, E7023, or 459836)
2. Deep well plates
3. Centrifuge with swinging bucket rotor capable of centrifuging deep well plates
4. Vacuum Source (capable of –16 to –21 inches of Hg)
5. Sterile breathable seals
6. 8 × 96 well, pyramidal bottom reservoir (Seahorse Bioscience formerly Innovative Microplates, S30014)

V. Instrument Requirements for the Biomek FX Workstation

Part Description	Qty
Multichannel Pod (96 Mandrel 200 µl Head) with integrated Gripper	1
Tip Loader	1
Orbital Shaker	1
Standard Passive ALPs (Four by Three)	1
Standard Passive ALPs (One by One)	3
SPE ALP with Manifold	1
Manifold Holder ALP	1
36 mm Manifold Collar	1
23 mm Spacer Collar	1
AP96 250 µl Tips	7

VI. Timer Software Installation

A countdown timer has been included in the automated method to show how long some of the steps have until completion. This requires the installation of additional software. A zip file containing all of the software has been included with the method file and is available for download from the website at www.sigma.com/automation. Please follow the steps below to install the timer software.

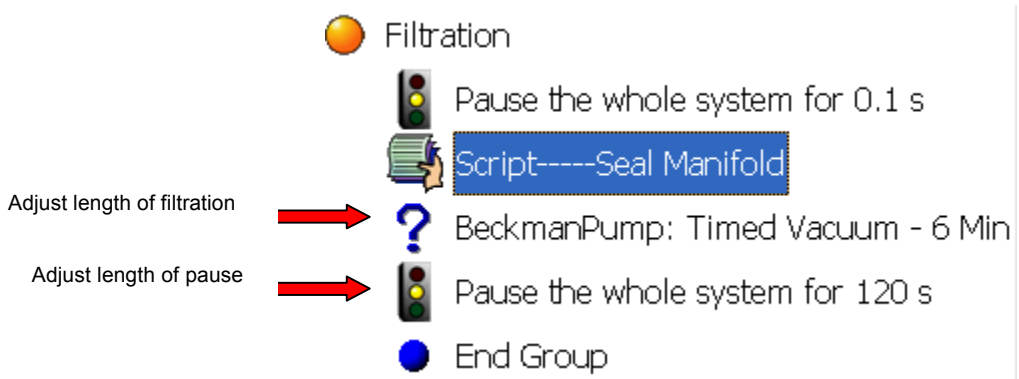
1. Open the file **GenElute HP 96 Well Plasmid Miniprep Kit.Zip**
2. Extract the file **FX Timer.Zip** using WinZip
3. Open and extract the files in **FX Timer.Zip**
4. Double click **Setup.exe** and follow instructions for installation of the Timer Software

VII. Vacuum Setup

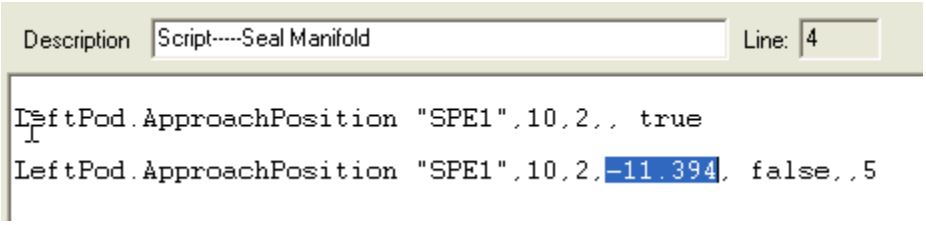
The automated protocol described here was optimized with a vacuum setting of –18 inches of Hg, but settings from –16 to –21 inches of Hg are recommended, depending upon vacuum configuration and density of cell pellets. It is recommended that the protocol be pre-run to verify that the vacuum pressure setting, length of vacuum steps, and length of pauses following filtration steps are sufficient. The pauses in the method are necessary to allow time for the vacuum to evacuate the lines in order for the manifold to vent. Some things to troubleshoot are listed below. Contact Beckman Coulter with any problems.

1. If having difficulties with vacuum seal the gaskets on the vacuum manifold components may need to be changed.

2. Slow filtration might require increasing the vacuum pressure setting or increasing the vacuum time. Slow filtration can occur during the filtration step in which the lysate must filter through both the Filter Plate and the Binding Plate. Where to make changes to this step can be seen below:



To make the filtration of the lysate more efficient, a step has been included in the method that will have the 96 channel pod push down on the filter plate just prior to the vacuum. The push down is accomplished using the step **Script-------Seal Manifold.** This script includes position information for the 96 channel pod. To avoid potential crashes or faulting of the motors, the Z (height)-position should be checked prior to running the method. To verify this position, go to **Instrument/Manual Control/Advanced Controls.** In the Advanced Controls screen, you will be able to manually adjust the Z-position so that the 96 channel pod rests on top of the filter plate located on top of the vacuum manifold. Make note of the Z-position before leaving the screen. Click on **Script-------Seal Manifold** and change the Z-position if necessary (see highlighted number below).



3. Plates sticking to the gaskets and slow venting of the vacuum manifold are signs that the vacuum pressure is too high. This can be a problem for subsequent gripper steps and cause crashes on the deck of the robot. If this problem is being observed, it is recommended that the vacuum pressure be reduced. It may also be necessary to increase the system pause that follows each filtration step (see Vacuum Setup, step 2).

VIII. Deck Setup

Deck Position From left to right	Equipment
Row 1-TL1	Tip Load with AP96 250 µl Tips
SPE1	Vacuum Manifold-protocol starts with Binding Plate on 36 mm collar on top of manifold
Holder1	Filter Plate on top of 23 mm collar
P7	96 well reservoir with Column Preparation Solution/ Elution microplate will end up in this location at end of protocol
Row 2- P1	96 well reservoir with Wash Solution 1
P4	96 well reservoir with Wash Solution 2
P8	96 well reservoir with Elution Solution
P11	AP96 250 µl Tips
Row 3-P14	AP96 250 µl Tips
P2	96 well reservoir with Resuspension Solution
P5	96 well reservoir with Neutralization Solution
P9	AP96 250 µl Tips
P12	AP96 250 µl Tips
Row 4-Orbital 1	Orbital shaker with 96-well deep well block containing cell pellets
P3	96 well reservoir with Lysis Solution
P6	96 well reservoir with Binding Solution
P10	AP96 250 µl Tips
P13	AP96 250 µl Tips

IX. Culture Preparation

1. Fill a 96 deep well plate with 1.3 ml of LB medium containing the appropriate antibiotic. If a rich media is used such as TB, then the medium volume may need to be reduced to avoid a cell mass that is too high, which could result in improper filtration of the bacterial lysates. It is not recommended to use cultures with an absorbance at 600 nm greater than 5.
2. Inoculate each well with a single colony from a freshly streaked agar plate. Cover the deep well block with a sterile breathable seal.
3. Incubate the deep well plate at 37 °C, shaking at 300 rpm for 18–22 hours.
4. Pellet the 96 deep well plate containing the overnight culture at 1,800 × *g* for 10 minutes.
5. Pour off supernatant and blot the deep well plate on an absorbent pad.

X. Reagent Preparation

1. *Resuspension Solution*
   Add 1.2 ml of RNase A Solution to the Resuspension Solution and mix. To process 96 samples, add 25 ml of the solution to a 96 well reservoir located at position P2.

2. *Lysis Solution*
   To process 96 samples, add 25 ml of the solution to the 96 well reservoir located at position P3.

3. *Neutralization Solution*
   To process 96 samples, add 25 ml of the solution to the 96 well reservoir located at position P5.

4. *Binding Solution*
   To process 96 samples, add 25 ml of the solution to the 96 well reservoir located at position P6

5. *Column Preparation Solution*
   To process 96 samples, add 70 ml of the solution to the 96 well reservoir located at position P7.

6. *Wash Solution 1*
   To process 96 samples, add 70 ml of the solution to the 96 well reservoir located at position P1.

7. *Wash Solution 2*
   Add 300 ml of 95–100% ethanol to Wash Solution 2 and mix. To process 96 samples, add 70 ml of the solution to the 96 well reservoir located at position P4.

8. *Elution Solution*
   To process 96 samples, add 15 ml of the solution to the 96 well reservoir located at position P8.

XI. Automated Method Description

This overview describes the general liquid handling steps required to execute the automated plasmid miniprep method and can be customized to a variety of applications. For custom applications see Section XII, Method Customization.

A. Getting Started

1. Set up the deck by placing the tip boxes, plates, and reservoirs at the appropriate positions on the deck as described in Section VIII, Deck Setup.
2. Add reagents to the appropriate reservoirs as described in Section X.
3. Place the Binding Plate on the 36 mm collar on top of the vacuum manifold. The Filter Plate should be located on the 23 mm manifold collar at the holder position.
4. Run the method using Biomek Software Version 3.1.
5. Turn vacuum pump on and adjust to the appropriate pressure setting
6. Prior to completion of the method, the robot will place the Binding Plate on top of a 96 well microplate and add Elution Solution. When the method is finished, the Binding Plate/Elution Plate is now ready to be placed in a centrifuge to complete the elution. The plate should be centrifuged at 1,800 × *g* for 5 minutes.

B. Method Overview

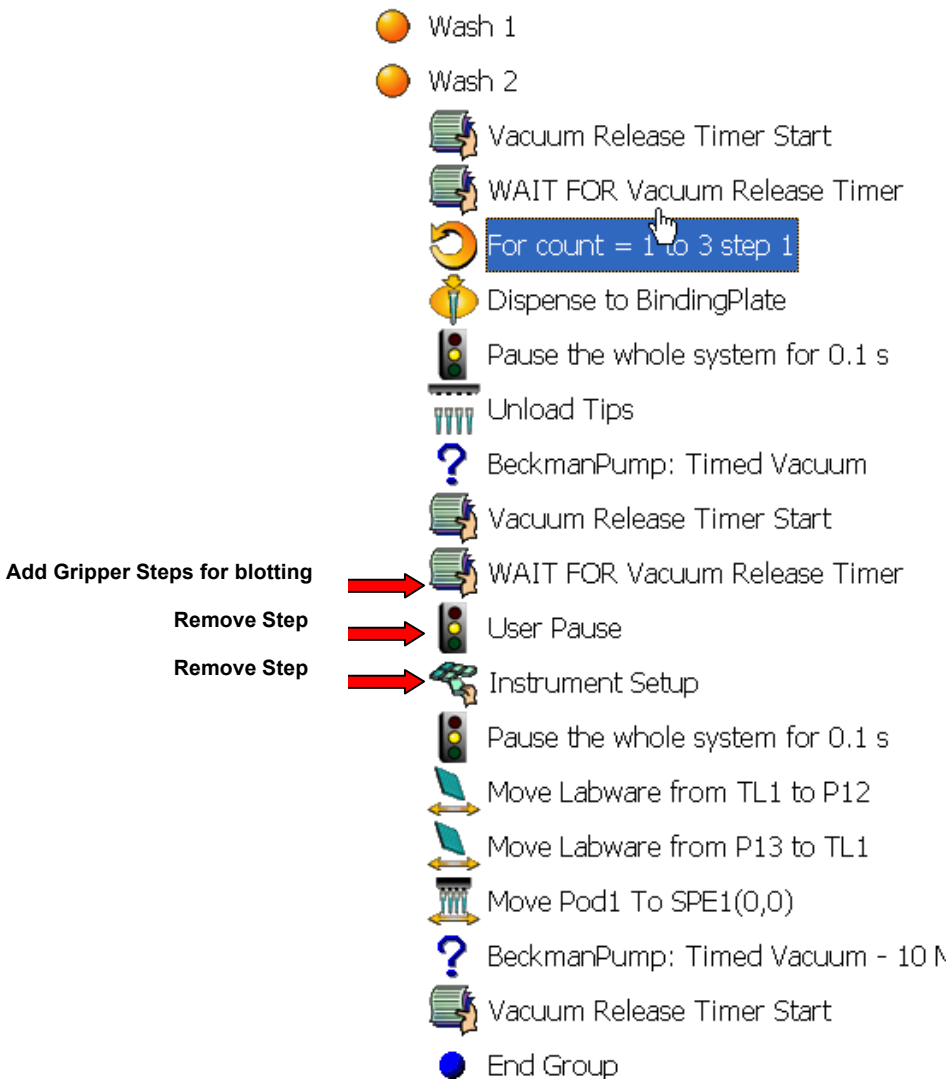
Below is a summary of the steps for the *GenElute HP 96 well Plasmid Miniprep* method. For complete program details, the automation program can be downloaded from www.sigmaaldrich.com/automation

1. Resuspension Solution (200 µl) is dispensed into the deep well plates containing the cell pellets.
2. The deep well plate is mixed using the orbital shaker and by pipette mixing.
3. Column Preparation Solution (600 µl) is dispensed to each well of the Binding Plate.
4. Lysis Solution (200 µl) is dispensed into each well of the of the deep well plate and samples are mixed using the orbital shaker for 3 minutes.
5. While the lysates are incubating, the Binding Plate containing Column Preparation Solution will be subjected to vacuum filtration for 40 seconds.
6. Neutralization Solution (200 µl) is dispensed into each well of the deep well plate and lysates are subjected to a pipette mix.
7. The Filter Plate on the 23 mm manifold collar is moved to the vacuum manifold and positioned on top of the Binding Plate.
8. Binding Solution (200 µl) is dispensed to each well of the deep well plate and lysates are subjected to a pipette mix.
9. Lysate (820 µl) is transferred to the Filter Plate located on the vacuum manifold and incubated for 5 minutes.
10. The Filter Plate is subjected to vacuum for 6 minutes.
11. A system pause of 90 seconds is in place to allow vacuum to evacuate the manifold.
12. The Filter Plate and 23 mm spacer collar are moved to the Holder position.
13. Wash Solution 1 (600 µl) is dispensed to each well of the Binding Plate.
14. The Binding Plate is subjected to vacuum for 40 seconds.
15. A system pause of 90 seconds is in place to allow vacuum to evacuate the manifold.
16. Wash Solution 2 is dispensed to each well of the Binding Plate.
17. The Binding Plate is subjected to vacuum for 40 seconds.
18. The method pauses while the user is prompted to blot the Binding Plate and replace the Reservoir containing the Column Preparation Solution with the final Elution Plate.
19. Click the “OK” button on the dialog box to finish the program.
20. The Binding Plate is subjected to vacuum for 10 minutes.
21. A system pause of 90 seconds is in place to allow vacuum to evacuate the manifold.
22. The Binding Plate is moved from the vacuum manifold to the top of the Elution Plate.
23. Elution Solution is dispensed to each well of the Binding Plate. The volume depends upon the amount entered by the user at the beginning of the method.

XII. Method Customization

A. Automating the Blotting Step

Blotting of the drip directors of the Binding Plate is currently performed off-line of the robot. Currently a user is prompted to blot the Binding Plate and place the Elution microplate on the deck of the robot due to lack of available deck space. If using a single pod Biomek FX, there will be additional open deck locations so that the blot step can be performed in an automated fashion. Place an upside down tip box lid onto an available ALP. Place a layer of absorbant material inside the tip box lid. Place an Elution microplate onto another open deck position. Gripper steps will need to be added to the **Wash 2 Node** (see figure) that will move the Binding Plate from the top of the vacuum manifold to the tip box lid. The blot should be repeated for a total of 3 blots before having the Gripper move the Binding Plate back to the vacuum manifold. The **User Pause** and **Instrument Setup** steps can be removed from the method. The **Move** and **Transfer step** under the **Elution Node** will need to be remapped to the updated deck location of the Elution microplate.



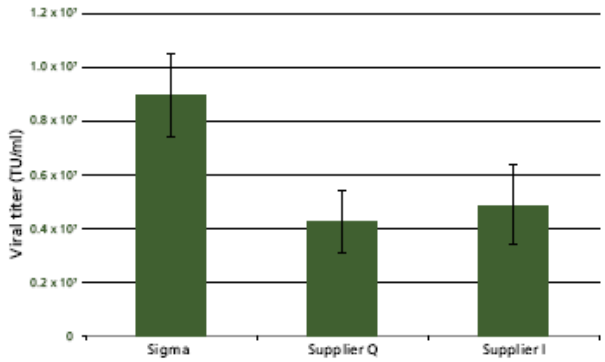
B. Elution using vacuum filtration

The automated method allows for the final elution of plasmid DNA to be performed by centrifugation off-line of the robot. It is possible to utilize vacuum filtration for the final elution. To do so the vacuum manifold will need to be reassembled. Following **Wash 2 Node**, steps will need to be added to the method to reassemble the vacuum manifold. The Binding Plate on the 36 mm manifold collar will need to be moved to the holder position. The Elution Microplate will need to be moved into the base of the manifold and the Gripper will then need to place the DNA Binding Plate/36 mm manifold collar back on top of the manifold.

Following the **Elution Node**, a timed vacuum step will also need to be added to the method. The length of vacuum will need to be evaluated, but it is recommended to apply the vacuum for at least 1 minute. Finally, check to make sure that the dispense in the **Transfer step** under the **Elution Node** is properly mapped to the Binding Plate positioned on top of the vacuum manifold.

XIII. Performance Characteristics

Robust viral titers from purified MISSION shRNA plasmid DNA clones:

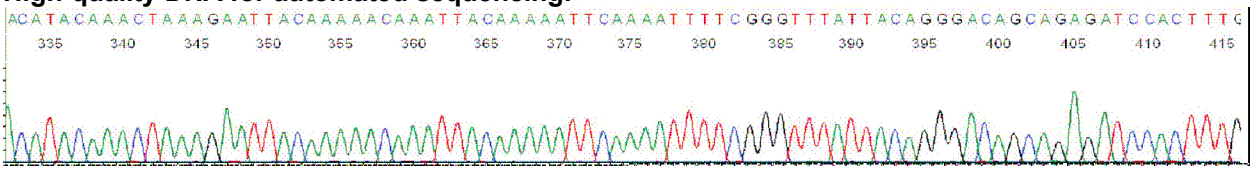


Titer measurements of lentiviral particles from cells transfected with purified MISSION shRNA plasmid DNA clones. Titer measurements of lentiviral particles from cells transfected with a purified MISSION shRNA plasmid DNA clone. MISSION shRNA plasmid DNA was isolated according to the protocol of each supplier. The purified plasmid DNA was then used to transfect HEK293T cells. The titer measurements of the resulting lentiviral particles were then determined using a p24-based ELISA. The final data represent the average of 12 experimental replicates for each manufacturer.

Purified plasmid DNA is suitable for restriction enzyme digestion:

Restriction digestion of plasmid DNA isolated with the GenElute HP 96 Well Plasmid Miniprep Kit. Restriction digestion of plasmid DNA isolated with the GenElute HP 96 Well Plasmid Miniprep Kit. MISSION shRNA Plasmid DNA was purified in a 96 well format. 40 µl *Pvu* II digests were set up using 4 µl from each recovered plasmid. The DNA was digested for 1 hour at 37 °C and then loaded onto the Caliper LabChip® instrument. The LabChip instrument analyzed the DNA and provided a visual image showing the correct digestion pattern for all samples.

High-quality DNA for automated sequencing:



Purified plasmid DNA is suitable for direct sequencing. Electropherogram showing purified plasmid DNA sequence. pLKO.1-puro plasmid DNA was purified using the GenElute HP 96 Well Plasmid Miniprep Kit. The product was then sequenced using BigDye® terminator v3.1 chemistry. Sequencing reactions were analyzed on an ABI 3730xl.

XIV. Troubleshooting

Problem	Cause	Solution
Poor or no recovery of plasmid DNA	Culture density is too low.	Confirm cell culture density. Grow cells to OD_{600} of 2–5.
	Culture density is too high. Wells of filter plate are clogged.	Confirm cell culture density. Grow cells to OD_{600} of 2–5. Depending on strain, plasmid, and culture medium used, cultures can reach high densities. Reduce starting volume of culture.
	Plasmid replication is poor.	Confirm that cells were grown in appropriate medium with a selective antibiotic under optimized conditions.
	Antibiotic activity is insufficient.	Use a fresh antibiotic solution for overnight cultures. Most antibiotics are light sensitive and degrade over long-term storage.
	Lysis is incomplete.	Allow lysis step to proceed 3–5 minutes; a noticeable clearing of the lysate mix should occur. Reduce starting cell culture volume if necessary.
	Wash Solution 2 is undiluted.	Confirm that the correct amount of ethanol has been added to the Wash Solution 2.
	The mixing of lysates prior to transfer to the Binding Plate is not sufficient.	Increase the aspiration and dispensing speed and/or cycle times in the mixing steps following the transfer of Binding Solution to lysates. Adjusting the tip height from the bottom of the well to the height of floating cell debris may also increase efficiency of mixing.
Absorbance of purified DNA does not accurately reflect quantity of plasmid DNA.	Plasmid DNA is contaminated with RNA	Confirm the RNase A Solution has been added to the Resuspension Solution prior to use. The RNase Solution may degrade due to high temperatures (>65 °C) or long-term storage (>6 months at room temperature).
	Plasmid DNA is contaminated with chromosomal DNA	Do not use cultures that have grown for more than 24 hours or are in the cell death phase. Reduce pipette mixing cycles during the Neutralization and Binding steps.

Slow or no filtration of lysates through the filter plates.	Wells of Filter Plate are clogged	Reduce starting culture volume or use a culture with a lower cell density.
	Vacuum pressure setting is too low	Increase the pressure setting on the vacuum pump prior to the start of the method.
	Poor vacuum seal	Contact Beckman Coulter. Gaskets on the vacuum manifold may need to be replaced.
Filter Plate or Binding Plate are sticking to gaskets on the vacuum manifold	Vacuum pressure setting is too high	Decrease vacuum pressure setting. Recommended range of pressure is between –16 and –21 inches of Hg. Increase time of system pauses following filtration steps to allow vacuum to completely evacuate the manifold.

XV. Contact Information

Technical Service
(800) 325-5832
email: techserv@sial.com

Customer Service
(800) 325-3010
(800) 588-9160
www.sigma-aldrich.com/order

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