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

GenElute™ HP 96 Well Plasmid Miniprep Kit

Catalog Number **NA9604**

TECHNICAL BULLETIN

Product Description

The GenElute HP 96 Well Plasmid Miniprep Kit offers a simple, rapid, cost-effective solution for high-throughput purification of plasmid DNA from recombinant *E. coli* cultures. By combining silica-binding technology and the convenience of a vacuum format, up to 10 µg of high copy plasmid DNA per well can be recovered from 1.3 ml of culture in less than 50 minutes. Actual yields and the optimum volume of culture depend on the plasmid and culture medium used. The procedure is compatible with automated liquid handling workstations and protocols can be downloaded from www.Sigma-Aldrich.com/automation.

Process Overview

Bacterial cells are harvested by centrifugation and resuspended in Resuspension Solution containing RNase. The cells are then lysed and neutralized followed by the addition of Binding Solution. The prepared lysate is then transferred to a Sigma HP Filter Plate assembled in a vacuum manifold where it is filtered and collected into a deep well plate. The vacuum manifold is rearranged and the filtered lysate is transferred to the Sigma HP Binding Plate. The plasmid DNA is captured onto the silica based membrane and impurities are removed by successive wash steps. Finally, the binding plate is dried and the plasmid DNA is eluted in Elution Solution. The purified plasmid is ready for immediate use in demanding downstream applications such as high throughput transfections.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Liquid handling workstations can vary dramatically based on vacuum system capabilities, available hardware, programming and deck configuration. Automated protocols should be optimized for each individual system.

Components Provided

Product	Catalog Number	4 x 96 preps
Column Preparation Solution	C2112	1 x 375 ml
RNase A solution	R6148	1 x 1.5 ml
Resuspension Solution	R1149	1 x 240 ml
Lysis Solution	L1912	1 x 240 ml
Neutralization Solution	N1285	1 x 240 ml
Binding Solution	B4683	1 x 280 ml
Wash Solution 1	W0263	1 x 375 ml
Wash Solution 2	W4639	1 x 75 ml
Elution Solution	E7777	2 x 115 ml
Filter Plate	F3555	4
Binding Plate	B6686	4
Elution Plate (Corning® Costar® cell culture plate)	CLS3790	4
AlumaSeal™ II film	A2350	12

Equipment and reagents required, but not provided

- Ethanol (95-100%), Catalog No. E7148, E7023 or 459836
- Deep Well Plates
- Centrifuge with a swinging bucket rotor capable of centrifuging deep well plates
- Vacuum Manifold capable of 16-21 in. Hg
- Vacuum Regulator
- Vacuum Source
- Sterile Breathable Seals

Preparation Instructions

1. Examine the reagents for precipitation. If any reagent forms a precipitate upon storage, warm at 37 °C until the precipitate dissolves.
2. Add 1.2 ml of RNase A Solution to the Resuspension Solution and mix.
   Once the RNase A Solution is added to the Resuspension Solution, store at 2-8 °C.
3. Add 300 ml of 95-100% ethanol to the Wash Solution 2 and mix.

Storage/Stability

Store the kit at room temperature (18-25 °C).

Vacuum Manifold Use
The vacuum manifold must accommodate the Sigma Filter and Binding Plates. Read the manufacturer's recommended instructions for proper vacuum manifold use. The manifold must be capable of capturing the filtered lysate in a deep well collection plate. The following protocol was tested and validated using a Qiagen QIAvac manifold, Catalog No. 19504.

Culture Preparation
1. Fill a 96 deep well plate with 1.3 ml of LB medium. If a rich medium is used, such as TB, cell densities can become very high. Reducing the cell culture volume may be necessary to avoid improper filtration of the bacterial lysates. It is not recommended to use cultures with an optical density greater than 5 at 600 nm.
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.

Procedure
1. Pellet the 96 deep well culture plate containing the overnight culture by centrifuging at 1800 x *g* for 10 minutes.
2. During centrifugation, assemble the manifold by placing an empty deep well collection plate in the manifold base. Place the manifold collar over the deep well collection plate so it fits securely on top of the manifold base. Place the filter plate on top of the manifold collar.
   Note: The tips of the filter plate should be positioned inside the wells of the deep well collection plate. It may be necessary to place something under the deep well collection plate to elevate it slightly.
3. Following centrifugation of the deep well culture plate, pour off the supernatant and blot the deep well culture plate upside down on an absorbent pad.
4. Using a multichannel pipette, add 200 µL of Resuspension Solution to the pelleted cells and resuspend by pipetting up and down.
5. Add 200 µL of Lysis Solution and pipette up and down 5-6 times to mix.
6. Allow the lysate to incubate at room temperature for 3 to 5 minutes; do not allow the lysis to exceed 5 minutes.
7. Add 200 µL of Neutralization Solution and pipette up and down 5-6 times to mix.
8. Add 200 µL of Binding Solution and pipette up and down 2-3 times to mix.
9. Transfer the lysate mix from the culture plate to the filter plate that is located on top of the manifold assembly and let sit for 5 minutes.
10. Apply vacuum.
    Note: To avoid spraying of the lysate into the deep well collection plate, adjust the vacuum using a vacuum regulator so that the lysate filters slowly through the filter plate (1-2 drops / second).
11. Allow the lysate to filter completely through the filter plate and into the deep well collection plate. Depending on the cell culture density this may take a few minutes. Once the lysate is completely filtered, turn off and release vacuum.
12. Discard filter plate and prepare the manifold by removing the manifold collar. Remove the deep well collection plate containing the filtered lysate from the manifold base and set aside. Place the waste tray in the manifold base. Place the manifold collar over the waste tray so it fits securely on top of the manifold base. Place the binding plate on top of the manifold collar.
13. Add 600 µL of Column Preparation Solution and apply vacuum until all the solution passes through the binding plate. Turn off vacuum.
14. Transfer the filtered lysate from the deep well collection plate to the binding plate.
15. Apply vacuum so that the solution slowly passes through the binding plate. Turn off vacuum.
16. Add 600 µL of Wash Solution 1 and apply vacuum until all the solution passes through the binding plate. Turn off vacuum.
17. Add 600 µL of Wash Solution 2 and apply vacuum until all the solution passes through the binding plate. Turn off vacuum.
18. Remove the binding plate from the vacuum manifold and thoroughly blot the plate on an absorbent pad, making sure all residual Wash Solution 2 is removed.
19. Place the binding plate back on the vacuum manifold, apply **maximum** vacuum and dry the binding plate for a minimum of 10 minutes.
20. Once the plate is dry, place the binding plate on top of the elution plate.
21. Add 100 µL of Elution Solution directly onto the membrane for each well of the binding plate and centrifuge the plate at 1800 x *g* for 5 minutes. The typical recovery is approximately 75-80 µL.
    Note: The concentration of eluted plasmid can be increased by eluting with less than 100 µL of Elution Solution. Overall plasmid yield may be reduced.
22. The process is complete, seal the elution plate using a foil seal and store at –20 °C.

Centrifugation Protocol.

1. Pellet the 96 deep well culture plate containing the overnight culture by centrifuging at 1800 x *g* for 10 minutes.
2. During centrifugation, place a filter plate on top of a deep well collection plate (not provided) in preparation of **Step 9**.
3. Following centrifugation of the deep well culture plate, pour off the supernatant and blot the deep well culture plate upside down on an absorbent pad.
4. Using a multichannel pipette, add 200 µL of Resuspension Solution to the pelleted cells and resuspend by pipetting up and down.
5. Add 200 µL of Lysis Solution and pipette up and down 5-6 times to mix.
6. Allow the lysate to incubate at room temperature for 3 to 5 minutes; do not allow the lysis to exceed 5 minutes.
7. Add 200 µL of Neutralization Solution and pipette up and down 5-6 times to mix.
8. Add 200 µL of Binding Solution and pipette up and down 2-3 times to mix.
9. Transfer the lysate mix from the culture plate to the filter plate.
10. Spin the filter plate assembly in swinging bucket centrifuge at 3500 – 4500 x *g* for 5 minutes.
11. If all lysate was not spun in previous step, then complete the transfer and centrifuge the filter plate assembly again at 3500 – 4500 x *g* for 5 minutes.
12. Discard the filter plate and place the deep well collection plate containing the filtered lyaste aside until **Step 14**. Place a binding plate on top of a clean deep well collection plate.
13. Add 600 µL of Column Preparation Solution to each well in the binding plate and centrifuge at 3500 – 4500 x *g* for 5 minutes. Discard the waste in the deep well collection plate and place back under binding plate.
14. Transfer the filtered lysate from the deep well collection plate in **Step 12** to the binding plate.
15. Centrifuge at 3500 – 4500 x *g* for 5 minutes. Discard the waste in the deep well collection plate and place back under binding plate.
16. Add 600 µL of Wash Solution 1 to each well in the binding plate and centrifuge at 3500 – 4500 x *g* for 5 minutes. Discard the waste in the deep well collection plate and place back under binding plate.
17. Add 600 µL of Wash Solution 2 to each well in the binding plate and centrifuge at 3500 – 4500 x *g* for 5 minutes. Discard the waste in the deep well collection plate and place back under binding plate.
18. **Optional:** Remove the binding plate and thoroughly blot the plate on an absorbent pad, if necessary, making sure all residual Wash Solution 2 is removed.
19. Place the binding plate back on the deep well collection plate, centrifuge at 3500 -4500 x *g* to dry the binding plate for a **minimum** of 10 minutes.
20. Once the plate is dry, place the binding plate on top of the elution plate.
21. Add 100 µL of Elution Solution directly onto the membrane for each well of the binding plate and centrifuge the plate at 1800 - 2800 x *g* for 5 minutes. The typical recovery is approximately 75-80 µL.
    Note: The concentration of eluted plasmid can be increased by eluting with less than 100 µL of Elution Solution. Overall plasmid yield may be reduced.
22. The process is complete, seal the elution plate using a foil seal and store at –20 °C.

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Troubleshooting Guide

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.
Absorbance of purified DNA does not accurately reflect quantity of plasmid DNA .	Plasmid DNA is contaminated with RNA	Confirm that RNase A 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. Do not vigorously mix the lysate mixture to avoid shearing of chromosomal DNA.
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 so you achieve a filtration rate of 1-2 drops / second.
	Poor vacuum seal	Gaskets on the vacuum manifold may need to be replaced.
Poor performance in downstream applications.	Ethanol present in eluted sample.	Confirm the Binding Plate has been dried on the manifold with full vacuum for a minimum of 10 minutes. Increase vacuum pressure and drying time if necessary
	High salt concentration in final elution.	Wash solution 2 was not added following Wash Solution 1.