December 2018

QIAprep® 96 *Plus* Miniprep Handbook

QIAprep 96 *Plus* Miniprep Kit
QIAprep 96 *Plus* BioRobot® Kit

For 96-well preparation of up to 50 µg of high-quality plasmid DNA from *E. coli*

Sample to Insight

QIAGEN

Contents

Kit Contents

Kit	QIAprep 96 *Plus* Miniprep Kit (4)	QIAprep 96 *Plus* BioRobot Kit (4)
Catalog no.	**27291**	**962241**
Number of reactions	**4**	**4**
Buffer P1	150 ml	2 x 250 ml
Buffer P2	140 ml	2 x 250 ml
Buffer S3	160 ml	2 x 160 ml
Buffer BB	160 ml	2 x 160 ml
Buffer PE (concentrate)	100 ml	2 x 100 ml
Buffer EB	55 ml	2 x 55 ml
RNase A (100 mg/ml)	15 mg	2 x 25 mg
TurboFilter® 96 Plates	4	4
Plasmid *Plus* 96 Plates	4	4
Tape pad	1	1
S-Blocks	8	8
Elution Microtubes (racked) (96)	4	4
Quick-Start Protocol	1	1

Storage

QIAprep 96 *Plus* Miniprep and BioRobot Kits should be stored dry at room temperature (15–25°C). Under these conditions, if no expiration date is mentioned on the kit label, kits can be stored for up to 1 year without showing any reduction in performance and quality. After adding RNase A, Buffer P1 should be stored at 2–8°C and is stable for 6 months. Other buffers and RNase A stock solution can be stored for 2 years at room temperature.

Intended Use

QIAprep 96 *Plus* Miniprep and BioRobot Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

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.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAprep 96 *Plus* Miniprep and BioRobot Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

QIAprep 96 *Plus* Miniprep and BioRobot Kits provide a novel method for plasmid preparation. The procedure is based on a nonchaotropic binding chemistry. Following lysate clearing, a simple bind-wash-elute procedure results in highly concentrated plasmid DNA, ready for direct use in subsequent applications. The unique kit chemistry and design of the Plasmid *Plus* 96 plates allow high binding capacities.

QIAprep 96 *Plus* Miniprep and BioRobot Kits provide highly pure DNA, suitable for transfection into robust cell lines, for preparation of short hairpin vectors (sh-vectors), as well as for routine applications such as enzymatic modifications, cloning, restriction digestion and in vitro transcription/translation.

Principle and procedure

The QIAprep 96 *Plus* Miniprep protocol is based on a modified alkaline lysis procedure. After neutralization, lysates are cleared by using a TurboFilter 96 plate. A nonchaotropic binding buffer (Buffer BB) is added to the cleared lysate to optimize plasmid DNA binding to the membrane of the Plasmid Plus 96 plate. The unique binding buffer provides very specific binding conditions leading to DNA quality that is comparable to anion-exchange preps.

Due to the proprietary binding chemistry, up to 50 µg plasmid DNA per well can be obtained from a 5 ml *E. coli* culture. The DNA yield depends on the plasmid copy number and the amount of *E. coli* culture used.

The procedure may be automated using QIAprep *Plus* 96 BioRobot Kit (4) (cat. no. 962241) on the BioRobot Universal System.

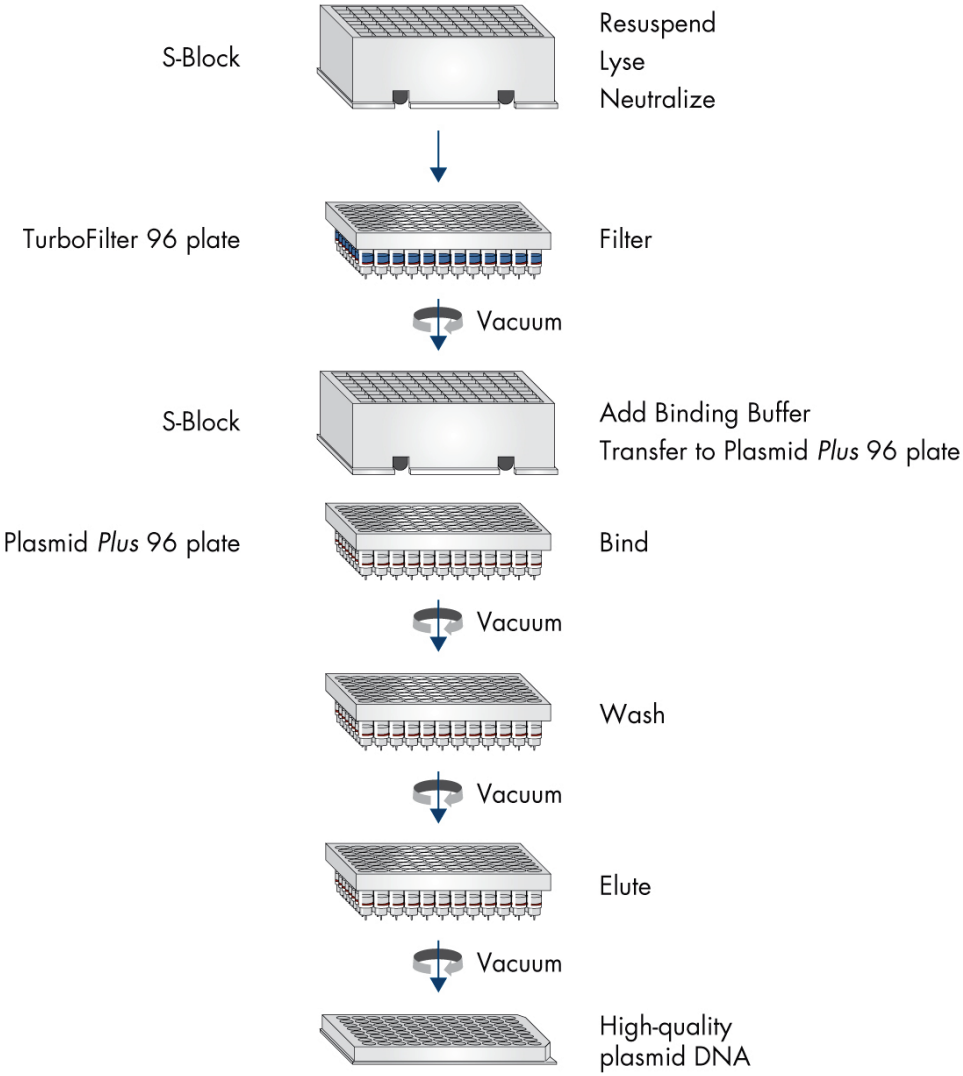
The QIAvac 96 is used for clearing lysate using the TurboFilter 96 plate, and DNA binding and wash steps using the Plasmid *Plus* 96 plate. Drying and elution steps may be performed either on the QIAvac 96 or using a compatible centrifuge. Alternatively, the complete procedure can be performed by using a compatible centrifuge.

Table 1. Typical yields of plasmid DNA from different volumes of *E. coli* culture

***E. coli* culture volume (LB medium)**	**Typical DNA yield**
1.25 ml	Up to 15 µg
2.5 ml	Up to 30 µg
5 ml*	Up to 50 µg

* When using a 5 ml culture, the volumes of the lysis buffers may be slightly increased. Use 350 µl Buffer P1, 350 µl Buffer P2, 350 µl Buffer S3 and 300 µl Buffer BB. If high DNA yields are expected, the elution volume may be increased to 100–120 µl.

QIAprep 96 *Plus* Miniprep Procedure



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 safety data sheets (SDSs), available from the product supplier.

- Standard microbiological equipment for growing and harvesting bacteria (e.g., inoculating loop, culture tubes and flasks, and 37°C shaking incubator)
- Centrifuge with rotor for 96-well blocks. We recommend QIAGEN's Centrifuge 4-16S (cat. no. 80510) for room-temperature centrifugation, and for refrigerated centrifugation, we recommend QIAGEN's Centrifuge 4-16KS (cat. no. 81610).
- 96–100% ethanol
- Vacuum pump (e.g., QIAGEN Vacuum Pump [cat. no. 84020])
- QIAvac 96 (cat. no. 19504)
- Elution Microtube Adapter (available from QIAGEN Technical Services) for QIAvac 96 for the elution step using the QIAvac 96. Alternatively, an empty microplate may be used to adjust the height of the elution microtubes, or the elution step could be performed in a suitable centrifuge where no adapter is necessary.
- If the drying step is performed in a suitable centrifuge (optional), additional S-Blocks (Square-Well Blocks) or elution microtubes are required.
- If the complete procedure is performed using a centrifuge, additional S-Blocks are required.

Important Notes

Please take a few moments to read this handbook carefully before beginning the DNA preparation. If QIAGEN plasmid purification kits are new to you, please visit "Growth Of Bacterial Cultures" at **www.qiagen.com/goto/plasmidinfo/GBC**. Also be sure to read and follow the appropriate detailed protocol.

Plasmid copy number

Plasmids and cosmids vary in copy number, depending on the origin of replication they contain, their size, and the size of insert. For more details, visit "Growth Of Bacterial Cultures" at **www.qiagen.com/goto/plasmidinfo/GBC**.

Host strains

The strain used to propagate a plasmid can have a substantial influence on quality of the purified DNA. Host strains such as DH1, DH5®α, and C600 yield high-quality DNA with QIAGEN protocols. The slower growing strain XL1-Blue® also yields DNA of very high quality.

Strain HB101 and its derivatives, such as TG1 and the JM100 series, contain large amounts of carbohydrates that are released during lysis and can inhibit enzyme activities if not completely removed. In addition, some strains, such as JM101, JM110 and HB101, have high levels of endonuclease activity and yield DNA of lower quality.

If the quality of purified DNA is not as expected, a change of host strain should be considered. If difficulty is encountered with strains such as TG1 and Top10F, we recommend reducing the amount of culture volume to improve the ratio of biomass to lysis buffers for optimized lysis conditions.

Culture media

QIAGEN plasmid purification protocols are optimized for use with cultures grown in Luria Bertani (LB) medium. Please note that a number of slightly different LB culture broths, containing different concentrations of NaCl, are commonly used. We recommend growing cultures in LB medium containing 10 g NaCl per liter, prepared as described on the following page, to obtain the highest plasmid yields.

In general, we do not recommend using rich media with our plasmid kits. Using rich media might lead to clogging of the filter. If this is the case, growth time must be optimized, and culture volumes reduced. For more details, visit "Growth Of Bacterial Cultures" at **www.qiagen.com/goto/plasmidinfo/GBC**.

Preparation of LB medium

Dissolve 10 g tryptone, 5 g yeast extract and 10 g NaCl in 800 ml distilled water. Adjust the pH to 7.0 with 1 N NaOH. Adjust the volume to 1 liter with distilled water. Sterilize by autoclaving.

Culture volume

Do not exceed the maximum recommended culture volumes. Using larger culture volumes will lead to an increase in biomass and can affect the efficiency of alkaline lysis, leading to reduced yield and purity of the preparation.

The QIAprep 96 *Plus* Miniprep protocol is optimized for use with cultures grown in Luria Bertani (LB) medium, grown to a cell density of approximately 3–4 x 10^9 cells/ml. It is best to assess the cell density of the culture and, if it is too high, reduce the culture volumes accordingly. A high ratio of biomass-to-lysis buffers will result in poor lysis conditions and, subsequently, low DNA yield and purity. For determination of cell density, calibration of each individual spectrophotometer is required to facilitate accurate conversion of OD_{600} measurements into the number of cells per milliliter. This can be achieved by plating serial dilutions of a culture onto LB-agar plates in the absence of antibiotics. The counted

colonies are used to calculate the number of cells per milliliter, which is then set in relation to the measured OD_{600} values.

Assembly of the QIAvac 96 vacuum manifold

The QIAvac 96 must be assembled before starting the QIAprep 96 *Plus* Miniprep protocol. Refer to Appendix A, page 23, for further details.

1. Place an S-Block inside the QIAvac base.
2. Place the QIAvac 96 top plate over the base.
3. Place the TurboFilter 96 plate into the QIAvac top plate.

   Make sure that the plate is seated securely. The S-Block should now be positioned under the TurboFilter 96 plate.
4. Attach the QIAvac 96 to a vacuum source.
5. Seal the TurboFilter 96 plate with tape and apply vacuum.
6. Using a vacuum regulator, adjust vacuum to –300 mbar.
7. Remove the tape.

Table 2. Pressure conversions

To convert from millibars (mbar) to	Multiply by
Millimeters of mercury (mm Hg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atm)	0.000987
Pounds per square inch (psi)	0.0145

Cell cultivation in a 96-well Flat-Bottom Block

1. Fill each well of a 96-well S-Block with 1.3 ml of growth medium containing the appropriate selective agent.

   Inoculate each well from a single bacterial colony or preculture.

2. Incubate the cultures for 20–24 h at 37°C, with vigorous shaking.

   The wells in the block may be protected against spillover by covering the block with an AirPore tape sheet (cat. no. 19571). AirPore microporous tape sheets promote gas exchange during culturing. If nonporous tape is used, pierce 2–3 holes in the tape with a needle above each well for aeration.

3. Harvest the bacterial cells in the block by centrifugation for 5 min at 2100 x *g* in a centrifuge with a rotor for a 96-well adapter (e.g., QIAGEN's Centrifuge 4-16KS or the Heraeus® Minifuge GL), preferably at 4–10°C. The block should be covered with adhesive tape during centrifugation. Remove media by inverting the block.

   To remove the media, peel off the tape and quickly invert the block over a waste container. Tap the inverted block firmly on a paper towel to remove any remaining droplets of medium.

   IMPORTANT: Ensure that the buckets on the rotor have sufficient clearance to accommodate the 2 ml S-Blocks before starting the centrifuge.

Cell cultivation in a 24-well block

To obtain high plasmid yields of up to 50 µg, a 5 ml LB culture volume per well is used for plasmid preparation. *E. coli* cultures can be grown and harvested in 24-well blocks. We do not recommend rich media here, because the higher amount of biomass might lead to clogging of the TurboFilter 96 plate. To ease the growth of *E. coli* cultures in parallel, cultures can be grown and harvested in 24-well blocks (e.g., QIAGEN's 24-Well Blocks RB [cat. no. 19583]).

Procedure

1. Fill each well of a 24-well block with 5 ml of LB medium containing the appropriate selective agent.
2. Inoculate each well from a single bacterial colony or preculture.
3. Carefully seal the block with an AirPore tape sheet (cat. no. 19571).
4. Incubate the cultures for 16–24 h at 37°C with vigorous but appropriate shaking.

   Harvest the bacterial cells in the block by centrifugation for 5 min at 2100 x *g* in a centrifuge with a rotor for a 96-well adapter (e.g., QIAGEN's Centrifuge 4-16KS or the Heraeus Minifuge GL), preferably at 4–10°C. The block should be covered with adhesive tape during centrifugation. Remove media by carefully inverting the block. Proceed as described in the section "Transfer from 24-well format to 96-well format".

   To remove the media, peel off the tape and quickly invert the block over a waste container. Tap the inverted block carefully on a paper towel to remove any remaining droplets of medium.

   Alternatively, the cultures may be harvested using an S-Block, where aliquots of each of the 5 ml cultures are collected per well by centrifugation with subsequent removal of medium.

   IMPORTANT: Ensure that the buckets on the rotor have sufficient clearance to accommodate the 24-well blocks before starting the centrifuge.

Transfer from 24-well format to 96-well format

To transfer from 24-well format to 96-well format, the samples may be transferred to a 96-well S-Block after resuspension of pelleted bacteria with Buffer P1 (step 1 of "Protocol: Plasmid DNA Purification using the QIAprep 96 *Plus* Miniprep Kit").

Alternatively, steps 1–3 of the purification protocol can be performed within the wells of the 24-well blocks. For subsequent lysate clearing, the lysates are directly transferred from the wells of the 24-well blocks to the wells of the TurboFilter 96 plate.

The following pipetting scheme may be used to transfer the lysates. Transfer from the 24-well block may be performed by using every second channel of an 8-channel pipet.

- Transfer lysates from the first block to rows 1–6, only to lanes A, C, E and G of the SBlock or TurboFilter 96 plate.
- Transfer lysates from the second block to rows 7–12, only to lanes A, C, E and G of the S-Block or TurboFilter 96 plate.
- Transfer lysates from the third block to rows 1–6, only to lanes B, D, F and H of the S-Block or TurboFilter 96 plate.
- Transfer lysates from the fourth block to the rows 7–12, only to lanes B, D, F and H of the S-Block or TurboFilter 96 plate.

Block 1	Block 2
Block 3	Block 4

Figure 1. 24-well blocks 1–4.

	Rows of the TurboFilter 96 plate											
Lanes	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	1	1	1	1	2	2	2	2	2	2
B	3	3	3	3	3	3	4	4	4	4	4	4
C	1	1	1	1	1	1	2	2	2	2	2	2
D	3	3	3	3	3	3	4	4	4	4	4	4
E	1	1	1	1	1	1	2	2	2	2	2	2
F	3	3	3	3	3	3	4	4	4	4	4	4
G	1	1	1	1	1	1	2	2	2	2	2	2
H	3	3	3	3	3	3	4	4	4	4	4	4

Figure 2. Lanes and rows of the 96-well TurboFilter 96 plate to which lysates from the 24-well blocks are transferred according to the given numerical scheme.

Protocol: Plasmid DNA Purification using the QIAprep 96 *Plus* Miniprep Kit

This protocol is designed for the preparation of up to 50 µg high-copy plasmid DNA using the QIAprep 96 *Plus* Miniprep Kit with a maximum culture volume (i.e., LB medium) of 5 ml per well.

Important points before starting

- Wear safety glasses when working near a vacuum manifold under pressure.
- Always place the QIAvac 96 manifold on a secure bench top or work area.
- For safety reasons, do not use 96-well plates that have been damaged in any way.
- To avoid the possibility of implosion, do not use any vessel/material that is not designed for use with a vacuum. Do not use any material that is cracked or scratched.
- **Optional**: samples can be removed after step 5 of the protocol to monitor the procedure on an analytical gel.
- Switch off the vacuum source between steps to ensure that a consistent, even vacuum is applied during vacuum steps.
- When using a 5 ml culture, the volumes of the lysis buffers may be slightly increased; use 350 µl Buffer P1, 350 µl Buffer P2, 350 µl Buffer S3 and 300 µl Buffer BB. If high DNA yields are expected, the elution may be increased to 100–120 µl.

Things to do before starting

- Add the provided RNase A solution to Buffer P1 before use. Use 1 vial RNase A (centrifuge briefly before use) per bottle of Buffer P1 for a final concentration of 100 µg/ml.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- Check Buffer P2 and Buffer BB for precipitation due to low storage temperature and, if necessary, dissolve by warming to 37°C.

- Close the bottle containing Buffer P2 immediately after use to avoid acidification of Buffer P2 from CO_2 in the air.
- Assemble the QIAvac 96 as described on page 11. The vacuum should be regulated to –300 mbar before beginning the procedure.

Procedure

1. Resuspend pelleted bacteria in 300 µl Buffer P1.

   IMPORTANT: Ensure that RNase A has been added to Buffer P1.

   Note: Resuspend the bacterial pellet completely by vortexing or pipetting up and down until no cell clumps remain. If frozen cells are used, make sure that the cells are completely thawed (also in the center of the S-Block). If cells were not harvested in an S-Block, transfer resuspended bacteria into the S-Block.

2. Add 300 µl Buffer P2 to each well. Dry the top of the S-Block thoroughly with a paper towel and seal the block with the tape provided. Gently invert the block upside down 6–8 times to mix and incubate at room temperature (15–25°C) for 5 min.

   Note: It is important to mix gently by inverting the block. Do not shake vigorously, as this will result in shearing of genomic DNA. If necessary, continue inverting the block until the solution becomes viscous and slightly clear.

   IMPORTANT: Do not allow the lysis reaction to proceed for more than 5 min.

3. Remove the tape from the block. Add 300 µl Buffer S3 to each well. Dry the top of the S-Block thoroughly with a paper towel and seal the block with new tape. Gently invert the block upside down 6–8 times to mix.

   Note: To avoid localized precipitation, mix the samples gently but thoroughly immediately after adding Buffer S3. The solutions should become cloudy.

4. Remove the tape from the block. Transfer the lysates from step 3 into the wells of the TurboFilter 96 plate on the assembled QIAvac 96. Apply vacuum (–300 mbar) until all samples have passed through the wells of the TurboFilter 96 plate into the S-Block.

   Note: Unused wells of the TurboFilter 96 plate should be sealed with tape.

5. After all liquid has been drawn through the TurboFilter 96 plate, switch off the vacuum source and ventilate the QIAvac 96 slowly.

   Note: Discard the TurboFilter 96 plate after this step.

6. Add 300 µl Buffer BB to the cleared lysate in each well of the S-Block. Dry the top of the S-Block thoroughly with a paper towel, and then seal the block with a new tape. Invert the block upside down 1–3 times to mix.

7. Place a waste tray into the base of the QIAvac 96. Place the QIAvac 96 top plate over the base. Place the Plasmid *Plus* 96 plate in the top plate. Transfer the lysates from the S-Block to the Plasmid *Plus* 96 plate.

   Note: Unused wells of the Plasmid *Plus* 96 plate should be sealed with tape.

8. Apply vacuum until all samples have passed through.

   Note: After the liquid has been drawn through all wells, switch off the vacuum source and ventilate the QIAvac 96 slowly.

9. To wash the DNA, add 900 µl Buffer PE to each well of the Plasmid *Plus* 96 plate. Apply vacuum until all samples have passed through.

   Note: After the liquid has been drawn through all wells, switch off the vacuum source and ventilate the QIAvac 96 slowly.

10. To dry the membranes of the plate, use the QIAvac 96 with the centrifuge or a vacuum manifold.

    Note: This step removes residual Buffer PE from the membrane by airflow going through the wells.

    If using a centrifuge, place the Plasmid *Plus* 96 plate onto an S-Block or elution microtube rack with elution microtubes and centrifuge at 6000 x *g* for 10 min.

    IMPORTANT: S-blocks or elution microtubes required here are not provided with the kit.

    If using vacuum, empty the waste tray and put it back into the QIAvac 96 and apply maximum vacuum for 10 min. Switch off the vacuum source, and ventilate the QIAvac 96 slowly. Lift the top plate from the base (but not the Plasmid *Plus* plate 96 from the top

plate), vigorously tap the top plate on a stack of absorbent paper until no more drops come out, and blot the nozzles of the Plasmid *Plus* 96 plate with clean absorbent paper.

Buffer PE removal is only effective when maximum vacuum is used (i.e., turn off vacuum regulator or leakage valves if they are used).

11. To elute the DNA from the plate, use a centrifuge or the QIAvac 96.

Note: Ensure that Buffer EB is dispensed directly onto the center of the Plasmid *Plus* 96 plate membrane for optimal elution of DNA. Average eluate volume is 65 µl from an elution buffer volume of 80 µl.

If using a centrifuge:

- Place the Plasmid *Plus* 96 plate onto a new elution microtube rack containing elution microtubes.
- Add 80 µl of Buffer EB to the center of each well of the Plasmid *Plus* 96 plate.
- Let it stand for 3 min and then centrifuge at 6000 x *g* for 1 min.

If using the QIAvac 96:

- Replace the waste tray with the Elution Microtube Adapter. Alternatively, if no Elution Microtube Adapter is available, an empty 96-well microplate may be used.
- Place the elution microtube rack containing elution microtubes onto the adapter.
- Place the top plate back on the base, making sure that the Plasmid *Plus* 96 plate is seated securely. If a microplate is used instead of the Elution Microtube Adapter, please ensure that the nozzles of the Plasmid *Plus* 96 plate extend into the elution microtubes.
- Add 80 µl of Buffer EB to the center of each well of the Plasmid *Plus* 96 plate.
- Let it stand for 3 min and then apply maximum vacuum for 1 min.
- Switch off the vacuum source and ventilate the QIAvac 96 slowly.

Use of a centrifuge for the QIAprep 96 *Plus* Miniprep procedure

The QIAprep 96 *Plus* Miniprep procedure may be performed using a centrifuge that is suitable for 96-well blocks. Before starting, check if the centrifuge can accommodate 96-well plates and the S-Block.

Replace the vacuum steps with the centrifugation steps as described below. For further details individual steps of the purification procedure, refer to "Protocol: Plasmid DNA Purification using the QIAprep 96 *Plus* Miniprep Kit" from page 15.

Lysate clearing with the TurboFilter 96 plate

1. After step 3 of "Protocol: Plasmid DNA Purification using the QIAprep 96 *Plus* Miniprep Kit", place the TurboFilter 96 plate on top of an S-Block.
2. Transfer the lysates onto the TurboFilter 96 plate.
3. Load the assembled components (TurboFilter 96 plate on the S-Block) into the rotor.
4. Centrifuge for 3 min at 3000 x *g*.

Binding

5. After step 6 of the protocol, place the Plasmid *Plus* 96 plate on top of an S-Block.

   IMPORTANT: S-Blocks required in this step are not provided with the kit.
6. Transfer the lysates onto the Plasmid *Plus* 96 plate.
7. Load the assembled components (Plasmid *Plus* 96 plate on the S-Block) into the rotor.
8. Centrifuge for 1 min at 160 x *g*.

Wash steps

9. Place the Plasmid *Plus* 96 plate on top of an S-Block.

   IMPORTANT: S-Blocks required in this step are not provided with the kit.
10. Add the respective wash buffers.

11. Load the assembled components (Plasmid *Plus* 96 plate on the S-Block) into the rotor.
12. Centrifuge for 1 min at 160 x *g*.

Drying

13. Place the Plasmid *Plus* 96 plate on top of an elution microtube rack or a clean S-Block.

    IMPORTANT: S-Blocks required in this step are not provided with the kit.
14. Centrifuge for 10 min at 6000 x *g*.

Elution

15. Place the Plasmid *Plus* 96 plate on top of a new elution microtube rack containing elution microtubes.
16. Add elution buffer (Buffer EB) as described in step 11 of the protocol (page 18).
17. Load the assembled components (Plasmid *Plus* 96 plate on the elution microtubes) into the rotor.
18. Centrifuge for 1 min at 6000 x *g*.

Determination of yield

To determine the yield, DNA concentration should be determined by both UV spectrophotometry at 260 nm and quantitative analysis on an agarose gel. For reliable spectrophotometric DNA quantification, A_{260} readings should lie between 0.1 and 1.0.

Agarose gel analysis

We recommend removing and saving an aliquot of the cleared lysate (step 5). If the plasmid DNA is of low yield or quality, the sample and eluate can be analyzed by agarose gel electrophoresis to determine the stage of the purification procedure where the problem occurred. See Appendix B (page 25) for more information.

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 at 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 and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Low or no yield	
No DNA in the cleared lysate before loading	
a) Plasmid did not propagate	Check that the conditions for optimal growth were met. For more details, see **www.qiagen.com/goto/plasmidinfo**.
b) Alkaline lysis was inefficient	If cells have grown to very high densities, or if a larger amount of culture medium than recommended was used, the ratio of the biomass to lysis reagent is shifted. This may result in poor lysis conditions because the volumes of Buffers P1, P2 and S3 are not sufficient for efficient release of plasmid DNA. Reduce the culture volume to improve the ratio of biomass to lysis buffer. Also, insufficient mixing of lysis reagents will result in reduced yield. Mix thoroughly after addition of Buffers P1, P2 and S3 to achieve homogeneous suspensions.
c) Buffer P2 or BB 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 a homogeneous suspension is obtained.

	Comments and suggestions
DNA is found in the wash flow-through	
Ethanol omitted from wash buffer	Repeat procedure with correctly prepared wash buffer (Buffer PE).
Low DNA quality	
Eluate contains residual ethanol omitted from wash buffer	Ensure that the membrane of the Plasmid *Plus* 96 plate is dried sufficiently using a centrifuge or vacuum (see step 10 on page 17 of the protocol).
TurboFilter 96 plate clogs during filtration	
a) Culture volume too large	Do not exceed the culture volume recommended in the protocol.
b) Inefficient mixing after addition of Buffer S3	Mix well until the solution is cloudy. Mix the samples immediately after adding Buffer S3 to avoid local precipitation.
c) Mixing too vigorously after addition of Buffer S3	After addition of Buffer S3, the lysate should be mixed immediately but gently. Vigorous mixing disrupts the precipitate into tiny particles.
d) Block was agitated	Gently mix after addition of Buffer S3. Agitation causes shearing of DNA.
e) The TurboFilter 96 plate was not loaded immediately after addition of Buffer S3	After adding Buffer S3, the lysate should be transferred to the TurboFilter 96 plate immediately.
f) Vacuum pressure was too low	Ensure that the vacuum generates a vacuum pressure of –200 to –600 millibars (–150 to –450 mm Hg).

Appendix A: Setup of the QIAvac 96

Guidelines for QIAvac manifolds

The following recommendations should be followed when handling QIAvac manifolds.

- QIAvac manifolds operate with a house vacuum or Vacuum Pump (e.g., Vacuum Pump, cat. no. 84010 [USA and Canada], 84000 [Japan] or 84020 [rest of the world]).
- Always store QIAvac manifolds clean and dry. To clean, simply rinse all components with water and dry with paper towels. Do not air dry, because the screws may rust and will need to be replaced. Do not use abrasives or solvents.
- Always place the QIAvac manifold on a secure bench top or work area. If dropped, the manifold may crack.
- The components of QIAvac manifolds are not resistant to ethanol, methanol, or other organic solvents (Table 3). 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 a QIAvac manifold. The spring lock on the top plate and the self-sealing gasket provide an airtight seal when vacuum is applied to the assembled unit. To maximize gasket lifetime, rinse the gasket free of salts and buffers after each use and dry with paper towels before storage.

Table 3. Chemical-resistance properties of QIAvac manifolds

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	

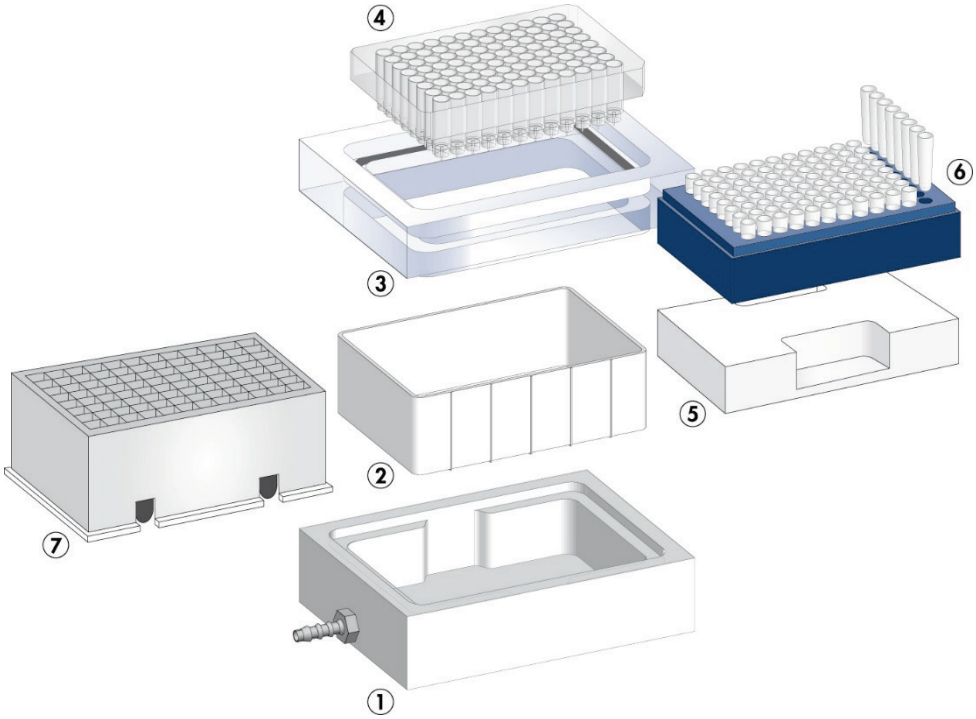


Figure 3. Components of the QIAvac 96 vacuum manifold.

1. QIAvac base, which holds a waste tray, S-Block, or an Elution Microtube Adapter
2. Waste tray
3. QIAvac 96 top plate
4. 96-well plate (i.e., TurboFilter 96 or Plasmid *Plus* 96 Plate)
5. Elution Microtube Adapter (not included with the QIAvac 96; please contact QIAGEN Technical Services)
6. Elution Microtube Rack with Elution Microtubes
7. S-Block

Appendix B: Agarose Gel Analysis of the Purification Procedure

DNA yields and quality can be readily analyzed by agarose gel electrophoresis. Poor yields and quality can be caused by a number of different factors. To determine the stage of the procedure where a problem occurred, save a fraction of the cleared lysate and analyze by agarose gel electrophoresis.

Preparation of samples

Remove an aliquot from the cleared lysate as indicated in the protocol. Precipitate the nucleic acids by adding 1 volume of isopropanol,* centrifuge for 15 min at maximum speed, and discard supernatant. Rinse the plasmid DNA pellets with 70% ethanol, drain well, and resuspend in 10 µl TE buffer, pH 8.0.

Agarose gel analysis

Run 2 µl of cleared lysate sample on a 1% agarose gel and compare to the eluted plasmid DNA as shown in Figure 4.

Lanes 1–5 illustrate some atypical results that may be observed in some preparations, depending on plasmid type and host strain.

Lane 1: Supercoiled (lower band) and open circular form (upper band) of the high-copy plasmid pUC18 with an additional band of denatured supercoiled DNA migrating

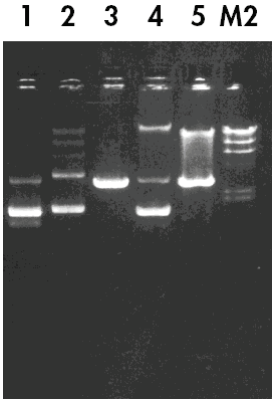


Figure 4. Agarose gel analysis of the plasmid purification procedure.

* 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.

just below the supercoiled form. This form may result from prolonged alkaline lysis with Buffer P2 and is resistant to restriction digestion.

Lane 2: Multimeric forms of supercoiled plasmid DNA (pTZ19), which may be observed with some host strains and should not be mistaken for genomic DNA. Multimeric plasmid DNA can easily be distinguished from genomic DNA by a simple restriction digestion — linearization of a plasmid sample displaying multimeric bands will yield a single defined band with the size of the linearized plasmid monomer (see lane 3).

Lane 3: Linearized form of plasmid pTZ19 after restriction digestion with *Eco*RI.

Lane 4: Sample contaminated with bacterial chromosomal DNA, which may be observed if the lysate is treated too vigorously (e.g., vortexing during incubation steps with Buffer P2). Genomic DNA contamination can easily be identified by digestion of the sample with *Eco*RI. A smear is observed, in contrast to the linear band seen after digestion of multimeric plasmid forms.

Lane 5: *Eco*RI digestion of a sample contaminated with bacterial genomic DNA, which gives a smear above the plasmid DNA.

M2: Lambda DNA digested with *Hin*dIII.

Ordering Information

Product	Contents	Cat. no.
QIAprep 96 *Plus* Miniprep Kit (4)*	For 4 x 96 plasmid minipreps: TurboFilter 96 Plates, Plasmid *Plus* 96 Plates, Buffers, Reagents, S-Blocks and Elution Microtubes	27291
QIAprep 96 *Plus* BioRobot Kit (4)†	For 4 x 96 plasmid minipreps: TurboFilter 96 Plates, Plasmid *Plus* 96 Plates, Buffers, Reagents, S-Blocks and Elution Microtubes	962241
QIAGEN Plasmid *Plus* Kits — for purification of up to 50 µg Transfection Grade plasmid DNA in 96-well format		
QIAGEN Plasmid *Plus* 96 Miniprep Kit (4)*	For 4 x 96 plasmid minipreps: TurboFilter 96 Plates, Plasmid *Plus* 96 Plates, Buffers, Reagents, S-Blocks and Elution Microtubes	16181
QIAGEN Plasmid *Plus* 96 BioRobot Kit (4)†	For 4 x 96 plasmid minipreps: TurboFilter 96 Plates and Plasmid *Plus* 96 Plates, Buffers, Reagents, S-Blocks and Elution Microtubes	960241
Accessories		
QIAvac 96	Vacuum manifold for processing QIAGEN 96-well plates: includes QIAvac 96 Top Plate, Base, Waste Tray, Plate Holder, Rack of Collection Microtubes (1.2 ml)	19504
BioRobot Universal System	Robotic workstation, computer-controlled vacuum pump, computer, QIAsoft 5 Operating System, installation, 1-year warranty on parts and labor	9001094

* Requires use of QIAvac 96 and Elution Microtube Adapter (contact QIAGEN Technical Services), or a centrifugation system suitable for 96-well blocks.

† For use with the BioRobot Universal System.

Product	Contents	Cat. no.
Centrifuge 4-16S	Universal laboratory centrifuge with brushless motor	81500* 81510† 81525‡ 81520§
Centrifuge 4-16KS	Refrigerated universal laboratory centrifuge with brushless motor	81600* 81610† 81625‡ 81620§
24-Well Blocks RB (24)	24-well blocks with 10 ml round-bottom wells, 24 per case	19583
48-Well Blocks (24)	48-well blocks with 5 ml wells, 24 per case	19577
Elution Microtubes CL (24 x 96)	Nonsterile polypropylene tubes (0.85 ml maximum capacity, less than 0.7 ml storage capacity, 0.4 ml elution capacity); 2304 in racks of 96; includes cap strips	19588
S-Blocks (24)	96-well blocks with 2.2 ml wells, 24 per case	19585
AirPore Tape Sheets (50)	Microporous tape sheets for covering 96-well blocks: 50 sheets per pack	19571

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.

* Japan.
† North America.
‡ UK.
§ Rest of the world.

Notes

Handbook Revision History

Document	Changes	Date
HB-1186-003	Replaced Flat-Bottom Blocks with S-Blocks. Added S-Block to Figure 3 and revised item numbering in image and in caption. Updated plasmid resource page links in Important Notes. Updated recommended centrifuges, from "4-16" to "4-16S", and from "4-16K" to "4-16KS".	December 2018

Limited License Agreement for QIAprep 96 *Plus* Miniprep and BioRobot Kits

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 handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, 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 kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit 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 kit 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 kit and/or its components.

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