Genesee Scientific
A Life Science Company

Product Manual

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Plasmid DNA Miniprep Kit

Table of Contents

Introduction

Gene Choice® Plasmid DNA Miniprep Kits combine the power of technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high-quality DNA in less than 30 minutes. DNA Mini Columns facilitate the binding, washing, and elution steps thus enabling multiple samples to be processed simultaneously.

Typically, a 1.5 ml overnight culture in LB medium produces 3-12 µg plasmid DNA; although yields may vary according to plasmid copy number, E. coli strain, and growth conditions. The Plasmid DNA Miniprep Kit is used to isolate plasmid DNA from1-5 ml cultures. Purified plasmid DNA can be directly used for most downstream applications including automated fluorescent DNA sequencing and restriction enzyme digestion.

Protocols

Gene Choice® Plasmid DNA Mini Kits are designed for fast and efficient processing. Depending on the protocol, the kits can be used with any microcentrifuge or vacuum manifold with standard luer connectors.

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Yield and Quality of DNA

Determine the absorbance of an appropriate dilution (20 to 50 - fold) of the sample at 260 nm and then at 280 nm.
The DNA concentration is calculated as follows:

$$\text{DNA Concentration} = \text{Absorbance } 260 \times 50 \times (\text{Dilution Factor}) \ \mu g/ml$$

A ratio greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) sometimes can be determined best by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations. Typically, the majority of the DNA eluted is in monomeric supercoil form, though concatemers may also be present.

Plasmid Copy Number and Expected Yield

Yield and quality of the plasmid DNA obtained depends on a number of factors including plasmid copy number, size of insert, host strain, culture volume, culture medium, and binding capacity of the kit. Of these factors, the vector copy number, culture volume, and kit binding capacity are most important.

Plasmid copy number ranges from one copy to several hundred copies per cell as dictated by their origin of replication. Very large plasmids often display a very low copy number per cell. The expected yield of 5 ml overnight cultures (LB medium) with the Plasmid Miniprep Kit are indicated in the following table.

Sample Yields from a 5 ml Starting Culture

Plasmid	Replicon	Copy Number	Expected Yield
pUC vectors	pMBI	500 - 700	15-25 µg
pBluescript® vectors	ColE14	300 - 500	10-18 µg
pGEM® vectors	pMB1	300 - 400	10-20 µg
pBR322 and its derivatives	pMB1	15 - 20	1-2 µg
ColE14	ColE14	15 - 20	1-2 µg
PACYC and its derivatives	p15A	37 - 40	0.5-1 µg
pSC101 and its derivatives	pSC101	~5	0.5 µg
pGEM	pMB1	300 - 700	10-20 µg

Centrifugation Protocol

Add GTC Lysis Buffer and Lyse

Transfer Lysate to RNA Homogenizer Mini Column

Add Ethanol Apply Sample to Column

Wash 3X

Dry

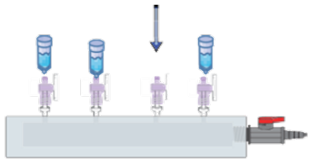
Elute

Vacuum Protocol

Add GTC Lysis Buffer and Lyse

Transfer Lysate to RNA Homogenizer Mini Column

Add Ethanol Apply Sample to Column



Wash 3X

Dry

Elute

Kit Contents

Product	SMP96-308	96-308	96-308B
Preps	5	50	200
DNA Mini Columns	5	50	200
2 ml Collection Tubes	5	50	200
Solution 1	3 ml	20 ml	60 ml
Solution 2	3 ml	20 ml	60 ml
Solution 3	3 ml	20 ml	80 ml
LB3 Buffer	5 ml	25 ml	80 ml
DNA Wash Buffer	2.5 ml	25 ml	3 x 25 ml
RNase A	N/A Pre-Added	100 µl	400 µl
Elution Buffer	2 ml	15 ml	30 ml
User Manual	✓	✓	✓

Storage and Stability

All kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Solution 1 (once RNase A is added) should be stored at 2-8˚C. All other materials should be stored at room temperature. Solution 2 must be tightly capped when not in use.

Preparing Reagents

1. Add the vial of RNase A to the bottle of Solution 1 and store at 2-8˚C. (50 and 200 prep size only)
2. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature

Kit	100% Ethanol to be Added
SMP96-308	10 ml
96-308	100 ml
96-308B	100 ml

3. Dilute LB3 Buffer with 100% isopropanol as follows and store at room temperature

Kit	100% Isopropanol to be Added
SMP96-308	2 ml
96-308	10 ml
96-308B	32 ml

3. Check Solution 2 and Solution 3 for precipitation before use. Redissolve any precipitation by warming to 37˚C

Guidelines for Vacuum Manifold

The following is required for use with the Vacuum/Spin Protocol:

A) **Vacuum Manifold**
Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector

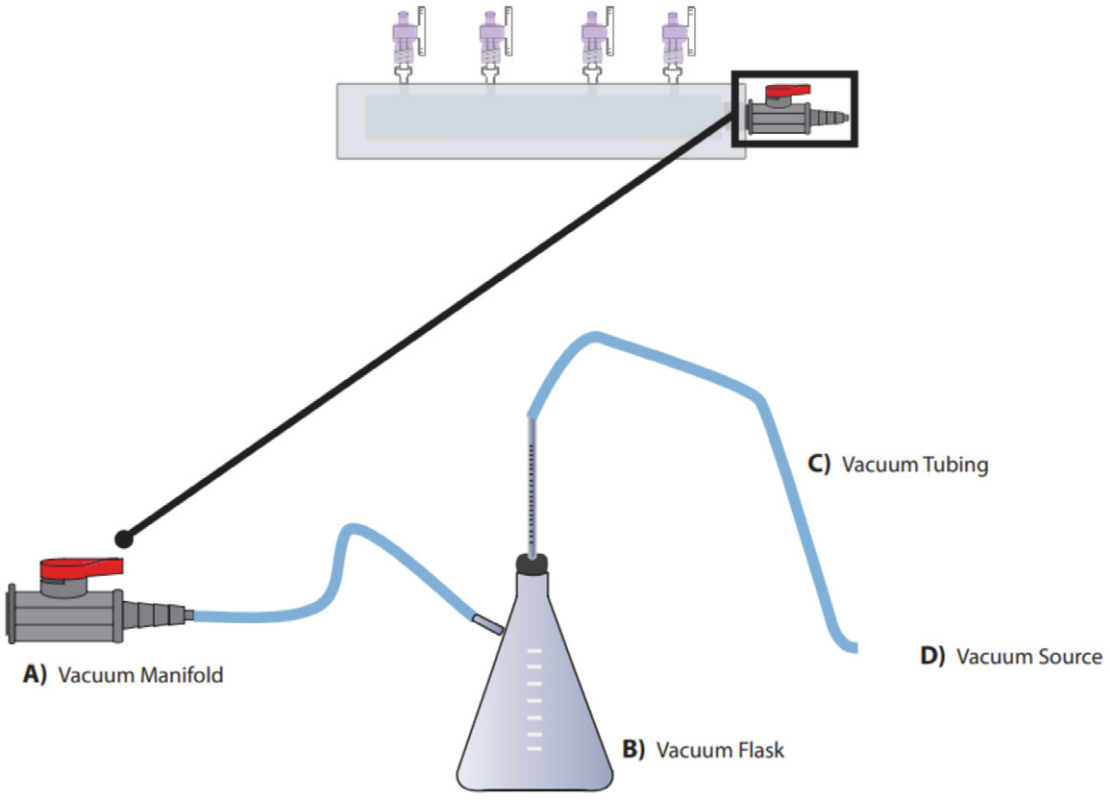
B) **Vacuum Flask**

C) **Vacuum Tubing**

D) **Vacuum Source** (review tables below for pressure settings)

Conversion from millibars:	Multiply by:
Millimeters of mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds Per Square Inch (psi)	0.0145

Vacuum Setup:



Recommended Settings

Growth and Culture of Bacteria

Bacterial Strain Selection

It is strongly recommended that an end A negative strain of E. coli be used for routine plasmid isolation. Examples of such strains include DH5α™, DH1, and C600. These host strains yield high-quality DNA with Plasmid DNA Mini Kit Protocols. XL1-Blue, although a slower growing strain is also recommended due to its yield of high-quality DNA.

Host strains derivatives from HB101 such as TG1 and the JM100 series release large amounts of carbohydrates during lysis, which may inhibit enzyme activity when not completely removed. Some strains may also lower DNA quality due to having high levels of endonuclease activity, and therefore are not recommended (i.e. JM101, JM110, HB101). One may reduce the amount of culture volume or double the volumes of Solution 1, Solution 2, and Solution 3, if problems are encountered with strains such as TG1 and Top10F.

Inoculation

Bacterial cultures for plasmid preparations should always be grown from a single colony picked from a freshly streaked plate. Subculturing directly from glycerol stock or liquid cultures may lead to uneven yields or plasmid loss. Optimal results are obtained by using one single isolated colony from a freshly transformed or freshly streaked plate to inoculate an appropriate volume of starter culture containing the appropriate antibiotic, and then incubated for 12-16 at 37°C with vigorous shaking (~300 rpm; shaking incubator).

Note: Aeration is especially important. The culture volume should not exceed 1/4 the volume of the container.

Culture Media

The Plasmid DNA Mini Kits are specially designed for use with cultures grown in Luria Bertani (LB) medium. Richer broths such as TB (Terrific Broth) or 2 x YT lead to high cell densities that can overload the purification system, and therefore are not recommended. If rich media must be used, growth times must be optimized, and the recommended culture volumes must be reduced to match the capacity of the DNA Column.

Note: As culture ages, DNA yield may begin to decrease due to cell death and lysis within the culture.

Culture Volume and Cell Density

Do Not Exceed Maximum Recommended Culture Volumes

For optimal plasmid yields, the starting culture volume should be based on culture cell density. A bacterial density between 2.0 and 3.0 at OD_{600} is recommended. When using nutrient-rich media, care should be taken to ensure that the cell density does not exceed an OD_{600} of 3.0. Using a high-density culture outside of the recommended OD range may overload the purification system.

Centrifugation Protocol

All centrifugations should be performed at room temperature unless otherwise noted. For low copy number plasmids refer "Low Copy Number Plasmid and BAC DNA Protocol." This protocol is designed to isolate plasmid DNA from E. coli grown in an overnight 1-5 mL LB culture.

Materials and Equipment to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 ml or 2 ml microcentrifuge tubes
- Culture tubes
- **Optional:** sterile deionized water
- **Optional:** water bath or incubator capable of 70°C
- **Optional:** 3M NaOH solution

Before Starting:

- Heat Elution Buffer to 70°C if plasmid DNA is >10 kb
- Prepare DNA Wash Buffer, LB3 Buffer, and Solution 1 according to the instructions in the "Preparing Reagents" section

1. Isolate a single colony from a freshly streaked selective plate and inoculate a culture of 1- 5 ml LB medium containing the appropriate selective antibiotic. Incubate for ~12-16 hours at 37°C with vigorous shaking (~ 300 rpm). Use a 10-20 ml culture tube or a flask with a volume of at least 4 times the volume of the culture. It is strongly recommended that an endA negative strain of E. coli be used for routine plasmid isolation. Examples of such strains include DH5a® and JM109®.

2. Centrifuge at 10,000 x *g* for 1 minute at room temperature.

3. Decant or aspirate and discard the culture media.

4. Add 250 µl Solution 1/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.
Note: RNase A must be added to Solution 1 before use. Please see the instructions in the "Preparing Reagents" section.

5. Transfer suspension into a new 1.5 ml microcentrifuge tube

6. Add 250 µl Solution 2. Invert and gently rotate the tube several times to obtain a clear lysate. A 2–3-minute incubation may be necessary
Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution 2 tightly capped when not in use to avoid acidification from CO_2 in the air.

7. Add 350 µl Solution 3. Immediately invert several times until a flocculent white precipitate forms.
Note: It is vital that the solution is mixed thoroughly and immediately after the addition of Solution 3 to avoid localized precipitation.

8. Centrifuge at maximum speed (≥13,000 x *g*) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.

9. Insert a DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

- Add 100 µL 3M NaOH to the DNA Mini Column
- Centrifuge at maximum speed for 30-60 seconds
- Discard the filtrate and reuse the collection tube

10. Transfer the cleared supernatant from Step 8 by CAREFULLY aspirating it into the DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the DNA Mini Column.

11. Centrifuge at maximum speed for 1 minute.

12. Discard the filtrate and reuse the collection tube.

13. Add 500 µL LB3 Buffer.
Note: LB3 Buffer must be diluted with 100% isopropanol before use. Please see "Preparing Reagents" section for instructions.

14. Centrifuge at maximum speed for 1 minute.

15. Discard the filtrate and reuse the collection tube.

16. Add 700 µL DNA Wash Buffer.
Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see "Preparing Reagents" section for instructions.

17. Centrifuge at maximum speed for 1 minute.

18. Discard the filtrate and reuse the collection tube.
Optional: Repeat **Steps 16-18** for a second DNA Wash Buffer wash step.

19. Centrifuge the empty DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.
Note: It is important to dry the DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

20. Transfer the DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

21. Add 30-100 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.
Note: The efficiency of eluting DNA from the DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

22. Let sit at room temperature for 1 minute.

23. Centrifuge at maximum speed for 1 minute.
Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

24. Store DNA at -20°C.

Vacuum Protocol

All centrifugations should be performed at room temperature unless otherwise noted. For low copy number plasmids refer to Page 14. This protocol is designed to isolate plasmid DNA from E. coli grown in an overnight 1-5 mL LB culture.

Materials and Equipment to be Supplied by User:

- Vacuum manifold
- 100% ethanol
- 100% isopropanol
- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 ml or 2 ml microcentrifuge tubes
- Appropriate centrifuge and centrifuge tube for **Step 1**
- **Optional:** sterile deionized water
- **Optional:** water bath or incubator capable of 70°C
- **Optional:** 3M NaOH solution

Before Starting:

- Heat Elution Buffer to 70°C if plasmid DNA is >10 kb
- Prepare DNA Wash Buffer, LB3 Buffer, and Solution 1 according to the instructions in the "Preparing Reagents" section

1. Isolate a single colony from a freshly streaked selective plate and inoculate a culture of 1- 5 ml LB medium containing the appropriate selective antibiotic. Incubate for ~12-16 hr at 37°C with vigorous shaking (~ 300 rpm). Use a 10-20 ml culture tube or a flask with a volume of at least 4 times the volume of the culture. It is strongly recommended that an endA negative strain of E. coli be used for routine plasmid isolation. Examples of such strains include DH5a® and JM109®

2. Centrifuge at 10,000 x *g* for 1 minute at room temperature

3. Decant or aspirate and discard the culture media

4. Add 250 µl Solution 1/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.
Note: RNase A must be added to Solution 1 before use. Please see the instructions in the "Preparing Reagents" section.

5. Transfer suspension into a new 1.5 ml microcentrifuge tube

6. Add 250 µl Solution 2. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary.
Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed for more than 5 minutes. Store Solution 2 tightly capped when not in use to avoid acidification from CO2 in the air.

7. Add 350 µL Solution 3. Immediately invert several times until a flocculent white precipitate forms
Note: It is vital that the solution is mixed thoroughly and immediately after the addition of Solution 3 to avoid localized precipitation.

8. Centrifuge at maximum speed (≥13,000 x *g*) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step

9. Prepare the vacuum manifold according to manufacturer's instructions

10. Connect the DNA Mini Column to the vacuum manifold

Optional Protocol for Column Equilibration:
Add 100 µl 3M NaOH to the DNA Mini Column
Turn on the vacuum source to draw the NaOH through the column
Turn off the vacuum

11. Transfer the cleared supernatant from **Step 8** by CAREFULLY aspirating it into the DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the DNA Mini Column

12. Turn on the vacuum source to draw the sample through the column

13. Turn off the vacuum

14. Add 500 µl LB3 Buffer
Note: LB3 Buffer must be diluted with 100% isopropanol before use. Please see "Preparing Reagents" section for instructions.

15. Turn on the vacuum source to draw the buffer through the column

16. Turn off the vacuum

17. Add 700 µl DNA Wash Buffer
Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see "Preparing Reagents" section for instructions.

18. Turn on the vacuum source to draw the buffer through the column

19. Turn off the vacuum

20. Repeat **Steps 17-19** for a second DNA Wash Buffer wash step

21. Transfer the DNA Mini Column to a 2 ml Collection Tube

22. Centrifuge the empty DNA Mini Column for 2 minutes at maximum speed to dry the column matrix
Note: It is important to dry the DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

23. Transfer the DNA Mini Column to a clean 1.5 ml microcentrifuge tube

24. Add 30-100 µl Elution Buffer or sterile deionized water directly to the center of the column membrane
Note: The efficiency of eluting DNA from the DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

25. Let sit at room temperature for 1 minute

26. Centrifuge at maximum speed for 1 minute
Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

27. Store DNA at -20°C

Low Copy Number Plasmid & BAC DNA Protocol

Low copy number plasmids generally give 0.1-1 µg DNA per ml overnight culture. For the isolation of plasmid DNA from low copy number plasmids (0.1-1 µg/ml culture) or low copy number plasmid (1-2 µg/ml culture) bacteria, use the following modified protocol.

Note: This Plasmid DNA Mini Kit comes with enough Solution 1, Solution 2, and Solution 3 to perform the standard protocols.

Additional Solution 1, Solution 2, and Solution 3 are needed to perform the "Low Copy Number Plasmid and BAC DNA Protocol" and are available for purchase separately.

1. Increase the volume of starting culture from that of high copy number plasmids. Use 5-10 ml bacterial culture

2. Pellet the bacterial cells by centrifugation

3. Decant or aspirate and discard the culture media

4. Perform **Steps 4-8** in the standard protocols with double the volumes of Solution 1, Solution 2, and Solution 3

5. Continue with **Step 9** of the standard protocols by following the wash, drying, and elution steps. There is no need to increase the volumes of LB3 Buffer, DNA Wash Buffer, or Elution Buffer

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, **please contact the technical support staff**.

Possible Problems and Suggestions

	Low DNA Yields
Poor cell lysis	• Only use LB or YT medium containing ampicillin. Do not use more than 5 ml (high copy number plasmids) or 10 ml (low copy number plasmids) culture with the basic protocols • Cells may not have been dispersed adequately prior to the addition of Solution II. Vortex to completely resuspend the cells • Increase Solution II incubation time to obtain a clear lysate • Solution II, if not tightly closed, may need to be replaced
Culture is overgrown or not fresh	Do not incubate cultures for more than 16 hours at 37°C. Storage of cultures for extended periods prior to plasmid isolation is detrimental
Low elution efficiency	The pH of Elution Buffer or water must be pH 8.0-9.0
Low copy-number plasmid used	Such plasmids may yield as little as 0.1 µg DNA from a 1 ml overnight culture. Double the culture volume and follow the low copy number plasmid protocol
Column matrix lost binding capacity during storage	Follow the Optional Protocol for Column Equilibration prior to transferring the cleared lysate to the DNA Mini Column. Add 100 µl 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x *g* for 30 seconds. Discard the filtrate
	No DNA eluted
DNA Wash Buffer not diluted with ethanol	Prepare DNA Wash Buffer according to the instructions
LB3 Buffer not diluted with isopropanol	Prepare LB3 Buffer according to instructions
	High molecular weight DNA contamination of product
Over mixing of cell lysate upon addition of Solution II	Do not vortex or mix aggressively after adding Solution 2
Culture overgrown	Overgrown cultures contain lysed cells and degraded DNA. Do not grow cell longer than 16 hours
	Plasmid DNA floats out of well while loading agarose gel
Ethanol was not completely removed from column following wash steps	Centrifuge column as instructed to dry the column before elution
	Absorbance of purified DNA does not accurately reflect quantity of the plasmid (A_{260}/A_{280} ratio is high or low)
DNA Wash Buffer is diluted with ethanol containing impurities	Check the absorbance of the ethanol between 250 nm and 300 nm. Do not use ethanol with high absorbance. Traces of impurities may remain on the binding column after washing and contribute to the absorbance in the final product.
Plasmid DNA is contaminated with RNA; RNase A treatment is insufficient	Confirm that the RNase A Solution was added to Solution I prior to first use. The RNase A solution may degrade due to high temperatures (>65 °C) or prolonged storage (> 6 months at room temperature)
Background reading is high due to silica fine particulates	Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings
Purification is incomplete due to column overloading	Reduce the initial volume of culture

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