March 2024

QIAwave Plasmid Miniprep Handbook

For purification of molecular biology–grade DNA

- Plasmid DNA
- Large plasmid DNA (>10kb)
- Low-copy plasmid DNA and cosmids
- Plasmid DNA prepared by other methods

Sample to Insight

Table of Contents

Kit Contents

QIAwave Plasmid Miniprep Kit Catalog no.	(50) 27204	(250) 27206
QIAprep® 2.0 Spin Columns	50	250
Buffer P1/C (concentrate)	2 mL	10 mL
Buffer P2	15 mL	1 x 20 mL, 1 x 50 mL
Buffer N3*	30 mL	140 mL
Buffer PB*	30 mL	150 mL
Buffer PE/C (concentrate)	1 mL	5 mL
Buffer EB/C (concentrate)	2 mL	5 mL
LyseBlue®	20 µL	1 x 20 µL, 1 x 50 µL
RNase A†	2 mg	1 x 2 mg, 1 x 5 mg
Waste Tubes (2 mL)	50	250

*Buffers N3 and PB contain chaotropic salts which are irritants and not compatible with disinfecting agents containing bleach. Take appropriate laboratory safety measures and wear gloves when handling. See page 6 for Safety Information

†Provided as a 10 mg/mL solution.

Storage

The QIAwave Plasmid Miniprep Kit should be stored dry at room temperature (15–25°C). Under these conditions, if no expiration date is mentioned on the kit label, the QIAwave Plasmid Miniprep Kit can be stored for up to 12 months.

After addition of RNase A and optional LyseBlue reagent, Buffer P1 is stable for 6 months when stored at 2–8°C. RNase A stock solution can be stored for 2 years at room temperature.

Intended Use

The QIAwave Plasmid Miniprep Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

QIAcube® Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

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

CAUTION

Do not add bleach or acidic solutions directly to the sample preparation waste.

Buffers N3 and PB contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

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

Introduction

The QIAwave Plasmid Miniprep system provides a fast, simple, and cost-effective plasmid DNA miniprep method for routine molecular biology laboratory applications. The QIAwave Plasmid Miniprep Kit uses silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. Plasmid DNA purified with the QIAwave Plasmid Miniprep Kit is immediately ready for use. Phenol extraction and ethanol precipitation are not required, and high-quality plasmid DNA is eluted in a small volume of Tris buffer or water. The QIAwave Plasmid Miniprep Kit is designed for quick and convenient processing of 1–24 samples simultaneously in less than 30 minutes. QIAprep 2.0 Spin Columns can be used in a microcentrifuge or on any vacuum manifold with luer connectors (e.g., QIAvac 24 Plus, cat. no. 19413).

Applications using QIAwave-purified DNA

Plasmid DNA prepared using the QIAwave Spin Miniprep Kit is suitable for a variety of routine applications, including:

- Restriction enzyme digestion
- Library screening
- In vitro translation
- Sequencing
- Ligation and transformation
- Transfection of robust cells

Automated purification of plasmid DNA on QIAcube instruments

Purification of plasmid DNA can be fully automated on QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using the QIAwave Plasmid Miniprep Kit for purification of high-quality plasmid DNA.

QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **www.qiagen.com/qiacubeprotocols**

The QIAwave kits can be run on the QIAcube Connect using either the QIAprep Spin Miniprep Kit protocols or the dedicated QIAwave Plasmid Miniprep Kit protocols. The protocol steps remain the same for both options. For added convenience, utilizing dedicated QIAwave protocols allows users to initiate run-setup by scanning the QR code on the kit label, with the kit name automatically appearing in the run reports. Please note, kit content is calculated for manual use. While automating on the QIAcube Connect, the processed sample number could be less than that stated in the kit handbook or on the kit label.

QIAcube Connect

Principle

The QIAwave Plasmid Miniprep procedure is based on alkaline lysis of bacterial cells followed by adsorption of DNA onto silica in the presence of high salt (1). The unique silica membrane used in the QIAwave Plasmid Miniprep Kit completely replaces glass or silica slurries for plasmid DNA minipreps.

The procedure consists of 3 basic steps:

- Preparation and clearing of a bacterial lysate
- Binding of DNA onto the QIAprep membrane
- Washing and elution of plasmid DNA

All steps are performed without the use of phenol, chloroform, CsCl or ethidium bromide, and without alcohol precipitation.

Preparation and clearing of bacterial lysate

The QIAwave Plasmid Miniprep procedure uses the modified alkaline lysis method of Birnboim and Doly (2). Bacteria are lysed under alkaline conditions, and the lysate is subsequently neutralized and adjusted to high-salt–binding conditions in one step. After lysate clearing, the sample is ready for purification on the QIAprep silica membrane. For more details on growth of bacterial cultures and alkaline lysis, please refer to Appendix A, page 42. In the QIAwave Plasmid procedure, lysates are cleared by centrifugation.

LyseBlue reagent

Use of LyseBlue is optional and not required to successfully perform plasmid DNA preparations. See section "Using LyseBlue reagent" on page 15

LyseBlue is a color indicator that provides visual identification of optimum buffer mixing. This prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. This makes LyseBlue ideal for use by researchers who are starting with plasmid DNA preparations, as well as experienced scientists who want to be assured of maximum product yield.

DNA binding to the QIAprep membrane

QIAprep 2.0 Spin Columns use a silica membrane for selective adsorption of plasmid DNA in high-salt buffer and elution in low-salt buffer. The optimized buffers in the lysis procedure, combined with the unique silica membrane, ensure that only DNA will be adsorbed, while RNA, cellular proteins, and metabolites are not retained on the membrane but are found in the flow-through.

Washing and elution of plasmid DNA

Endonucleases are efficiently removed by a brief wash step with Buffer PB. This step is essential when working with endA+ strains, such as the JM series, HB101 and its derivatives, or any wild-type strain, to ensure that plasmid DNA is not degraded. The Buffer PB wash step is also necessary when purifying low-copy plasmid DNA, where large culture volumes are used.

Salts are efficiently removed by a brief wash step with Buffer PE. High-quality plasmid DNA is then eluted from the QIAprep 2.0 Spin Column with 50–100 µL of Buffer EB or water. The purified DNA is ready for immediate use in a range of applications — no need to precipitate, concentrate, or desalt.

Note: Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH value is within this range. Store DNA at –30 to –15°C when eluted with water because DNA may degrade in the absence of a buffering agent.

DNA yield

Plasmid DNA yield with the QIAwave Plasmid Miniprep Kit varies depending on plasmid DNA copy number per cell (see Appendix A, page 42), the individual insert in a plasmid DNA, factors that affect growth of the bacterial culture (see page 42), the elution volume Figure 1), and the elution incubation time (Figure 2). A 1.5 mL overnight culture can yield 5–15 µg of plasmid DNA (Table 1 on page 14). To obtain the optimum combination of DNA quality, yield, and concentration, we recommend using Luria Bertani (LB) medium for growth of cultures (for composition see Table 6 on page 46), eluting plasmid DNA in a volume of 50 µL and performing a short incubation after addition of the elution buffer.

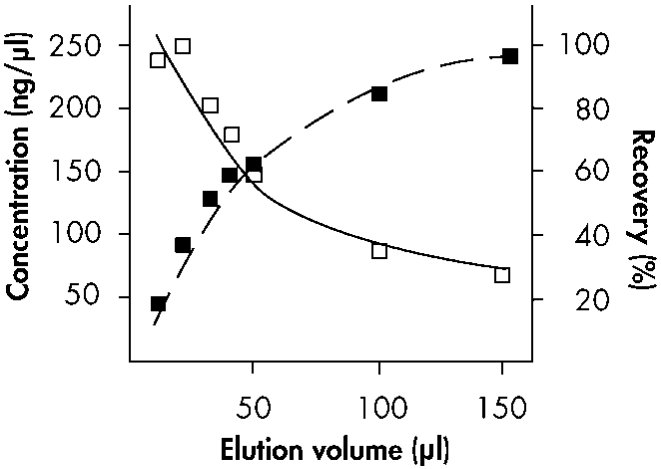


Figure 1. Elution volume versus DNA concentration and recovery. Using the QIAwave Plasmid Miniprep protocol, 10 µg pUC18 DNA was purified and eluted with the indicated volumes of Buffer EB. The standard protocol uses 50 µL Buffer EB for elution, because this combines high yield with high concentration. However, the yield can be increased by increasing the elution volume.

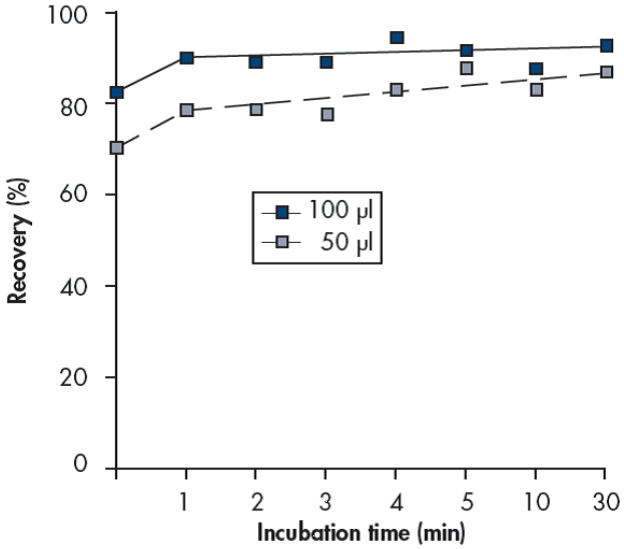


Figure 2. Incubation time versus DNA recovery. Using the QIAwave Plasmid Miniprep protocol, 10 µg pBluescript®DNA was purified and eluted after the indicated incubation times with either 50 or 100 µL Buffer EB. The graph shows that an incubation time of 1 minute and doubling the elution buffer volume increase yield.

Table 1. Effect of different compositions of growth medium LB on DNA yield

Culture media	Yield (µg)
LB (containing 10 g/liter NaCl)	11.5
LB (containing 5 g/liter NaCl)	9.5

The QIAprep Spin Miniprep Kit was used to purify DNA from 1.5 mL LB overnight cultures of XL1 Blue containing pBluescript. Elution was performed according to the standard protocol (50 µL Buffer EB and 1 minute incubation). Use of the recommended LB composition (with 10 g/liter NaCl; see also Appendix A, page 42) provides optimal plasmid DNA yield.

Using LyseBlue reagent

Using a simple visual identification system, LyseBlue reagent prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, cell debris, and genomic DNA.

LyseBlue can be added to the reconstituted resuspension buffer (Buffer P1) bottle before use. Alternatively, smaller amounts of LyseBlue can be added to aliquots of reconstituted Buffer P1, enabling visual control of single plasmid DNA preparations.

LyseBlue reagent should be added to reconstituted Buffer P1 at a ratio of 1:1000 to achieve the required working concentration (e.g., 10 µL LyseBlue into 10 mL reconstituted Buffer P1). Make sufficient LyseBlue/Buffer P1 working solution for the number of plasmid DNA preps being performed.

LyseBlue precipitates after addition into reconstituted Buffer P1. This precipitate will completely dissolve after addition of Buffer P2. Shake Buffer P1 before use to resuspend LyseBlue particles.

The plasmid DNA preparation procedure is performed as usual. After addition of Buffer P2 to Buffer P1, the color of the suspension changes to blue. Mixing should result in a homogeneously colored suspension. If the suspension contains localized regions of colorless solution, or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

Upon addition of neutralization buffer (Buffer N3), LyseBlue turns colorless. The presence of a homogeneous solution with no traces of blue indicates that SDS from the lysis buffer has been effectively precipitated.

Quantification of DNA fragments

DNA fragments can be quantified by running a sample alongside standards containing known quantities of the same-sized DNA fragment. The amount of sample DNA loaded can be estimated by visual comparison of the band intensity with that of the standards (please see Using LyseBlue reagent).

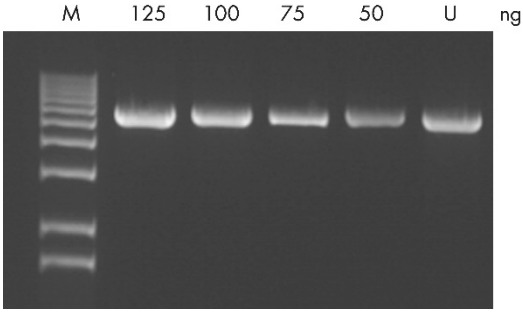


Figure 3. Agarose gel analysis. An unknown amount of a 5.5 kb DNA fragment (U) was run alongside known quantities (as indicated in ng) of the same DNA fragment. The unknown sample contained 75–100 ng DNA, as estimated by visual comparison with the standards. **M**: 1 kb DNA ladder.

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, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

- Standard microbiological equipment for growing and harvesting bacteria (e.g., inoculating loop, culture tubes and flasks, and 37°C shaking incubator)
- 96–100% ethanol*
- Microcentrifuge tubes for elution (1.5 mL or 2 mL)
- Ultrapure water
- Glassware for reconstitution of buffers (see "Important Notes" for more information)
- **Optional**: Vacuum pump (e.g., Vacuum Pump)
- **Optional**: Waste Tubes (2 mL) (cat. no. 19211), in case the user prefers to use a new Waste Tube for each washing step. We recommend reusing the same Waste Tube throughout the procedure to reduce plastic consumption.

*Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Important Notes

Working with QIAwave products

Preparation of functional buffers

Kit (cat. no.)	Final buffer	Buffer concentrate*	Ultrapure water (mL)	Ethanol (96–100%) (mL)	Final volume (mL)
27204	PE	PE/C	10	44	55
	P1	P1/C	12	–	14
	EB	EB/C	20	–	22
27206	PE	PE/C	50	220	275
	P1	P1/C	60	–	70
	EB	EB/C	50	–	55

*Use entire volume.

Selected buffers are provided as concentrates in 15 mL bottles or 2 mL tubes to shrink buffer bottles and reduce the amount of plastics used. Before using the kit for the first time, concentrates have to be reconstituted to receive the functional buffer. This is done with either water or water and ethanol. To reconstitute, the entire volume of the buffer concentrate should be transferred from the 15 mL bottle or 2 mL tube into a suitably sized glass bottle, either by using a pipette or by pouring. Subsequently, the appropriate volume of water or water and ethanol should be added as indicated in the table above. Afterwards, the glass bottle should be capped tightly and the reconstituted buffer mixed thoroughly by inverting.

For detailed instructions see “Things to do before starting ” on page 25 or watch our educational “how-to video” **www.qiagen.com/qiawavebuffer**

Water quality used for preparation of functional buffers

We strongly recommend using highly pure water for reconstitution. Ultrapure water (also known as type 1 water) with a resistivity of 18.2 MΩ-cm at 25°C, such as from a Milli-Q® system, works well. In case users do not have access to type 1 water, QIAGEN offers Nuclease-Free Water (5 liters, cat. no. 129117; 1000 mL, cat. no. 129115)*. Please note that these items need to be purchased separately.

Important: Avoid using tap water as this can have detrimental impact on the extraction of the target analyte.

Glassware

We suggest the use of glass bottles for the reconstitution of buffers. Glass bottles can be cleaned, sterilized, and reused more easily than plastic bottles, which will further reduce the plastic footprint of the kit.

Glassware should be treated before use to ensure that it is DNase free. Glassware used for DNA work should be cleaned with a detergent, thoroughly rinsed, and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many DNases.

Labeling of functional buffers in glass bottles

Reconstituted buffers from buffer concentrates can be labelled with the additional label supplied with the kit. Use the enclosed label and transfer it onto the glass bottle containing the functional buffer prepared before using the kit for the first time.

*This item needs to be purchased separately.

Waste Tubes

The newly introduced Waste Tube is made of recycled plastic recovered from post-consumer plastic waste and can differ in color from lot to lot due to slight differences in composition of the raw material. This however, has no effect on its intended use to collect the flow-through from sample binding and membrane washing. After each of these steps, the flow-through is discarded and the Waste Tube is reused. The Waste Tube is only used for processing waste and never comes into direct contact with the analyte of interest.

For detailed instructions watch our educational "how- to video" **www.qiagen.com/qiawavewastetube**

Elution tubes

Elution tubes are not included in the kit. This allows the flexibility to use elution tubes of one´s own choice and purchase them in, for example, eco-friendlier big packs.

Recycling information

Please visit **www.qiagen.com/recycling- card** to learn more about how to recycle kit components.

Growth of bacterial cultures in tubes or flasks

1. Pick a single colony from a freshly streaked selective plate and inoculate a culture of 1–5 mL LB medium containing the appropriate selective antibiotic. Incubate for 12–16 hours at 37°C with vigorous shaking.

   Growth for more than 16 hours is not recommended because cells begin to lyse and plasmid DNA yields may be reduced. Use a tube or flask with a volume of at least 4 times the volume of the culture.

2. Harvest the bacterial cells by centrifugation at >8000 rpm (6800 x g) in a conventional table-top microcentrifuge for 3 minutes at room temperature.

   The bacterial cells can also be harvested in 15 mL centrifuge tubes at 5400 x g for 10 min at 4°C. Remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.

Elution Notes

- If water is used for elution, make sure that its pH is between 7.0 and 8.5. Elution efficiency is dependent on pH, and the maximum elution efficiency is achieved within this range. A pH <7.0 can decrease yield.
- **Note**: Store DNA at –30 to –15°C when eluted with water, as DNA may degrade in the absence of a buffering agent.
- DNA can also be eluted in Buffer TE (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

Guidelines for QIAvac 24 Plus

QIAvac 24 Plus facilitate DNA minipreps by providing a convenient modular vacuum manifold for use with the QIAwave Plasmid Miniprep Kit. The following recommendations

should be followed when handling QIAvac 24 Plus:

- QIAvac 24 Plus operate with a house vacuum or Vacuum Pump (e.g., cat. no. 84010 [USA and Canada], 84000 [Japan], or 84020 [rest of world]).
- Always store QIAvac 24 Plus clean and dry. To clean, simply rinse all components with water and dry with paper towels. Do not air dry, as the screws may rust and need to be replaced. Do not use abrasives or solvents.
- Always place the QIAvac 24 Plus on a secure bench top or work area. If dropped, the manifold may crack.
- The components of QIAvac 24 Plus are not resistant to ethanol, methanol, or other organic solvents (Guidelines for QIAvac 24 Plus). Do not bring solvents into contact with the vacuum manifold. If solvents are spilled on the unit, rinse thoroughly with distilled water. Ensure that no residual Buffer PE remains in the vacuum manifold.
- To ensure consistent performance, do not apply silicone or vacuum grease to any part of the QIAvac 24 Plus manifold.

Table 2. Chemical-resistance properties of QIAvac 24 Plus

Resistant to	Not resistant to	
Chlorine bleach (12%)	Acetic acid*	Benzene
Hydrochloric acid	Acetone	Chloroform
Sodium chloride	Chromic acid*	Ethers
Sodium hydroxide	Phenol	Toluene
Urea	Concentrated alcohols*	

* QIAvac 24 Plus is resistant to these chemicals.

QIAvac vacuum manifolds

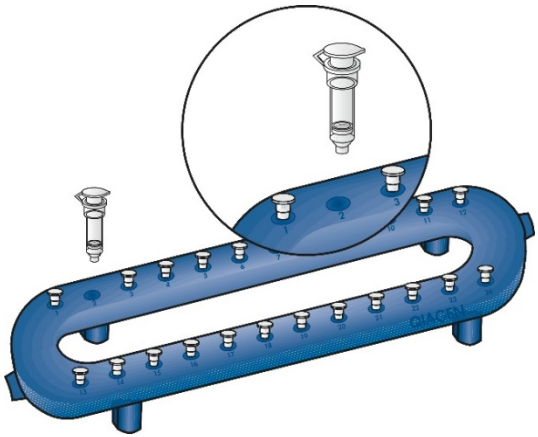
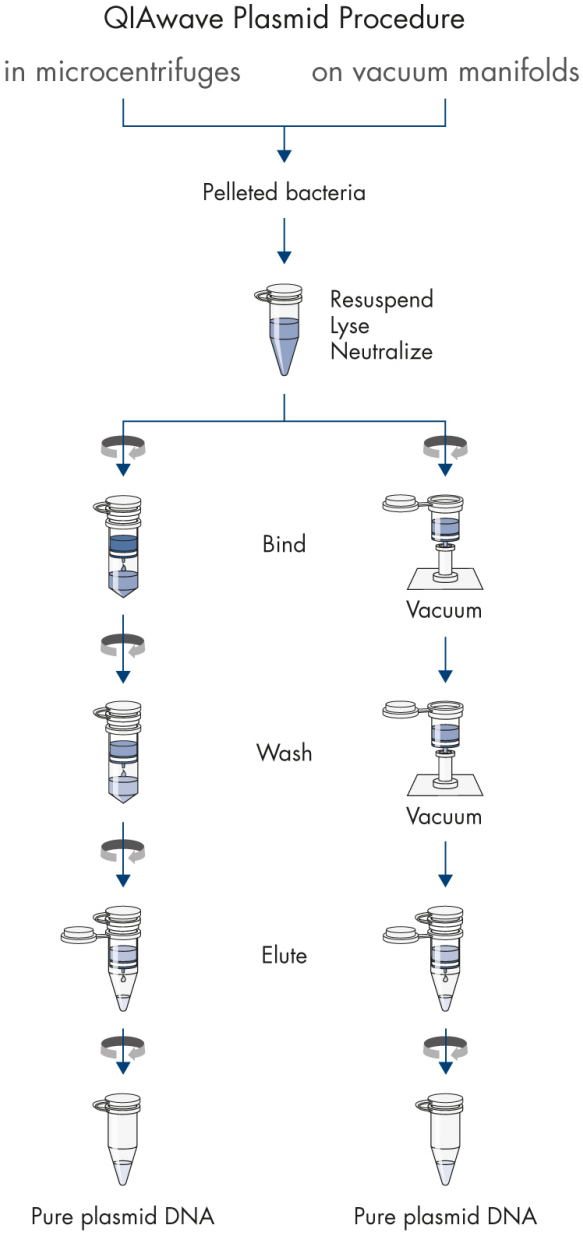


Figure 4. Components of the QIAvac 24 Plus manifold. 1: QIAvac 24 Plus vacuum manifold. **2**: Luer slot closed with luer plug. **3**: Spin column.


QIAwave Plasmid Procedure
in microcentrifuges
on vacuum manifolds
Pelleted bacteria
Resuspend
Lyse
Neutralize
Bind
Vacuum
Wash
Vacuum
Elute
Pure plasmid DNA
Pure plasmid DNA

Protocol: Plasmid DNA Purification using the QIAwave Plasmid Miniprep Kit and a Microcentrifuge

Important points before starting

- This protocol is designed for the purification of up to 20 µg of high-copy plasmid DNA from 1 to 5 mL overnight cultures of *E. coli* in LB medium. For purification of low-copy plasmid DNA and cosmids, large plasmid DNA (>10 kb), and DNA prepared using other methods, refer to Appendix C, page 49.
- If using the QIAwave Plasmid Miniprep Kit for the first time, read "Important Notes" (page 18).
- All centrifugation steps are carried out at 13,000 rpm (approx. 17,900 x *g*) in a conventional table-top microcentrifuge.

Things to do before starting

Table 3. Preparation of final buffers from buffer concentrates

Kit (cat. no.)	Final buffer	Buffer concentrate*	Ultrapure water (mL)	Ethanol (96–100%) (mL)	Final volume (mL)
27204	PE	PE/C	10	44	55
	P1	P1/C	12	–	14
	EB	EB/C	20	–	22
27206	PE	PE/C	50	220	275
	P1	P1/C	60	–	70
	EB	EB/C	50	–	55

*Use entire volume.

- **Preparation of Buffer P1 (Kit cat. no. 27204)**: Transfer the entire volume of Buffer P1/C from the 2 mL tube into a glass bottle larger than 14 mL, either by using a pipette or by pouring. Add 12 mL ultrapure water such as Nuclease-free Water (5 liters, cat. no. 129117; 1000 mL, cat. no. 129115) to obtain a final volume of 14 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer P1 (Kit cat. no. 27206)**: Transfer the entire volume of Buffer P1/C from the 15 mL bottle into a glass bottle larger than 70 mL, either by using a pipette or by pouring. Add 60 mL ultrapure water such as Nuclease-free Water (5 liters, cat. no. 129117; 1000 mL, cat. no. 129115) to obtain a final volume of 70 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **For cat. no. 27204**: Add 140 µL of the provided RNase A solution (conc.10mg/mL) to the 14 mL reconstituted Buffer P1 for a final concentration of 100 µg/mL. Mix and store at 2–

8°C.

- **For cat. no. 27206**: Add 700 µL of the provided RNase A solution (conc.10 mg/mL) to the 70 mL reconstituted Buffer P1 for a final concentration of 100 µg/mL. Mix and store at 2–8°C.
- **Optional**: Add the provided LyseBlue reagent to Buffer P1 at a ratio of 1:1000 (e.g., 10 µL LyseBlue into 10 mL Buffer P1) and mix before use. LyseBlue provides visual identification of optimum buffer mixing, thereby preventing the common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. For more details, see "Using LyseBlue reagent" on page 15.
- **Preparation of Buffer PE (Kit cat. no. 27204)**: Transfer the entire volume of Buffer PE/C from the 2 mL tube into a glass bottle larger than 55 mL, either by using a pipette or by pouring. Add 10 mL ultrapure water such as Nuclease-free Water (5 liters, cat. no. 129117; 1000 mL, cat. no. 129115) and 44 mL ethanol (96–100%) to obtain a final volume of 55 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer PE (Kit cat. no. 27206)**: Transfer the entire volume of Buffer PE/C from the 15 mL bottle into a glass bottle larger than 275 mL, either by using a pipette or by pouring. Add 50 mL ultrapure water such as Nuclease-free Water (5 liters, cat. no. 129117; 1000 mL, cat. no. 129115) and 220 mL ethanol (96–100%) to obtain a final volume of 275 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer EB (Kit cat. no. 27204)**: Transfer the entire volume of Buffer EB/C from the 2 mL tube into a glass bottle larger than 22 mL, either by using a pipette or by pouring. Add 20 mL ultrapure water such as Nuclease-free Water (5 liters, cat. no. 129117; 1000 mL, cat. no. 129115) to obtain a final volume of 22 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.

- **Preparation of Buffer EB (Kit cat. no. 27206)**: Transfer the entire volume of Buffer EB/C from the 15 mL bottle into a glass bottle larger than 55 mL, either by using a pipette or by pouring. Add 50 mL ultrapure water such as Nuclease-free Water (5 liters, cat. no. 129117; 1000 mL, cat. no. 129115) to obtain a final volume of 55 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- Check Buffers P2 and N3 before use for salt precipitation. Redissolve any precipitate by warming to 37°C. Do not shake Buffer P2 vigorously.
- Close the bottle containing Buffer P2 immediately after use to avoid acidification of Buffer P2 from CO_2 in the air.
- Buffers P2, N3, and PB contain irritants. Wear gloves when handling these buffers.
- Preassemble QIAprep 2.0 Spin Columns with Waste Tubes.
- **Note**: All protocol steps should be carried out at room temperature.

Procedure

1. Resuspend pelleted bacterial cells in 250 µL Buffer P1 and transfer to a microcentrifuge tube.

   Ensure that RNase A has been added to Buffer P1. Cell clumps should not be visible after resuspension of the pellet.

   If LyseBlue reagent has been added to Buffer P1, before use, shake the buffer bottle vigorously to ensure LyseBlue particles are completely dissolved. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

2. Add 250 µL Buffer P2 and mix thoroughly by inverting the tube 4–6 times.

   Mix gently by inverting the tube. Do not vortex, because this will result in shearing of genomic DNA and contamination of plasmid. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

   If LyseBlue has been added to Buffer P1, the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions, or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

3. Add 350 µL Buffer N3. Mix immediately and thoroughly by inverting the tube 4–6 times.

   To avoid localized precipitation, mix the solution thoroughly immediately after addition of Buffer N3. Large culture volumes (e.g., ≥5 mL) may require inverting up to 10 times. The solution should become cloudy.

   If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue is gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

4. Centrifuge for 10 min at 13,000 rpm (approx. 17,900 x *g*) in a table-top microcentrifuge.

   A compact white pellet will form.

5. Apply 800 µL of the supernatant from step 4 to the QIAprep 2.0 Spin Column placed in a 2 mL Waste Tube (provided) by pipetting.

6. Centrifuge for 30–60 s. Discard the flow-through and reuse the Waste Tube.

7. **Recommended**: Wash the QIAprep 2.0 Spin Column by adding 0.5 mL Buffer PB and centrifuging for 30–60 s. Discard the flow-through and reuse the Waste Tube.

   This step is necessary to remove trace nuclease activity when using *end*A+ strains, such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of

nuclease activity or high carbohydrate content. Host strains, such as XL-1 Blue and DH5 α, do not require this additional wash step.

8. Wash QIAprep 2.0 Spin Column by adding 0.75 mL Buffer PE and centrifuging for 30–60 s.
9. Discard the flow-through reuse the Waste Tube, and centrifuge at full speed for an additional 1 min to remove residual wash buffer.

   Important: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

10. Place the QIAprep 2.0 Spin Column in a clean 1.5 or 2.0 mL microcentrifuge tube (not provided). To elute DNA, add 50 µL Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep 2.0 Spin Column, let stand for 1 min, and centrifuge for 1 min.

Protocol: Plasmid DNA Purification using the QIAwave Plasmid Miniprep Kit and 5 mL Collection Tubes

The QIAwave Plasmid Miniprep procedure can be performed using 5 mL centrifuge tubes (e.g., Greiner Bio-One®, cat. no. 115101 or 115261) as collection tubes to decrease handling. The standard protocol on pages 22–26 should be followed with the following modifications:

Step 4:	Place a QIAprep 2.0 Spin Column in a 5 mL centrifuge tube instead of a 2 mL collection tube.
Step 6	Centrifuge at 3000 x *g* for 1 min using a suitable rotor (e.g., Beckman® GS-6KR centrifuge at approx. 4000 rpm). (The flow-through does not need to be discarded.)
Steps 7 and 8:	For washing steps, centrifugation should be performed at 3000 x *g* for 1 min. (The flow-through does not need to be discarded.)
Step 9:	Transfer the QIAprep 2.0 Spin Column to a microcentrifuge tube. Centrifuge at maximum speed for 1 min. Continue with step 10 of the protocol.

Protocol: Plasmid DNA Purification using the QIAwave Plasmid Miniprep Kit and a Vacuum Manifold

Important points before starting

- This protocol is designed for purification of up to 20 µg high-copy plasmid DNA from 1 to 5 mL overnight cultures of *E. coli* grown in LB medium, using QIAprep 2.0 Spin Columns on QIAvac 24 Plus or other vacuum manifolds with luer connectors. For purification of low-copy plasmid DNA and cosmids, large plasmid DNA (>10 kb), and DNA prepared using other methods, refer to Appendix C, page 49.
- If using the QIAwave Plasmid Miniprep Kit for the first time, read Important Notes (page 18).
- Switch off the vacuum between steps to ensure that a consistent, even vacuum is applied during manipulations.
- Wear safety glasses when working near a manifold under pressure.

Things to do before starting

Kit (cat. no.)	Final buffer	Buffer concentrate*	Ultrapure water (mL)	Ethanol (96–100%) (mL)	Final volume (mL)
27204	PE	PE/C	10	44	55
	P1	P1/C	12	–	14
	EB	EB/C	20	–	22
27206	PE	PE/C	50	220	275
	P1	P1/C	60	–	70
	EB	EB/C	50	–	55

*Use entire volume.

- **Preparation of Buffer P1 (Kit cat. no. 27204):** Transfer the entire volume of Buffer P1/C from the 2 mL tube into a glass bottle larger than 14 mL, either by using a pipette or by pouring. Add 12 mL ultrapure water such as Nuclease-free Water (5 liters, cat. no. 129117; 1000 mL, cat. no. 129115) to obtain a final volume of 14 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer P1 (Kit cat. no. 27206):** Transfer the entire volume of Buffer P1/C from the 15 mL bottle into a glass bottle larger than 70 mL, either by using a pipette or by pouring. Add 60 mL ultrapure water such as Nuclease-free Water (5 liters, cat. no. 129117; 1000 mL, cat. no. 129115) to obtain a final volume of 70 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
-

- **For cat. no. 27204**: Add 140 µL of the provided RNase A solution (conc.10mg/mL) to the 14 mL reconstituted Buffer P1 for a final concentration of 100 µg/mL. Mix and store at 2–8°C.
- **For cat. no. 27206**: Add 700 µL of the provided RNase A solution (conc.10 mg/mL) to the 70 mL reconstituted Buffer P1/C for a final concentration of 100 µg/mL. Mix and store at 2–8°C.
- **Optional**: Add the provided LyseBlue reagent to Buffer P1 at a ratio of 1:1000 (e.g., 10 µL LyseBlue into 10 mL Buffer P1) and mix before use. LyseBlue provides visual identification of optimum buffer mixing, thereby preventing the common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. For more details, see "Using LyseBlue reagent" on page 15
- **Preparation of Buffer PE (Kit cat. no. 27204):** Transfer the entire volume of Buffer PE/C from the 2 mL tube into a glass bottle larger than 55 mL, either by using a pipette or by pouring. Add 10 mL ultrapure water such as Nuclease-free Water (5 liters, cat. no. 129117; 1000 mL, cat. no. 129115) and 44 mL ethanol (96–100%) to obtain a final volume of 55 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer PE (Kit cat. no. 27206)**: Transfer the entire volume of Buffer PE/C from the 15 mL bottle into a glass bottle larger than 275 mL, either by using a pipette or by pouring. Add 50 mL ultrapure water such as Nuclease-free Water (5 liters, cat. no. 129117; 1000 mL, cat. no. 129115) and 220 mL ethanol (96–100%) to obtain a final volume of 275 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer EB (Kit cat. no. 27204)**: Transfer the entire volume of Buffer EB/C from the 2 mL tube into a glass bottle larger than 22 mL, either by using a pipette or by pouring. Add 20 mL ultrapure water such as Nuclease-free Water (5 liters, cat. no. 129117; 1000 mL, cat. no. 129115) to obtain a final volume of 22 mL. Cap the glass bottle tightly and

mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.

- **Preparation of Buffer EB (Kit cat. no. 27206)**: Transfer the entire volume of Buffer EB/C from the 15 mL bottle into a glass bottle larger than 55 mL, either by using a pipette or by pouring. Add 50 mL ultrapure water such as Nuclease-free Water (5 liters, cat. no. 129117; 1000 mL, cat. no. 129115) to obtain a final volume of 55 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- Check Buffers P2 and N3 before use for salt precipitation. Redissolve any precipitate by warming to 37°C. Do not shake Buffer P2 vigorously.
- Close the bottle containing Buffer P2 immediately after use to avoid acidification of Buffer P2 from CO_2 in the air.
- Buffers P2, N3, and PB contain irritants. Wear gloves when handling these buffers
- **Note**: All protocol steps should be carried out at room temperature.

Procedure

1. Resuspend pelleted bacterial cells in 250 µL Buffer P1 and transfer to a microcentrifuge tube.

   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.

   If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle to ensure LyseBlue particles are completely dissolved. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

2. Add 250 µL Buffer P2 and mix thoroughly by inverting the tube gently 4–6 times.

   Do not vortex, because this will result in shearing of genomic DNA and gDNA contamination. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

   If LyseBlue has been added to Buffer P1, the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions, or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

3. Add 350 µL Buffer N3. Mix immediately and thoroughly by inverting the tube 4–6 times.

   To avoid localized precipitation, mix the solution gently but thoroughly, immediately after addition of Buffer N3. Large culture volumes (e.g., ≥5 mL) may require inverting up to 10 times. The solution should become cloudy.

   If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue is gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

4. Centrifuge for 10 min at 13,000 rpm (approx. 17,900 x *g*) in a table-top microcentrifuge.

   A compact white pellet will form.

   During centrifugation, prepare the vacuum manifold and QIAprep 2.0 Spin Columns.

For QIAvac 24 Plus (see pages 21–23):

- Ensure that the vacuum source is connected to the upper threaded hole of the QIAvac 24 Plus and the lower threaded hole is tightly sealed using the screw cap.
- If using the QIAvac Connecting System, connect the system to the manifold and vacuum soured as described in the *QIAvac 24 Plus Handbook*.
- Insert up to 24 spin columns into the luer slots of the QIAvac 24 Plus. Close unused luer slots with luer plugs.

For other vacuum manifolds: Follow the supplier's instructions. Insert each QIAprep 2.0 Spin Column into a luer connector.

5. Apply 800 µL of the supernatant from step 4 to the QIAprep 2.0 Spin Column by pipetting.
6. Switch on the vacuum source to draw the solution through the QIAprep 2.0 Spin Columns, and then switch off the vacuum source.
7. **Recommended**: Wash the QIAprep 2.0 Spin Column by adding 0.5 mL Buffer PB. Switch on the vacuum source. After the solution has moved through the column, switch off the vacuum source.

   This step is necessary to remove trace nuclease activity when using endA+ strains, such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains, such as XL-1 Blue and DH5α, do not require this additional wash step.
8. Wash the QIAprep 2.0 Spin Column by adding 0.75 mL Buffer PE. Switch on the vacuum source to draw the wash solution through the column, and then switch off the vacuum source.
9. Transfer the QIAprep 2.0 Spin Columns to a Waste Tube. Centrifuge for 1 min.

   Important: This extra spin is necessary to remove residual Buffer PE. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

10. Place the QIAprep 2.0 Spin Column in a clean 1.5 or 2.0 mL microcentrifuge tube (not provided). To elute DNA, add 50 µL Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the QIAprep 2.0 Spin Column, let stand for 1 min, and centrifuge for 1 min.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page in our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx** The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook (for contact information, visit **support.qiagen.com**).

	Comments and suggestions
Low or no yield	
a. 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 (e.g., Figure 5, page 48). A small amount of the cleared 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 7,000 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.
b. Buffer PE or Buffer P1 or Buffer EB prepared incorrectly	Make sure that ultrapure water and/or ethanol has been added to Buffer PE/C, Buffer P1/C, and Buffer EB/C before use (see "Things to do before starting", pages 25 and 32.
No DNA in the cleared lysate before loading	
a. Plasmid DNA did not propagate	Read ""Growth of bacterial cultures" on page 42" (page 42) and check that the conditions for optimal growth were met.
b. Lysate prepared incorrectly	Check storage conditions and age of buffers.
c. Buffer P2 precipitated	Redissolve by warming to 37°C.
d. Cell resuspension incomplete	Pelleted cells should be completely resuspended in Buffer P1. Do not add Buffer P2 until an even suspension is obtained.
DNA is found in the flow-through of cleared lysate	

	Comments and suggestions
a. QIAprep membrane overloaded	If rich culture media, such as Terrific Broth (TB) or 2x YT, are used, culture volumes must be reduced. It may be necessary to adjust LB culture volume if the plasmid DNA and host strain show extremely high copy number or growth rates. See ""Culture media" on page 45" on page 42.
b. RNase A digestion omitted	Ensure that RNase A is added to Buffer P1 before use.
c. RNase A digestion insufficient	Reduce culture volume if necessary. If Buffer P1 containing RNase A is older than 6 months, add additional RNase A.
DNA is found in the wash flow-through	
Ethanol omitted from wash buffer	Repeat procedure with correctly prepared wash buffer (Buffer PE).
Little or no DNA in eluate	
a. Elution buffer incorrect	DNA is eluted only in the presence of low-salt buffer (e.g., Buffer EB [10 mM Tris·Cl, pH 8.5]) or water. Elution efficiency is dependent on pH. The maximum efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH value is within this range.
b. Elution buffer incorrectly dispensed onto membrane	Add elution buffer to the center of the QIAprep membrane to ensure that the buffer completely covers the surface of the membrane for maximum elution efficiency.
Low DNA quality	
DNA does not perform well in downstream applications	
a. Eluate salt concentration	For the QIAprep 2.0 Spin Column, modify the wash step by adding 0.75 mL of Buffer PE into the column, centrifuging, and then incubating the column for 5 min at room temperature. For QIAprep 96 Turbo preparations, ensure that 2 wash steps are carried out prior to elution.
b. Nuclease contamination	When using *end*A+ host strains – such as HB101 and its derivatives, the JM series, or any wild-type strain – ensure that the wash step with Buffer PB is performed.
c. Eluate contains residual ethanol	Ensure that step 9 in the QIAwave Plasmid Miniprep protocol (page 25) is performed.
RNA in the eluate	
a. RNase A digestion omitted	Ensure that RNase A is added to Buffer P1 before use.

	Comments and suggestions
b. RNase A digestion insufficient	Reduce culture volume if necessary. If Buffer P1 containing RNase A is older than 6 months, add additional RNase A.
Genomic DNA in the eluate	
a. Buffer P2 added incorrectly	The lysate must be handled gently after addition of Buffer P2 to prevent shearing. Reduce culture volume if lysate is too viscous for gentle mixing.
b. Buffer N3 added incorrectly	Upon addition of Buffer N3 in step 3 (page 25), mix immediately but gently.
c. Lysis too long	Lysis in step 2 (page 25) must not exceed 5 min.
d. Culture overgrown	Overgrown cultures contain lysed cells and degraded DNA. Do not grow cultures for longer than 12–16 h.

Appendix A: Background Information

Growth of bacterial cultures

Plasmid DNA are generally prepared from bacterial cultures grown in the presence of a selective agent, such as an antibiotic (3,4). The yield and quality of plasmid DNA may depend on factors, such as plasmid DNA copy number, host strain, inoculation, antibiotic, and type of culture medium.

Plasmid DNA copy number

Plasmid DNA vary widely in their copy number per cell (Table 4) 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 DNA and its associated insert. Some plasmid DNA, such as the pUC series and derivatives, have mutations that allow them to reach very high copy numbers within the bacterial cell. Plasmid DNA based on pBR322 and cosmids are generally present in lower copy numbers. Very large plasmid DNA and cosmids are often maintained at very low copy numbers per cell.

Table 4. Origins of replication and copy numbers of various plasmid DNA

DNA construct	Origin of replication	Copy number	Classification
Plasmid DNA			
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	10–20	Low copy

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

Host strains

Most *E.coli* strains can be used successfully to isolate plasmid DNA, although the strain used to propagate a plasmid DNA has an effect on the quality of the purified DNA. Host strains, such as DH1, DH5 α, and C600, give high-quality DNA. The slower-growing strain XL1-Blue also yields DNA of very high quality, which works extremely well for sequencing. Strain HB101 and its derivatives, such as TG1 and the JM series, produce large amounts of carbohydrates, which are released during lysis and can inhibit enzyme activities if not completely removed (4). In addition, these strains have high levels of endonuclease activity which can reduce DNA quality. The methylation and growth characteristics of the strain

should also be taken into account when selecting a host strain. XL1-Blue and DH5 α are highly recommended for reproducible and reliable results.

Inoculation

Bacterial cultures for plasmid DNA 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 DNA yield or loss of the plasmid DNA. Inoculation from plates that have been stored for a long time may also lead to loss or mutation of the plasmid DNA.

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. This procedure should then be repeated to ensure that a single colony of an antibiotic-resistant clone can be picked. A single colony should be inoculated into 1–5 mL of media containing the appropriate selective agent and grown with vigorous shaking for 12–16 hours. Growth for more than 16 hours is not recommended, because cells begin to lyse and plasmid DNA 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 that ensures that the plasmid DNA segregate equally during cell division. Daughter cells that do not receive plasmid DNA will replicate much faster than plasmid DNA-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 DNA-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 Appendix A: Background Information are based on these considerations.

Table 5. Concentrations of commonly used antibiotics

	Stock solutions		
Antibiotic	**Concentration**	**Storage (°C)**	**Working concentration (dilution**
Ampicillin (sodium salt)	50 mg/mL in water	–30 to –15	100 µg/mL (1/500)
Chloramphenicol	34 mg/mL in ethanol	–30 to –15	170 µg/mL (1/200)
Kanamycin	10 mg/mL in water	–30 to –15	50 µg/mL (1/200)
Streptomycin	10 mg/mL in water	–30 to –15	50 µg/mL (1/200)
Tetracycline HCl	5 mg/mL in ethanol	–30 to –15	50 µg/mL (1/100)

Culture media

LB broth is the recommended culture medium for use with the QIAwave Plasmid Miniprep Kit because richer broths, such as TB or 2x YT, lead to extremely high cell densities, which can overload the purification system. It should be noted that cultures grown in TB may yield 2–5 times the number of cells compared to cultures grown in LB broth. If these media are used, recommended culture volumes must be reduced to match the capacity of the membrane of the QIAprep 2.0 Spin columns. If excess culture volume is used, alkaline lysis will be inefficient, the QIAprep membrane will be overloaded, and the performance of the system will be unsatisfactory. Furthermore, the excessive viscosity of the lysate will require vigorous mixing, which may result in shearing of bacterial genomic DNA and contamination of the plasmid DNA. Care must also be taken if strains are used which grow unusually fast or to very high cell densities. In such cases, doubling the volumes of Buffers P1, P2, and N3 may be beneficial. It is best to calculate culture cell density and adjust the volume accordingly.

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 DNA yields can vary significantly.

Table 6. Recommended composition of LB medium

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

Preparation of cell lysates

Bacteria are lysed under alkaline conditions. After harvesting and resuspension, the bacterial cells are lysed in NaOH/SDS (Buffer P2) in the presence of RNase A (2, 5). SDS solubilizes the phospholipid and protein components of the cell membrane, leading to lysis and release of the cell contents, while the alkaline conditions denature the chromosomal and plasmid DNA, as well as proteins. The optimized lysis time allows maximum release of plasmid DNA without release of chromosomal DNA, while minimizing the exposure of the plasmid DNA to denaturing conditions. Long exposure to alkaline conditions may cause the plasmid DNA to become irreversibly denatured (2). This denatured form of the plasmid DNA runs faster on agarose gels and is resistant to restriction enzyme digestion.

The lysate is neutralized and adjusted to high-salt–binding conditions in one step by the addition of Buffer N3. The high salt concentration causes denatured proteins, chromosomal DNA, cellular debris, and SDS to precipitate, while the smaller plasmid DNA renatures correctly and stays in solution. It is important that the solution is thoroughly and gently mixed to ensure complete precipitation.

To prevent contamination of plasmid DNA with chromosomal DNA, vigorous stirring and vortexing must be avoided during lysis. Separation of plasmid DNA from chromosomal DNA is based on coprecipitation of the cell-wall–bound chromosomal DNA with insoluble complexes containing salt, detergent, and protein. Plasmid DNA remains in the clear supernatant. Vigorous treatment during the lysis procedure will shear the bacterial chromosome, leaving free chromosomal DNA fragments in the supernatant. Since chromosomal fragments are chemically indistinguishable from plasmid DNA under the conditions used, the two species will not be separated on QIAprep membrane and will elute under the same low-salt conditions. Mixing during the lysis procedure must therefore be carried out by slow, gentle inversion of the tube.

Appendix B: Agarose Gel Analysis of Plasmid DNA

The QIAwave Plasmid Miniprep procedure can be analyzed using agarose gel electrophoresis as shown in Appendix B: Agarose Gel Analysis of Plasmid DNA. Samples can be taken from the cleared lysate and its flow-through, precipitated with isopropanol and resuspended in a minimal volume of Buffer TE. In Appendix B: Agarose Gel Analysis of Plasmid DNA, the cleared lysate shows closed circular plasmid DNA and degraded RNase A-resistant RNA. The flow-through contains only degraded RNA, and no plasmid DNA is present. The eluted pure plasmid DNA shows no contamination with other nucleic acids.

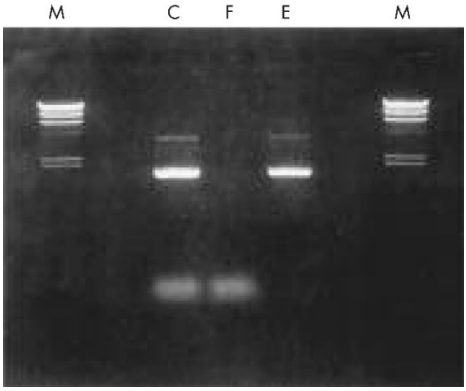


Figure 5. Agarose gel analysis of the QIAprep Miniprep procedure.C: cleared lysate; **F**: flow-through; **E**: eluted plasmid DNA; **M**: markers.

Appendix C: Special Applications

Purification of low-copy plasmid DNA and cosmids

All QIAwave Plasmid Miniprep protocols in this handbook can be used for preparation of low-copy-number plasmid DNA or cosmids from 1 to 10 mL overnight *E. coli* cultures grown in LB medium.

Only two slight modifications to the protocols are required:

- The wash step with Buffer PB is required for all strains.
- When plasmid DNA or cosmids are >10 kb, preheat Buffer EB (or water) to 70°C prior to eluting DNA from the QIAprep membrane. A 10 mL overnight LB culture typically yields 5–10 µg DNA.

  Note: When using 10 mL culture volume, it is recommended to double the volumes of Buffers P1, P2, and N3 used.

Purification of very large plasmid DNA (>50 kb)

Plasmid DNA that are >50 kb in size elute less efficiently from silica than smaller plasmid DNA but do elute efficiently from the QIAGEN anion-exchange resin. QIAGEN provides the anion-exchange–based QIAGEN Large-Construct Kit for efficient large-scale purification of ultrapure genomic DNA-free BAC, PAC, P1, or cosmid DNA. For high-throughput, small-scale purification of BACs, PACs, and P1s, an optimized alkaline lysis protocol in R.E.A.L.® Prep 96 Kits yields DNA suitable for sequencing and screening. Call QIAGEN Technical Services or your local distributor for more information on these kits, or see “Ordering Information” on page 52.

Purification of plasmid DNA prepared by other methods

Plasmid DNA isolated by other methods can be further purified using QIAprep modules and any of the QIAwave protocols in this handbook.

1. Add 5 volumes of Buffer PB to 1 volume of the DNA solution and mix (e.g., add 500 µL Buffer PB to 100 µL of DNA sample).
2. Apply the samples to QIAprep 2.0 Spin Columns or to the wells of a QIAprep 96-well plate. Draw the samples through the QIAprep membrane by centrifugation or vacuum and continue the appropriate protocol at the Buffer PE wash step. The optional wash step with Buffer PB is not necessary.

References

1. Vogelstein, B., and Gillespie, D. (1979) Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. U S A. 76, 615–619.
2. Birnboim, H.C., and Doly, J. (1979) A rapid alkaline lysis procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7, 1513–1523.
3. Sambrook, J., Fritsch, E.F., and Maniatis, T., eds. (1989) *Molecular Cloning: A Laboratory Manual*. 2nd ed., New York: Cold Spring Harbor Laboratory Press.
4. Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Smith, J.A., and Strahl, K., eds. (1991) *Current Protocols in Molecular Biology*. New York: Wiley Interscience.
5. Birnboim, H.C. (1983) A rapid alkaline extraction method for the isolation of plasmid DNA. Methods Enzymol. 100, 243–255.

Ordering Information

Product	Contents	Cat. no.
QIAwave Plasmid Miniprep Kit (50)	50 QIAprep 2.0 Spin Columns, Waste Tubes (2 mL), buffers	27204
QIAwave Plasmid Miniprep Kit (250)	250 QIAprep 2.0 Spin Columns, Waste Tubes (2 mL), buffers	27206
QIAwave RNA Mini Kit (50)	50 RNeasy Mini Spin Columns, Waste Tubes (2 mL), RNase-free Reagents and Buffers	74534
QIAwave RNA Mini Kit (250)	250 RNeasy Mini Spin Columns, Waste Tubes (2 mL), RNase-free Reagents and Buffers	74536
QIAwave DNA Blood & Tissue Kit (50)	50 DNeasy Mini Spin Columns, Waste Tubes (2 mL), Proteinase K, Buffers	69554
QIAwave DNA Blood & Tissue Kit (250)	250 DNeasy Mini Spin Columns, Waste Tubes (2 mL), Proteinase K, Buffers	69556
QIAvac and accessories		
QIAvac 24 Plus	Vacuum manifold for processing 1–24 spin columns: includes QIAvac 24 Plus Vacuum Manifold, luer plugs, and quick couplings	19413
QIAvac Luer Adapter Set *	For processing 1–24 QIAprep 2.0 Spin Columns: 6 adapters, each with 4 luer connectors, and 24 plugs	19541
Vacuum Regulator	For use with QIAvac manifolds	19530
Vacuum Pump (100 V, 50/60 Hz)†	Universal vacuum pump (capacity 34 liters/min, 8 mbar vacuum abs.)	84000
Vacuum Pump (115 V, 60 Hz)‡	Universal vacuum pump (capacity 34 liters/min, 8 mbar vacuum abs.)	84010

*Compatible only with QIAvac top plates containing flip-up lid.
†Japan
‡North America

Product	Contents	Cat. no.
Vacuum Pump (230 V, 50 Hz)§	Universal vacuum pump (capacity 34 liters/min, 8 mbar vacuum abs.)	84020
Automated low-throughput plasmid DNA purification		
QIAcube Connect ¶	Instrument, connectivity package, 1 year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µL (1024), 1000 µL filter-tips (1024), 30 mL reagent bottles (12), rotor adapters (240), elution tubes (240), and rotor adapter holder	990395
Individual buffers and accessories		
Waste Tubes (2 mL)	1000 Waste Tubes (2 mL)	19211
Nuclease-Free Water (1000 mL)	1000 mL nuclease-free water prepared without the use of diethylpyrocarbonate (DEPC); provided in a plastic bottle	129115
Nuclease-Free Water (5 liters)	5 liters nuclease-free water prepared without the use of diethylpyrocarbonate (DEPC); Provided in five 1 liter bottles, delivered in a cardboard box	129117
Buffer N3	500 mL Buffer N3	19064
Buffer PB	500 mL Buffer PB	19066
Buffer PE (concentrate)	100 mL Buffer PE (concentrate)	19065
RNase A	2.5 mL (100 mg/mL; 7000 units/mL solution)	19101

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

§Rest of world.

¶All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Furthermore, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training, and preventive subscription. Contact your local sales representative to learn about your options.

Document Revision History

Date	Changes
01/2022	Initial release.
06/2023	Updated component list and instructions. Updated Ordering Information section
03/2024	Changed the last paragraph of the "Automated purification of DNA on QIAcube instruments" section to further explain the details on how the QIAwave Kits can be used on either the QIAprep Spin Miniprep Kit protocols or the dedicated QIAwave Plasmid Miniprep Kit protocols.

Limited License Agreement for QIAwave Plasmid Miniprep Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this Instructions for Use and for use with components contained in the panel only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this panel with any components not included within this panel except as described in the protocols provided with the product, this Instructions for Use, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this panel and/or its use(s) do not infringe the rights of third-parties.
3. This panel and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the panel agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the panel and/or its components.

For updated license terms, see **www.qiagen.com**.