October 2006

QuickLyse Miniprep Handbook

For purification of sequencing grade plasmid DNA

Contents

Kit Contents

QuickLyse Miniprep Kit	**(100)**	**(250)**
Catalog no.	**27405**	**27406**
Number of preps	**100**	**250**
QuickLyse Spin Columns	100	250
QuickLyse Lysis Tubes (2 ml)	100	250
Buffer QLL	56 ml	140 ml
RNase A	0.7 ml	1.75 ml
Lysozyme	60 mg	150 mg
Buffer QLW	18 ml	45 ml
Buffer QLE	10 ml	25 ml
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Storage

QuickLyse Spin Columns should be stored at room temperature (15–25°C) upon arrival.

Buffer QLL, RNase Solution, and lyophilized lysozyme should be stored at 2–8°C for maximum stability.

Complete Lysis Solution (Buffer QLL, RNase A, and Lysozyme mix) should be stored at 2–8°C and is stable for 4 months.

Buffer QLW should be stored in a tightly sealed container at room temperature (15–25°C) to prevent evaporation of the isopropanol.

Store all other QuickLyse Miniprep Kit components at room temperature (15–25°C). Do not freeze.

All QuickLyse Miniprep Kit components are stable for at least 12 months when stored unopened.

Quality Control

In accordance with QIAGEN's ISO-certified Total Quality Management System, each lot of QuickLyse Miniprep Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

The QuickLyse Miniprep Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN® product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QuickLyse Miniprep Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

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Introduction

The QuickLyse Miniprep system provides a very fast and simple plasmid miniprep method for routine molecular biology laboratory applications. This innovative technology has dramatically transformed traditional lysis-based preps by significantly reducing the number of steps associated with conventional processes. Plasmid purification can be performed in as little as 9 minutes, representing a significant time saving.

QuickLyse Miniprep Kits provide true one-step lysis, using enzymatic and osmotic driven processes to enable bacterial cell lysis in just one 3-minute step. The cleared lysate then binds directly to the membrane of the QuickLyse spin column without an additional lysate clearing centrifugation step.

Plasmid DNA purified with QuickLyse Miniprep Kits is immediately ready for use. Sequencing grade plasmid DNA is eluted in a small volume of elution buffer (included in each kit).

Plasmid DNA can be used immediately in amplification reactions or stored at –20°C.

Applications using QuickLyse purified DNA

- Restriction enzyme digestion
- Cloning
- Ligation and transformation
- PCR
- Sequencing

Principle

The QuickLyse Miniprep Kit is based on a proprietary technology providing a rapid, nonorganic means of isolating sequencing grade plasmid DNA from 1.5–3 ml of *E. coli* bacterial cultures.

QuickLyse technology uses a single solution for cell resuspension, lysis, and DNA binding. After lysing the cells, plasmid DNA is captured on a membrane housed in the QuickLyse spin column. The bound DNA is then washed with an isopropanol-containing buffer, and eluted in a low-salt buffer.

DNA yield

Plasmid yield with the QuickLyse miniprep system varies depending on plasmid copy number per cell (see page 18), the individual insert in a plasmid, factors that affect growth of the bacterial culture (see pages 18–21), and the elution volume. A 1.5 ml overnight culture of high-copy plasmids typically yields 3–8 µg of plasmid DNA. The QuickLyse Miniprep Kit has been optimized for use with high-copy plasmids. Low-copy plasmids will deliver considerably lower DNA yields and quality, and are not recommended for use with this kit. To obtain the optimum combination of DNA quality, yield, and concentration, we strongly recommend using Luria-Bertani (LB) medium for growth of cultures (for composition see page 20), and eluting plasmid DNA in a volume of 50 µl.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- Liquid bacterial growth medium supplemented with the appropriate antibiotic
- Incubator and basic microbiology equipment for bacterial culture
- Ice
- Microcentrifuge
- Vortexer*
- Isopropanol (95–100%)
- 2 ml microcentrifuge tubes
- Pipets and pipet tips

* A vortexer with multiple microtube wells (e.g., Eppendorf Thermomixer®) will considerably simplify the handling if performing multiple parallel preparations.

Important Notes

Please read the following notes before starting the QuickLyse procedure.

Growth of bacterial cultures in tubes or flasks

1. **Pick a single colony from a freshly streaked selective plate and inoculate a culture of 1–3 ml LB medium containing the appropriate selective antibiotic. Incubate for 12–16 h at 37°C with vigorous shaking until an OD_{600} of 2.0–4.0 is achieved.**

   The *E. coli* cells should not have entered the stationary phase. Growth for more than 16 h is not recommended since cells begin to lyse and plasmid yields may be reduced. Use a tube or flask with a volume of at least 4 times the volume of the culture.

   Important: Growth in LB culture medium is strongly recommended (see "Culture media" on page 20 for more information).

2. **Using a 2 ml QuickLyse Lysis Tube (provided), pellet bacterial cells from 1.5 ml of culture by centrifugation at >13,000 rpm (approximately 17,000 x *g*) in a conventional, table-top microcentrifuge for 1 min at room temperature (15–25°C).**

   Note: If necessary (i.e., OD_{600} is <4), a maximum of 3 ml culture can be processed. However, a culture volume of 1.5 ml should be used for cells with an OD_{600} >2 as excessive biomass will cause inefficient lysis leading to poor yield and DNA quality.

3. **Remove medium by decanting or pipetting.**

   Inverting the tubes on a paper towel may improve removal of the medium.

Buffer notes

Complete Lysis Solution

To ensure optimum lysis conditions, store Buffer QLL, RNase A, and the lyophilized lysozyme at 2–8°C prior to use.

1. **Briefly centrifuge the RNase A to collect all of the liquid in the bottom of the tube.**
2. **Resuspend the lyophilized lysozyme using the entire volume of the RNase A.**
3. **Mix thoroughly by pipeting up and down. Take care to ensure that all of the powder is dissolved. Some foaming will occur.**
4. **Pipet the entire contents of the resuspended lysozyme/RNase mixture to the Buffer QLL bottle to make the Complete Lysis Solution. Mix thoroughly and check the "RNase A/lysozyme added" box on the label.**
5. **Store Complete Lysis Solution at 2–8°C.**

   Note: It may be necessary to rinse the lysozyme tube with a small volume of Buffer QLL in order to collect the entire volume of the RNase/lysozyme mixture.

   Note: Complete Lysis Solution should be stored at 2–8°C for optimum performance. Before use, chill the Complete Lysis Solution to <4°C on ice. Complete Lysis Solution can be incubated on ice for an indefinite period of time without affecting kit performance.

Diluted Buffer QLW (wash buffer)

Add the appropriate volume of isopropanol (95–100%) to the entire bottle of Buffer QLW Concentrate as indicated in the table below. Mix thoroughly and check the "Isopropanol Added" box on the label.

Number of preps	Volume of isopropanol
100	38 ml
250	95 ml

Centrifugation notes

All centrifugation steps are carried out at 13,000 rpm (approximately 17,000 x *g*).

Elution notes

- Ensure that Buffer QLE (elution buffer) is dispensed directly onto the center of the QuickLyse membrane for optimal elution of DNA.
- For slightly increased DNA yield, use a higher elution-buffer volume. For increased DNA concentration, use a lower elution-buffer volume (see "DNA yield", page 9).

Protocol: Plasmid DNA Purification Using the QuickLyse Miniprep Kit

This protocol is designed for purification of 3–8 µg high-copy plasmid DNA from 1.5 ml overnight cultures of *E. coli* in LB (Luria-Bertani) medium.

Please read "Important Notes" on pages 11–13 before starting.

Things to do before starting

- Ensure that the Complete Lysis Solution has been prepared according to the instructions on page 12.
- Chill the Complete Lysis Solution on ice until it is <4°C.
- Ensure that Diluted Buffer QLW has been prepared according to instructions on page 13.

Procedure

1. **Using a 2 ml QuickLyse Lysis Tube (provided), pellet bacterial cells from 1.5 ml of culture (OD_{600} 2.0–4.0) by centrifugation at >13,000 rpm (approximately 17,000 x *g*) in a conventional, table-top microcentrifuge for 1 min at room temperature (15–25°C).**

   A culture volume of 1.5 ml should be used for cells with an OD_{600} >2 as excessive biomass will cause inefficient lysis leading to poor yield and DNA quality.

2. **Remove medium by decanting or pipetting.**

   Inverting the tubes on a paper towel may improve removal of the medium.

3. **Add 400 µl ice cold Complete Lysis Solution to the pelleted bacterial cells.**

   Complete Lysis Solution must be ice cold (<4°C) to ensure maximum DNA yield.

4. **Mix thoroughly by vortexing at the highest setting for 30 s.**

   This step is critical to obtaining maximum DNA yield.

   If the pellet is not completely resuspended, continue vortexing until no cell clumps are visible.

5. **Incubate at room temperature (15–25°C) for 3 min.**

   Lysate should appear nonviscous and slightly cloudy, with no precipitate.

6. **Transfer the lysate to a QuickLyse spin column by decanting or pipetting.**

7. **Centrifuge for 30–60 s at 13,000 rpm (approximately 17,000 x *g*) in a table-top microcentrifuge.**

   It is not necessary to decant the flow-through.

6. **Wash the QuickLyse spin column by adding 400 µl diluted Buffer QLW and centrifuge for 30–60 s at 13,000 rpm (approximately 17,000 x *g*). Discard the flow-through.**
7. **Place the QuickLyse spin column back into the waste tube and return it to the centrifuge.**
8. **Centrifuge for 1 min at 13,000 rpm (approximately 17,000 x *g*) to dry the QuickLyse spin column.**
9. **Transfer the QuickLyse spin column into a clean collection tube. To elute DNA, pipet 50 µl Buffer QLE directly onto the center of the QuickLyse spin column. Centrifuge for 30–60 s at 13,000 rpm (approximately 17,000 x *g*).**

   To avoid inconsistent elution volumes, ensure that Buffer QLE is pipetted onto the center of the column, taking care to avoid the walls of the column.

   Eluted DNA can be used immediately in downstream reactions or stored at –20°C.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this handbook or molecular biology applications (see back cover for contact information).

	Comments and suggestions
Low or no yield	
General	Low yields may be caused by a number of factors. To find the source of the problem, analyze fractions saved from each step in the procedure on an agarose gel. A small amount of the lysate and the entire flow-through can be precipitated by adding 0.7 volumes isopropanol and centrifuging at maximum speed (13,000 rpm or approximately 17,000 x *g*) for 30 min. The entire wash flow-through can be precipitated by adding 0.1 volumes of 3 M sodium acetate,* pH 5.0, and 0.7 volumes of isopropanol.
Little or no DNA in eluate	
a) Plasmid did not propagate	Make sure that the appropriate antibiotic was included during all stages of growth. Read "Growth of bacterial cultures" (pages 18–21) and check that the conditions for optimal growth were met.
b) Cell resuspension incomplete	Ensure vortexing is performed for 30 seconds. If the pellet is not completely resuspended, continue vortexing until no cell clumps remain.
c) Lysate incubation not long enough	Ensure lysate is incubated for at least 3 min (step 3). Longer incubations (up to 5 min) may increase yield.
d) Buffer QLL incorrectly stored or old	Check storage conditions and age of buffers.
e) Buffer QLW prepared incorrectly	Check isopropanol is added according to the instructions.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

	Comments and suggestions
f) Vector/host combination	Addition of isopropanol to the lysate may improve yields in certain vector/host combinations. Add 125 µl isopropanol (95–100%) to the lysate after the incubation in step 6.
g) System overloaded	Ensure that the culture has an OD_{600} of 2.0–4.0.
h) Buffer QLE incorrectly dispensed onto membrane	Add Buffer QLE to the center of the QuickLyse membrane to ensure that the buffer completely covers the surface of the membrane for maximum elution efficiency.
Low DNA quality	
General	Complete Lysis Solution may be too warm. Ensure that Complete Lysis Solution is equilibrated on ice to 0–4°C. See also "Cell resuspension incomplete" and "Buffer QLW prepared incorrectly" above.
RNA in the eluate	
a) RNase A digestion omitted	Ensure that RNase A is added to the lyophilized lysozyme and then to the Complete Lysis Solution before use.
b) RNase A digestion insufficient	Reduce culture volume if necessary.

Appendix: Growth of Bacterial Cultures

Plasmids are generally prepared from bacterial cultures grown in the presence of a selective agent such as an antibiotic (1, 3). The yield and quality of plasmid DNA may depend on factors such as plasmid copy number, host strain, inoculation, type of culture medium, and antibiotic.

Plasmid copy number

Plasmids vary widely in their copy number per cell (Table 1), depending on their origin of replication (e.g., pMB1, ColE1, or pSC101), which determines whether they are under relaxed or stringent control; and depending on the size of the plasmid and its associated insert. Some plasmids, such as the pUC series and derivatives, have mutations that allow them to reach very high copy numbers within the bacterial cell.

Plasmids based on pBR322 and cosmids are generally present in lower copy numbers. Very large plasmids and cosmids are often maintained at very low copy numbers per cell. The QuickLyse Miniprep Kit has been optimized for use with high-copy plasmids. Low-copy plasmids will deliver considerably lower DNA yields and quality and are not recommended for use with this kit.

Table 1. Origins of Replication and Copy Numbers of Various Plasmids

DNA construct	Origin of replication	Copy number	Classification
Plasmids			
pUC vectors	pMB1*	500–700	high copy
pBluescript® vectors	ColE1	300–500	high copy
pGEM® vectors	pMB1*	300–400	high copy
pTZ vectors	pMB1*	>1000	high copy
pBR322 and derivatives	pMB1*	15–20	low copy
pACYC and derivatives	p15A	10–12	low copy
pSC101 and derivatives	pSC101	~5	very low copy
Cosmids			
SuperCos	ColE1	10–20	low copy
pWE15	ColE1	150–20	low copy

Information from reference 2.

* The pMB1 origin of replication is closely related to that of ColE1 and falls in the same incompatibility group. The high-copy-number plasmids listed here contain mutated versions of this origin.

Host strains

Several strains of *E coli* have proven themselves as reliable propagators of plasmids. The most common strains are DH5α™, DH10B, DH12S, and XL1-Blue.

Culture media

Maximum plasmid DNA yields are obtained when optimal growth conditions are employed. These conditions are achieved by using a single isolated colony from a freshly transformed or freshly plated *E. coli* bacterial strain and inoculating in culture medium.

Growth in LB culture medium is strongly recommended. Incubate culture to an OD_{600} of 2.0–4.0 (12–16 hours at 37°C with sufficient aeration). Dilute the sample to obtain an OD_{600} in the linear range of the instrument. Please note that a number of slightly different LB culture broths, containing different concentrations of NaCl, are in common use. Although different LB broths produce similar cell densities after overnight culture, plasmid yields can vary significantly. See Table 2 for recommended composition.

Growth in nutrient-rich media, such as 2x YT and TB, is not recommended as this can produce significantly higher cell densities and overload the purification system.

If using a small amount of a frozen glycerol stock as inoculum, streak it onto an agar plate containing the appropriate antibiotic for single colony isolation. After overnight incubation, a well-separated colony should be picked and used to inoculate the culture medium.

Table 2. Recommended Composition of Luria Bertani (LB) Medium

Contents	Per liter
Tryptone	10 g
Yeast extract	5 g
NaCl	10 g

Inoculation

Bacterial cultures for plasmid preparation should always be grown from a single colony picked from a freshly streaked selective plate. Subculturing directly from glycerol stocks, agar stabs, and liquid cultures may lead to uneven plasmid yield or loss of the plasmid. Inoculation from plates that have been stored for a long time may also lead to loss or mutation of the plasmid. The desired clone should be streaked from a glycerol stock onto a freshly prepared agar plate containing the appropriate selective agent so that single colonies can be isolated.

A single colony should be inoculated into liquid culture media containing the appropriate selective agent, and grown with vigorous shaking for 12–16 hours. The bacteria should still be in growth phase for optimal results. Growth for more than 16 hours is not recommended since cells begin to lyse and plasmid yields may be reduced.

Antibiotics

Antibiotic selection should be applied at all stages of growth. Many plasmids in use today do not contain the *par* locus, which ensures that the plasmids segregate equally during cell division. Daughter cells that do not receive plasmids will replicate much faster than plasmid-containing cells in the absence of selective pressure and can quickly take over the culture.

The stability of the selective agent should also be taken into account. Resistance to ampicillin, for example, is mediated by β-lactamase, which is encoded by the plasmid linked *bla* gene and which hydrolyzes ampicillin. Levels of ampicillin in the culture medium are thus continually depleted. This phenomenon is clearly demonstrated on ampicillin plates, where "satellite colonies" appear as the ampicillin is hydrolyzed in the vicinity of a growing colony. Ampicillin is also very sensitive to temperature and, when in solution, should be stored frozen in single-use aliquots. The recommendations given in Table 3 are based on these considerations.

Table 3. Concentrations of Commonly Used Antibiotics

	Stock solutions		
Antibiotic	**Concentration**	**Storage**	**Stock solutions**
Ampicillin (sodium salt)	50 mg/ml in water	–20°C	100 µg/ml (1/500)
Chloramphenicol	34 mg/ml in ethanol	–20°C	170 µg/ml (1/200)
Kanamycin	10 mg/ml in water	–20°C	50 µg/ml (1/200)
Streptomycin	10 mg/ml in water	–20°C	50 µg/ml (1/200)
Tetracycline HCl	5 mg/ml in ethanol	–20°C	50 µg/ml (1/200)

Cited References

1. Birnboim, H.C., and Doly, J. (1979) A rapid alkaline lysis procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**, 1513.
2. Sambrook, J. and Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
3. Ausubel, F.M. et al., eds. (1991–2006) *Current Protocols in Molecular Biology*. New York: Wiley Interscience.

Ordering Information

Product	Contents	Cat. no.
QuickLyse Miniprep Kit (100)	For 100 minipreps: 100 QuickLyse Spin Columns, Buffers, QuickLyse Lysis Tubes, Collection Tubes	27405
QuickLyse Miniprep Kit (250)	For 250 minipreps: 250 QuickLyse Spin Columns, Buffers, QuickLyse Lysis Tubes, Collection Tubes	27406
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