

HiSpeed® Plasmid Purification Handbook

HiSpeed Plasmid Midi and Maxi Kit
For rapid purification of transfection grade
plasmid DNA



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Kit Contents

	HiSpeed Plasmid Midi Kit (25)	HiSpeed Plasmid Maxi Kit (10)	HiSpeed Plasmid Maxi Kit (25)
Catalog no.	12643	12662	12663
HiSpeed Tips	25 HiSpeed Midi Tips	10 HiSpeed Maxi Tips	25 HiSpeed Maxi Tips
QIAfilter Cartridges	25 QIAfilter Midi Cartridges	10 QIAfilter Maxi Cartridges	25 QIAfilter Maxi Cartridges
Caps for QIAfilter	25	10	25
20 ml Syringes	25	–	–
30 ml Syringes	–	10	25
5 ml Syringes	25	10	25
QIAprecipitator	25	10	25
	QIAprecipitator Midi Modules	QIAprecipitator Maxi Modules	QIAprecipitator Maxi Modules
Buffer P1	1 x 220 ml	1 x 110 ml	1 x 280 ml
Buffer P2	1 x 220 ml	1 x 110 ml	1 x 280 ml
Buffer P3	1 x 220 ml	1 x 110 ml	1 x 280 ml
Buffer QBT	1 x 110 ml	1 x 110 ml	1 x 280 ml
Buffer QC	3 x 190 ml	3 x 240 ml	2 x 850 ml
Buffer QF	1 x 170 ml	1 x 170 ml	1 x 420 ml
Buffer TE	1 x 30 ml	1 x 30 ml	1 x 30 ml
LyseBlue	1 x 220 µl	1 x 110 µl	1 x 280 µl
RNase A*	1 x 22 mg	1 x 11 mg	1 x 28 mg
Handbook	1	1	1

* Provided as a 10 mg/ml or 100 mg/ml solution.

Storage Conditions

HiSpeed Plasmid Kits should be stored dry and at room temperature (15–25°C). HiSpeed Tips, QIAfilter Cartridges, and QIAprecipitator Modules can be stored for at least two years without showing any reduction in performance, capacity or quality of separation. After addition of RNase A, Buffer P1 should be stored at 8°C and is stable for six months. Other buffers and RNase A stock solution can be stored for two years at room temperature.

Technical Assistance

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

QIAGEN customers are also a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore also encourage you to contact us if you have any suggestions about product performance or new applications and techniques. For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Comprehensive background information on plasmid preparation procedures and common plasmid applications — in addition to kit selection guides, frequently asked questions, and information about our purification technologies — can be found on our plasmid Web page www.qiagen.com/goto/plasmidinfo .

Quality Control

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

Product Use Limitations

HiSpeed Plasmid Midi and Maxi Kits are developed, designed, and sold for research purposes only. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

Product Warranty and Satisfaction Guarantee

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

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

Safety Information

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

The following risk and safety phrases apply to components of the HiSpeed Midi and Maxi Kits.

Buffer P2

Contains sodium hydroxide: irritant. Risk and safety phrases: * R36/38, S13-26-36-46

Buffer P3

Contains acetic acid: irritant. Risk and safety phrases: * R36/38, S13-26-36-46

Buffers QBT, QC, QF

Contain isopropanol: flammable. Risk and safety phrases: * R10

RNase A

Contains ribonuclease: sensitizer. Risk and safety phrases: * R42/43, S23-24-26-36/37

24-hour emergency information

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

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R10: Flammable; R36/38: Irritating to eyes and skin; R41: Risk of serious damage to eyes; R42/43: May cause sensitization by inhalation and skin contact; S13: Keep away from food, drink, and animal feedings; S23: Do not breathe vapor; S24: Avoid contact with skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S36/37: Wear suitable protective clothing and gloves; S46: If swallowed, seek medical advice immediately and show this container or label.

Introduction

HiSpeed Plasmid Purification Kits will dramatically change the way you isolate nucleic acids. The ultrafast purification protocol, based on the remarkable selectivity of patented QIAGEN Resin, allows the isolation of ultrapure, supercoiled plasmid DNA with high yields in less than one hour, and without centrifugation. No expensive equipment such as ultracentrifuges and HPLC, or toxic reagents such as phenol and ethidium bromide are required. Plasmid and cosmid DNA purified with HiSpeed Tips is suitable for use in demanding applications such as transfection, automated or manual sequencing, and enzymatic modifications. QIAGEN continually strives to streamline and further develop nucleic acid purification to offer a complete plasmid purification system, for all requirements. Select the optimum plasmid kit for your requirements by visiting our online selection guide at www.qiagen.com/products/plasmid/selectionguide. For transfection, QIAGEN also offers the advanced PolyFect[®], SuperFect[®], and Effectene[®] transfection reagents. These reagents, combined with the high-quality plasmid DNA obtained from QIAGEN, QIAfilter, HiSpeed[®], and EndoFree[®] Plasmid Kits, provide optimal transfection results (for ordering information, see pages 33–36).

Principle and procedure

QIAGEN plasmid purification protocols are based on a modified alkaline lysis procedure, followed by binding of plasmid DNA to QIAGEN Anion-Exchange Resin under appropriate low-salt and pH conditions. RNA, proteins, dyes, and low-molecular-weight impurities are removed by a medium-salt wash. Plasmid DNA is eluted in a high-salt buffer and then concentrated and desalted by isopropanol precipitation. No expensive equipment such as ultracentrifuges and HPLC, or toxic reagents, such as phenol and ethidium bromide are required.

Each disposable HiSpeed Tip packed with QIAGEN Resin HS is designed to operate by gravity flow, reducing the amount of hands-on time required for the purification procedure. DNA binding, washing, and elution steps proceed markedly faster than with conventional QIAGEN-tips and the increased capacity enables higher yields from high-copy plasmids.

QIAfilter Cartridges enable rapid and efficient clearing of bacterial lysates without centrifugation. They have a syringe format and lysates are cleared by pushing the liquid through a filter (Figure 3, page 15). QIAfilter Cartridges completely remove KDS* precipitates for efficient clearing in a fraction of the time needed for conventional centrifugation. Plasmid DNA from the filtered lysate is then efficiently purified using a HiSpeed Tip. The QIAprecipitator Module revolutionizes the isopropanol precipitation step, making it fast, easy, and risk-free. Plasmid DNA eluted from the HiSpeed Tip is mixed with isopropanol and applied to the QIAprecipitator Module using the syringe provided. The precipitated DNA is trapped in the QIAprecipitator as a thin layer, which allows thorough drying and removal of alcohol by simply pushing air through the QIAprecipitator with a syringe. The DNA is then simply eluted from the QIAprecipitator into a microcentrifuge tube with Buffer TE or water (Figure 4, page 15).

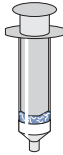
* Potassium Dodecyl Sulfate

HiSpeed Procedure

Pelleted bacteria



Alkaline lysate



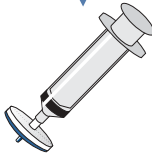
Clear lysate
by filtration using
QIAfilter Cartridge



Bind DNA,
wash, elute on
HiSpeed Tip



Add isopropanol



Collect DNA using
QIAprecipitator

Elute



Ultrapure plasmid DNA

Equipment and Reagents to Be Supplied by User

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

For all protocols:

- Standard microbiological equipment for growing and harvesting bacteria (e.g., inoculating loop, culture tubes and flasks, 37°C shaking incubator, and centrifuge with rotor and tubes or bottles for harvesting cells)
- QIArack or equivalent holder (see “Setup of HiSpeed Tips”, page 15)
- Isopropanol
- 70% ethanol

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 our plasmid resource page at www.qiagen.com/goto/plasmidinfo and click on the link "General Considerations for Optimal Results". Also be sure to read and follow the appropriate detailed protocol.

Plasmid size

Plasmids and cosmids up to 50 kb in size can be purified using HiSpeed Plasmid Kit. For larger constructs QIAGEN Plasmid Kits or the Large-Construct Kit are recommended

Plasmid/cosmid copy number

Plasmid and cosmids vary in copy number, depending on the origin of replication they contain, their size, and the size of insert.

The HiSpeed Kit is suitable for isolation of both high- and low-copy plasmids. However, in the case of low-copy plasmids, the quantity of plasmid in the lysate can be limited and may lead to lower yields. Furthermore, since the QIAprecipitator requires a minimum volume of 500 μ l for elution, the final DNA concentration may be considerably lower than for high-copy plasmids. For more details, visit our plasmid resource page at www.qiagen.com/goto/plasmidinfo and click on the link "General Considerations for Optimal Results".

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 either reducing the amount of culture volume or doubling the volumes of Buffers P1, P2, and P3 in order to improve the ratio of biomass to lysis buffers for optimized lysis conditions.

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

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

Culture media

QIAGEN plasmid purification protocols are optimized for use with cultures grown in standard Luria Bertani (LB) medium to a cell density of approximately $3\text{--}4 \times 10^9$ cells/ml, which typically corresponds to a pellet wet weight of approximately 3 g/liter 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 (Table 2) to obtain the highest plasmid yields.

Rich media are not recommended for plasmid preparation with QIAGEN-tips. If rich media must be used, growth time must be optimized, and culture volumes reduced. For more details, visit our plasmid resource page at www.qiagen.com/goto/plasmidinfo and click on the link “General Considerations for Optimal Results”.

Table 2. Composition of Luria Bertani Medium

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

Please refer to Appendix B on page 32 for preparation of LB medium.

Culture volume

Do not exceed the maximum recommended culture volumes given at the beginning of each protocol. 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 protocol for HiSpeed Midi and Maxi Kits is optimized for use with cultures grown in standard Luria Bertani (LB) medium (see page 13), grown to a cell density of approximately $3\text{--}4 \times 10^9$ cells per ml. We advise harvesting cultures after approximately 12–16 hours of growth, which typically is the transition from logarithmic into stationary growth phase. It is best to assess the cell density of the culture, and if that is too high, reduce the culture volumes accordingly or increase the volumes of Buffers P1, P2, and P3. 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 OD₆₀₀ measurements into the number of cells per ml. 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 ml, which is then set in relation to the measured OD₆₀₀ values.

Capacity of HiSpeed Tips

The maximum binding capacity of HiSpeed Midi and Maxi Tips is 200 µg and 750 µg respectively. Actual yields will depend on a number of variables such as, culture volume, culture medium, plasmid copy number (see Table 1, page 13), size of insert, and host strain. Expected yields are given in the first section of the protocol. A final DNA concentration of up to 0.4 µg/µl (Midi) or 1.5 µg/µl (Maxi) can be expected if eluting a high-copy plasmid with 500 µl of Buffer TE. However, the final concentration of low-copy vectors may be considerably lower. Low-copy vectors or dilute samples may be concentrated by vacuum centrifugation or ethanol precipitation. For more details, visit our plasmid resource page at www.qiagen.com/goto/plasmidinfo and click on the link “General Considerations for Optimal Results”.

Setup of HiSpeed Tips

HiSpeed Tips can be held upright in a suitable collection vessel such as a tube or flask, using the tip holders provided with the kit (Figure 2A). Alternatively, HiSpeed Tips can be placed in the QIArack (cat. no. 19015), which has a removable collection tray for collecting liquid flow-through (Figure 2B). HiSpeed Maxi Tips fit in the QIArack and not in standard 50 ml collection tubes.



Figure 1 HiSpeed Midi and Maxi Tips.

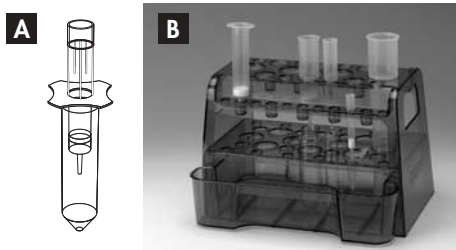


Figure 2 Setup of HiSpeed Tips **A** with tip holder or **B** with the QIArack.



Figure 3 The syringe format QIAfilter in use.



Figure 4 The QIAprecipitator in use.

Analytical gel analysis

The success of the plasmid purification procedure can be monitored on an analytical gel (see Figure 5, page 29). We recommend removing and saving aliquots where indicated during the purification procedure (samples 1–4). If the plasmid DNA is of low yield or quality, the samples can be analyzed by agarose gel electrophoresis to determine at what stage of the purification the problem occurred (see page 29).

Convenient stopping points in protocols

The purification procedure can be stopped and continued later by freezing the cell pellets obtained by centrifugation. The frozen cell pellets may be stored at -20°C for several weeks. In addition, the DNA eluted from the HiSpeed Tip may be stored overnight at $2-8^{\circ}\text{C}$,* after which the protocol can be continued. These stopping points are indicated by the symbol ⊗.

Using LyseBlue reagent

LyseBlue is a color indicator which 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 have not had much experience with plasmid preparations as well as experienced scientists who want to be assured of maximum product yield.

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

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

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

The plasmid 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 P3 or 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.

* Longer storage is not recommended.

Protocol: Plasmid or Cosmid DNA Purification Using HiSpeed Plasmid Midi and Maxi Kits

This protocol is for preparation of up to 200 µg of high- or low-copy plasmid or cosmid DNA using the **HiSpeed Plasmid Midi Kit** or 750 µg using the **HiSpeed Plasmid Maxi Kit**.

A final DNA concentration of up to 0.4 µg/µl (Midi) or 1.5 µg/µl (Maxi) can be expected, if eluting a high-copy plasmid with 500 µl of Buffer TE. However, the final concentration of low-copy vectors may be considerably lower. If higher yields of low-copy plasmids are desired, the lysates from two QIAfilter Cartridges can be loaded onto one HiSpeed Tip. In addition, eluates from low-copy vectors or dilute samples can be concentrated by spinning in a centrifuge under vacuum, or ethanol precipitation.

Low-copy plasmids that have been amplified in the presence of chloramphenicol should be treated as high-copy plasmids when choosing the appropriate culture volume.


Maximum Recommended Culture Volumes*

	HiSpeed Plasmid Midi Kit	HiSpeed Plasmid Maxi Kit
High-copy plasmids	50 ml	150 ml
Low-copy plasmids	150 ml	250 ml

* For high-copy plasmids, expected yields are 100–200 µg for the HiSpeed Plasmid Midi Kit and 300–750 µg for the HiSpeed Plasmid Maxi Kit. For low-copy plasmids, expected yields are 30–100 µg for the HiSpeed Plasmid Midi Kit and 50–250 µg for the HiSpeed Plasmid Maxi Kit using these culture volumes.

† The maximum recommended culture volume applies to the capacity of the QIAfilter Cartridge. If higher yields of low-copy plasmids are desired, the lysates from two QIAfilter Cartridges can be loaded onto one HiSpeed Tip.

Important notes before starting

- New users are advised to familiarize themselves with the detailed protocol provided in this handbook. In addition, extensive background information is provided on our plasmid resource page www.qiagen.com/goto/plasmidinfo.
- Optional: Remove samples at the steps indicated with the symbol  in order to monitor the procedure on an analytical gel (see page 29)
- **Blue** (marked with a ▲) denotes values for the **HiSpeed Plasmid Midi Kit**; **red** (marked with a ●) denotes values for the **HiSpeed Plasmid Maxi Kit**.

Things to do before starting

- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (centrifuge briefly before use) per bottle of Buffer P1, to give a final concentration of 100 µg/ml.
- Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.
- Pre-chill Buffer P3 at 4°C.
- Optional: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use one vial LyseBlue (centrifuge briefly before use) per bottle of Buffer P1 to achieve a 1:1000 dilution. 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 16.

Procedure

1. **Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic. Incubate for approx. 8 h at 37°C with vigorous shaking (approx. 300 rpm).**

Use a tube or flask with a volume of at least 4 times the volume of the culture.

2. **Dilute the starter culture 1/500 to 1/1000 into selective LB medium. For high-copy plasmids inoculate ▲ 50 ml or ● 150 ml medium. For low-copy plasmids, inoculate ▲ 150 ml or ● 250 ml medium. Grow at 37°C for 12–16 h with vigorous shaking (approx. 300 rpm).**

Use a flask or vessel with a volume of at least 4 times the volume of the culture. The culture should reach a cell density of approximately $3\text{--}4 \times 10^9$ cells per milliliter, which typically corresponds to a pellet wet weight of approximately 3 g/liter.

3. **Harvest the bacterial cells by centrifugation at 6000 x g for 15 min at 4°C.**

Remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.

⊗ If you wish to stop the protocol and continue later, freeze the cell pellet at -20°C.

4. **Resuspend the bacterial pellet in ▲ 6 ml or ● 10 ml Buffer P1.**

For efficient lysis it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. Ensure that RNase A has been added to Buffer P1.

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

5. **Add ▲ 6 ml or ● 10 ml Buffer P2, mix thoroughly by vigorously inverting the sealed tube 4–6 times, and incubate at room temperature (15–25°C) for 5 min.**

Do not vortex, as this will result in shearing of genomic DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min. After use, the bottle containing Buffer P2 should be closed immediately to avoid acidification from CO₂ in the air.

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.

During the incubation prepare the QIAfilter Cartridge:

Screw the cap onto the outlet nozzle of the QIAfilter Midi or QIAfilter Maxi Cartridge.

Place the QIAfilter Cartridge into a convenient tube or a QIArack.

6. **Add ▲ 6 ml or ● 10 ml chilled Buffer P3 to the lysate, and mix immediately and thoroughly by vigorously inverting 4–6 times. Proceed directly to step 7. Do not incubate the lysate on ice.**

Precipitation is enhanced by using chilled Buffer P3. After addition of Buffer P3, a fluffy white precipitate containing genomic DNA, proteins, cell debris, and KDS becomes visible. The buffers must be mixed completely. If the mixture appears still viscous and brownish, more mixing is required to completely neutralize the solution. It is important to transfer the lysate into the QIAfilter Cartridge immediately in order to prevent later disruption of the precipitate layer.

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

7. **Pour the lysate into the barrel of the QIAfilter Cartridge. Incubate at room temperature for 10 min. Do not insert the plunger!**

Important: This 10 min incubation at room temperature is essential for optimal performance of the QIAfilter Cartridge. Do not agitate the QIAfilter Cartridge during this time. A precipitate containing proteins, genomic DNA, and detergent will float and form a layer on top of the solution. This ensures convenient filtration without clogging. If, after the 10 min incubation, the precipitate has not floated to the top of the solution, carefully run a sterile pipet tip around the walls of the cartridge to dislodge it.

8. **Equilibrate a ▲ HiSpeed Midi or ● HiSpeed Maxi Tip by applying ▲ 4 ml or ● 10 ml Buffer QBT and allow the column to empty by gravity flow.**

Flow of buffer will begin automatically by reduction in surface tension due to the presence of detergent in the equilibration buffer. Allow the HiSpeed Tip to drain completely. HiSpeed Tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the upper frit in the column.

9. **Remove the cap from the QIAfilter outlet nozzle. Gently insert the plunger into the ▲ QIAfilter Midi or ● QIAfilter Maxi Cartridge and filter the cell lysate into the previously equilibrated HiSpeed Tip.**

Filter until all of the lysate has passed through the QIAfilter Cartridge, but do not apply extreme force. Approximately ▲ 15 ml or ● 25 ml of the lysate is generally recovered after filtration.

☞ Remove a ▲ 300 μ l or ● 120 μ l sample of the filtered lysate and save for an analytical gel (sample 1) in order to determine whether growth and lysis conditions were optimal.

10. **Allow the cleared lysate to enter the resin by gravity flow.**

☞ Remove a ▲ 300 μ l or ● 120 μ l sample of the flow-through and save for an analytical gel (sample 2) in order to determine the efficiency of DNA binding to the QIAGEN Resin.

11. **Wash the ▲ HiSpeed Midi or ● HiSpeed Maxi Tip with ▲ 20 ml or ● 60 ml Buffer QC.**

Allow Buffer QC to move through the HiSpeed Tip by gravity flow.

☞ Remove a ▲ 400 μ l or ● 240 μ l sample of the wash fraction and save for an analytical gel (sample 3).

12. **Elute DNA with ▲ 5 ml or ● 15 ml Buffer QF.**

Collect the eluate in a tube with a minimum capacity of ▲ 10 ml or ● 30 ml.

☞ Remove a ▲ 100 μ l or ● 60 μ l sample of the eluate and save for an analytical gel (sample 4).

⊗ If you wish to stop the protocol and continue later, store the eluate at 4°C. Storage periods longer than overnight are not recommended.

13. **Precipitate DNA by adding ▲ 3.5 ml or ● 10.5 ml (0.7 volumes) room-temperature isopropanol to the eluted DNA. Mix and incubate at room temperature for 5 min.**

All solutions should be at room temperature in order to minimize salt precipitation.

14. During the incubation remove the plunger from a ▲ 20 ml or ● 30 ml syringe and attach the ▲ QIAprecipitator Midi Module or ● QIAprecipitator Maxi Module onto the outlet nozzle. Do not use excessive force, bending, or twisting to attach the QIAprecipitator!

Important: Always remove the QIAprecipitator from the syringe before pulling up the plunger!

15. Place the QIAprecipitator over a waste bottle, transfer the eluate/isopropanol mixture into the ▲ 20 ml or ● 30 ml syringe, and insert the plunger. Filter the eluate/isopropanol mixture through the QIAprecipitator using constant pressure.

Alternatively, the QIAprecipitator attached to the ▲ 20 ml or ● 30 ml syringe can be placed on a QIAvac 24 Plus or QIAvac 6S manifold. Use of VacConnectors (cat. no. 19407) is recommended for vacuum processing, in order to raise the QIAprecipitator above the level of adjacent luer extensions. Switch on vacuum to draw the eluate/isopropanol mixture through the QIAprecipitator. Switch off the vacuum once all the liquid has been drawn through.

Important: Complete the QIAprecipitator procedure (steps 16–21) within 10 min. To prevent detachment of the QIAprecipitator and subsequent loss of DNA and alcohol, do not use excessive force when pushing liquid through the QIAprecipitator.

16. Remove the QIAprecipitator from the ▲ 20 ml or ● 30 ml syringe and pull out the plunger. Re-attach the QIAprecipitator and add 2 ml 70% ethanol to the syringe. Wash the DNA by inserting the plunger and pressing the ethanol through the QIAprecipitator using constant pressure.

Alternatively, if you are using the vacuum procedure, add 2 ml 70% ethanol, and switch on vacuum to draw the ethanol through the QIAprecipitator. Keep vacuum on for 3 min. Proceed to step 18.

17. Remove the QIAprecipitator from the ▲ 20 ml or ● 30 ml syringe and pull out the plunger. Attach the QIAprecipitator to the ▲ 20 ml or ● 30 ml syringe again, insert the plunger, and dry the membrane by pressing air through the QIAprecipitator quickly and forcefully. Repeat this step.

18. Dry the outlet nozzle of the QIAprecipitator with absorbent paper to prevent ethanol carryover.

- 19. Remove the plunger from a new 5 ml syringe and attach the QIAprecipitator onto the outlet nozzle. Hold the outlet of the QIAprecipitator over a 1.5 ml collection tube. Add 1 ml of Buffer TE to the 5 ml syringe. Insert the plunger and elute the DNA into the collection tube using constant pressure.**

Ensure that the outlet of the QIAprecipitator is held over the collection tube when Buffer TE is poured into the syringe, as eluate can drip through the QIAprecipitator before the syringe barrel is inserted.

Be careful, as residual elution buffer in the QIAprecipitator tends to foam when expelled.

Alternatively, if a higher DNA concentration is desired and a reduction in yield of up to 10% is acceptable, elute with 500 μ l Buffer TE. Lower volumes of elution buffer are not recommended, since incomplete wetting of the QIAprecipitator membrane will lead to reduced DNA yields.

Water or buffers commonly used to dissolve DNA (e.g., Tris, may also be used for elution).

Note: TE Buffer contains EDTA which may inhibit downstream enzymatic or sequencing reactions.

Note: Store DNA at -20°C when eluted with water as DNA may degrade in the absence of buffering and chelating agents.

- 20. Remove the QIAprecipitator from the 5 ml syringe, pull out the plunger and reattach the QIAprecipitator to the 5 ml syringe.**
- 21. Transfer the eluate from step 19 to the 5 ml syringe and elute for a second time into the same 1.5 ml tube.**

This re-elution step ensures that the maximum amount of DNA in the QIAprecipitator is solubilized and recovered.

Be careful, as residual elution buffer in the QIAprecipitator tends to foam when expelled.

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 aliquots during the purification procedure (samples 1–4). If the plasmid DNA is of low yield or quality, the samples can be analyzed by agarose gel electrophoresis to determine at what stage of the purification procedure the problem occurred (see page 29).

Troubleshooting Guide

Poor yields and quality can be caused by a number of different factors. For optimal plasmid preparation conditions, particular attention should be paid to the lysis conditions as described in the protocol. In addition, adhering to our recommendations with respect to plasmid copy number, capacity of QIAGEN-tip, culture volume, and culture media will ensure consistent and optimal results.

The following troubleshooting guide as well “General Considerations for Optimal Results” provided on our Web page www.qiagen.com/goto/plasmidinfo may be helpful in solving any problems that may arise. The scientists at QIAGEN Technical Service are always happy to answer any questions you may have about either the information and protocols in this handbook or molecular biology applications (see back cover for contact information).

This troubleshooting guide is divided into three sections: The first details general troubleshooting relevant to all HiSpeed Plasmid Kits, the second is specifically for QIAfilter Cartridges, and the third for QIAprecipitator Modules.

Comments and suggestions

Low or no DNA yield

No DNA in lysate (sample 1)

- | | |
|---|---|
| a) Plasmid did not propagate | Please read “Growth of Bacterial Cultures” on our Web page www.qiagen.com/goto/plasmidinfo , and check that the conditions for optimal growth were met. |
| b) Alkaline lysis was inefficient | If cells have grown to very high densities or a larger amount of cultured medium than recommended was used, the ratio of biomass to lysis reagent is shifted. This may result in poor lysis conditions, because the volumes of Buffers P1, P2, and P3 are not sufficient for setting the plasmid DNA free efficiently. Reduce culture volume or increase volumes of Buffers P1, P2, and P3. Also, insufficient mixing of lysis reagents will result in reduced yield. Mix thoroughly after addition of Buffers P1, P2, and P3 to achieve homogeneous suspensions. Use LyseBlue to visualize efficiency of mixing. |
| c) Insufficient lysis for low-copy plasmids | For low copy-plasmid preparations, doubling the volumes of lysis buffers P1, P2, and P3 may help to increase plasmid yield and quality (see page 12 and background on our Web page www.qiagen.com/goto/plasmidinfo). |

Comments and suggestions

- d) Lysate incorrectly prepared
- Check Buffer P2 for SDS precipitation resulting from low storage temperatures and dissolve the SDS by warming. The bottle containing Buffer P2 should always be closed immediately after use. Lysis buffers prepared in the laboratory should be prepared according to the instructions on page 32.
If necessary, prepare fresh Buffers P1, P2, and P3.

DNA in flow-through fraction (sample 2)

- a) Column was overloaded
- Check the culture volume and yield against the capacity of the HiSpeed Tip, as detailed at the beginning of the protocol. Reduce the culture volume accordingly.
- b) SDS (or other ionic detergent) was in lysate
- Chill Buffer P3 before use. If the lysate is cleared by centrifugation, load onto HiSpeed Tip promptly after centrifugation. If lysate is too viscous for effective mixing of Buffer P3, reduce culture volume or increase volumes of Buffers P1, P2, and P3.
Use LyseBlue to visualize efficiency of mixing.
- c) Inappropriate salt or pH conditions in buffers
- Ensure that any buffers prepared in the laboratory were prepared according to the instructions provided on page 32.
- d) Column flow was uneven
- Store HiSpeed Tips at room temperature (15–25°C). If stored under cold, damp conditions for prolonged periods of time, the resin may clump. This problem can be overcome by shaking the column before use.

DNA in Buffer QC wash fraction (sample 3)

- a) Column was overloaded
- Check the culture volume and yield against the capacity of the HiSpeed Tip, as detailed at the beginning of the protocol. Reduce the culture volume accordingly.
- b) Buffer QC was incorrect
- Check pH and salt concentration of Buffer QC. Recover DNA by precipitation, and purify on a new HiSpeed Tip as detailed in "Purification of Plasmid DNA Prepared by Other Methods" on our Web page www.qiagen.com/goto/plasmidinfo.

Comments and suggestions

No DNA in eluate (sample 4)

- a) No DNA in the lysate See section "No DNA in lysate" page 23.
- b) Elution Buffer QF was incorrect Check pH and salt concentration of Buffer QF. Recover DNA by eluting with fresh buffer.
- c) DNA passed through in the flow-through or wash fraction See previous two sections.

Contaminated DNA/poor-quality DNA

- a) Genomic DNA in the eluate Mixing of bacterial lysate was too vigorous. The lysate should not be vortexed after addition of Buffers P2 and P3 to prevent shearing of chromosomal DNA. Reduce culture volume if lysate is too viscous for gentle mixing.
- b) RNA in the eluate RNase A digestion was insufficient. Check culture volume against recommended volumes, and reduce if necessary. Check that the RNase A provided with the kit has been used. If Buffer P1 is more than 6 months old, add more RNase A. Recover DNA by precipitating the eluate, digesting with RNase A, and purifying on a new HiSpeed Tip.
- c) Nuclease contamination Check buffers for nuclease contamination and replace if necessary. Use new glass- and plasticware, and wear gloves.
- d) Lysis time was too long Ensure that the lysis step (Buffer P2) does not exceed 5 min.
- e) Too much cell material in the alkaline lysis Check the culture volume and yield against the capacity of the HiSpeed Tip. Reduce the culture volume accordingly or alternatively increase the volumes of Buffers P1, P2, and P3.
- f) Plasmid DNA is nicked/sheared/degraded DNA was poorly buffered. Redissolve DNA in TE buffer, pH 8.0, to inhibit nuclease activity and maintain stable pH during storage.
- g) Endonuclease-containing host Refer to background information on our Web page (www.qiagen.com/goto/plasmidinfo), and consider changing *E. coli* host strain.

Comments and suggestions

Poor DNA performance

- a) Residual protein Check culture volume against the recommended volumes and reduce if necessary. Ensure that the bacterial lysate is cleared properly by centrifugation at $\geq 20,000 \times g$ for 45 min, or using a QIAfilter Cartridge.

Extra DNA bands on analytical gel

- a) Dimer form of plasmid Dimers or multimers of supercoiled plasmid DNA are formed during replication of plasmid DNA. Typically, when purified plasmid DNA is electrophoresed, both the supercoiled monomer and dimer form of the plasmid are detected upon ethidium bromide staining of the gel (see Figure 5, page 29). The ratio of these forms is often host dependent.
- b) Plasmid has formed denatured supercoils This species runs faster than closed circular DNA on a gel and is resistant to restriction digestion (see Figure 5, page 29). Do not incubate cells for longer than 5 min in Buffer P2. Mix immediately after addition of Buffer P3.
- c) Possible deletion mutants Some sequences are poorly maintained in plasmids. Check for deletions by restriction analysis. Cosmid clones, in particular, should always be prepared from freshly streaked, well-isolated colonies, since cosmids are not stable in *E. coli* for long periods of time.

Blocked QIAGEN-tip

- Lysate was turbid Ensure that the lysate is clear before it is loaded onto the column. Ensure that Buffer P3 is chilled before use. To clear a blocked HiSpeed Tip, positive pressure may be applied, (e.g., by using a syringe fitted into a rubber stopper with a hole).

QIAfilter Cartridges

QIAfilter Cartridge clogs during filtration

- a) Too large culture volume used Use no more than the culture volume recommended in the protocol.
- b) Inefficient mixing after addition of Buffer P3 Mix well until a fluffy white material has formed and the lysate is no longer viscous.

Comments and suggestions

- c) Mixing too vigorous after addition of Buffer P3
After addition of Buffer P3 the lysate should be mixed immediately but gently. Vigorous mixing disrupts the precipitate into tiny particles which may clog the QIAfilter Cartridge.
- d) QIAfilter Cartridge was not loaded immediately after addition of Buffer P3
After addition of Buffer P3 the lysate should be poured immediately into the QIAfilter Cartridge. Decanting after incubation may disrupt the precipitate into tiny particles which may clog the QIAfilter Cartridge.
- e) QIAfilter Cartridge was agitated during incubation
Pour the lysate into the QIAfilter Cartridge immediately after addition of Buffer P3 and do not agitate during the 10 min incubation.
Agitation causes the precipitate to be disrupted into tiny particles, instead of forming a layer.
- f) Incubation after addition of Buffer P3 on ice instead of at RT
Ensure incubation is performed at room temperature Buffer P3 on ice instead of in the QIAfilter Cartridge. Precipitate flotation is more efficient at room temperature than on ice.
- g) Incubation time after addition of Buffer P3 too short
Incubate with Buffer P3 as indicated in the protocol. If the precipitate has not risen to the top after the 10 min incubation, carefully run a sterile pipet tip or sterile spatula around the cartridge wall to dislodge the precipitate before continuing with the filtration.

Lysate not clear after filtration

Precipitate was forced through the QIAfilter Cartridge
Filter until all of the lysate has passed through the QIAfilter Midi or Maxi Cartridge, but do not apply extreme force. Approximately 15 ml (Midi) or 25 ml (Maxi) of the lysate are typically recovered.

QIAprecipitator Modules

DNA does not perform well

Eluate contains residual alcohol
Ensure that the membrane is dried by pressing air through the QIAprecipitator at least twice. Dry the outlet nozzle of the QIAprecipitator with absorbent paper.

Comments and suggestions

QIAprecipitator clogs during use

- a) Too much DNA applied to the QIAprecipitator Do not load eluate from several columns on the QIAprecipitator.
- b) QIAprecipitator Midi Module was used for precipitation of eluate from a HiSpeed Maxi Tip Use the size of QIAprecipitator corresponding to the HiSpeed Tip being used.
- c) Ethanol was used for precipitation instead of isopropanol Use of ethanol instead of isopropanol for precipitation leads to a finer precipitate that can clog the module.

QIAprecipitator casing breaks, causing leakage

- a) Excessive exposure of QIAprecipitator to alcohol Prolonged incubation with alcohol may weaken the joint between upper and lower part of the QIAprecipitator. Complete steps 15–21 within 10–15 min.
- b) QIAprecipitator attached with excessive force Do not apply excessive force, bending, or twisting when attaching the QIAprecipitator to the syringe.
- c) QIAprecipitator inlet was bent during processing Do not stress the inlet by resting one edge of the QIAprecipitator on a hard surface (e.g., the edge of a sink) and depressing the syringe plunger. Always apply gentle, even, pressure perpendicularly to the QIAprecipitator.

Appendix A: 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 at what stage of the procedure any problem occurred, save fractions from different steps of the purification procedure (see below and Table 3), and analyze by agarose gel electrophoresis.

Preparation of samples

Remove aliquots from the cleared lysate (sample 1), flow-through (sample 2), combined Buffer QC wash fractions (sample 3), and Buffer QF eluate (sample 4), as indicated in each protocol and in Table 3. Precipitate the nucleic acids with 1 volume of isopropanol, rinse the pellets with 70% ethanol, drain well, and resuspend in 10 μ l TE buffer, pH 8.0.

Table 3. Sample Volumes Required for Agarose Gel Analysis

Sample	Protocol step	Midi	Maxi
1	Cleared lysate	300 μ l	120 μ l
2	Flow-through	300 μ l	120 μ l
3	Wash fraction	400 μ l	240 μ l
4	Eluate	100 μ l	60 μ l

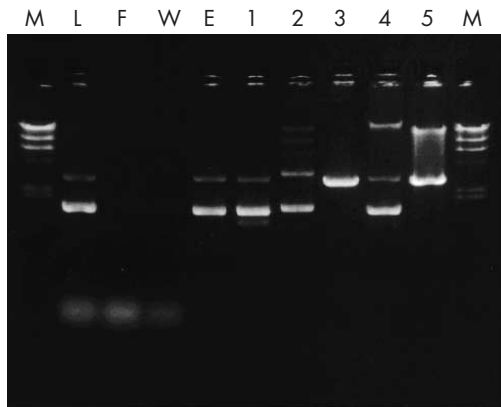


Figure 5 Agarose gel analysis of the plasmid purification procedure.

Agarose gel analysis

Run 2 μ l of each sample on a 1% agarose gel for analysis of the fractions at each stage of the plasmid purification procedure. Figure 5 shows an analytical gel of the different fractions, together with examples of problems that can arise at each step. If you find that you have a problem with a particular step of the protocol, turn to the hints in the relevant section of the troubleshooting guide on pages 23–28. If the problem remains unresolved, or if you have any further questions, please call QIAGEN Technical Service.

L: Cleared lysate containing supercoiled and open circular plasmid DNA and degraded RNA (sample 1).

F: Flow-through fraction containing only degraded RNA is depleted of plasmid DNA which is bound to the QIAGEN Resin (sample 2).

W: Wash fraction, in which the remaining traces of RNA are removed without affecting the binding of the DNA (sample 3).

E: The eluate containing pure plasmid DNA with no other contaminating nucleic acids (sample 4).

M: Lambda DNA digested with *Hind*III.

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 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 or P3). 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.

Appendix B: Composition of Buffers

Buffer	Composition	Storage
Buffer P1 (resuspension buffer)	50 mM Tris-Cl, pH 8.0; 10 mM EDTA; 100 µg/ml RNase A	2–8°C, after addition of RNase A
Buffer P2 (lysis buffer)	200 mM NaOH, 1% SDS (w/v)	15–25°C
Buffer P3 (neutralization buffer)	3.0 M potassium acetate, pH 5.5	15–25°C or 2–8°C
Buffer FWB2 (QIAfilter wash buffer)	1 M potassium acetate pH 5.0	15–25°C
Buffer QBT (equilibration buffer)	750 mM NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol (v/v); 0.15% Triton® X-100 (v/v)	15–25°C
Buffer QC (wash buffer)	1.0 M NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol (v/v)	15–25°C
Buffer QF (elution buffer)	1.25 M NaCl; 50 mM Tris-Cl, pH 8.5; 15% isopropanol (v/v)	15–25°C
Buffer QN (elution buffer)	1.6 M NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol (v/v)	15–25°C
TE	10 mM Tris-Cl, pH 8.0; 1 mM EDTA	15–25°C
STE	100 mM NaCl; 10 mM Tris-Cl, pH 8.0; 1 mM EDTA	15–25°C

Preparation of buffers

Buffer compositions are given per liter of solution. Do not autoclave MOPS- or isopropanol-containing buffers; sterilize by filtration instead.

Buffer calculations are based on Tris base adjusted to pH with HCl (Tris-Cl). If using Tris-Cl reagent, the quantities used should be recalculated.

- P1: Dissolve 6.06 g Tris base, 3.72 g $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$ in 800 ml distilled water. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with distilled water. Add 100 mg RNase A per liter of P1.
- P2: Dissolve 8.0 g NaOH pellets in 950 ml distilled water, 50 ml 20% SDS (w/v) solution. The final volume should be 1 liter.
- P3: Dissolve 294.5 g potassium acetate in 500 ml distilled water. Adjust the pH to 5.5 with glacial acetic acid (~110 ml). Adjust the volume to 1 liter with distilled water.
- FWB2: Dissolve 98.2 g potassium acetate in 500 ml distilled water. Adjust the pH to 5.0 with glacial acetic acid (~36 ml). Adjust the volume to 1 liter with distilled water.
- QBT: Dissolve 43.83 g NaCl, 10.46 g MOPS (free acid) in 800 ml distilled water. Adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol and 15 ml 10% Triton X-100 solution (v/v). Adjust the volume to 1 liter with distilled water.
- QC: Dissolve 58.44 g NaCl and 10.46 g MOPS (free acid) in 800 ml distilled water. Adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with distilled water.
- QF: Dissolve 73.05 g NaCl and 6.06 g Tris base in 800 ml distilled water and adjust the pH to 8.5 with HCl. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with distilled water.
- QN: Dissolve 93.50 g NaCl and 10.46 g MOPS (free acid) in 800 ml distilled water and adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with distilled water.
- STE: Dissolve 5.84 g NaCl, 1.21 g Tris base, and 0.37 g $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$ in 800 ml distilled water. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with distilled water.

Note: Always recheck pH of buffers after preparation.

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.

Ordering Information

Product	Contents	Cat. no.
HiSpeed Plasmid Kits		
HiSpeed Plasmid Midi Kit (25)	25 HiSpeed Midi Tips, 25 QIAfilter Midi Cartridges, 25 QIAprecipitator Midi Modules, plus Syringes, Reagents, Buffers	12643
HiSpeed Plasmid Maxi Kit (10)	10 HiSpeed Maxi Tips, 10 QIAfilter Maxi Cartridges, 10 QIAprecipitator Maxi Modules, plus Syringes, Reagents, Buffers	12662
HiSpeed Plasmid Maxi Kit (25)	25 HiSpeed Maxi Tips, 25 QIAfilter Maxi Cartridges, 25 QIAprecipitator Maxi Modules, plus Syringes, Reagents, Buffers	12663
QIAGEN Plasmid Kits		
QIAGEN Plasmid Midi Kit (25)	25 QIAGEN-tip 100, Reagents, Buffers	12143
QIAGEN Plasmid Midi Kit (100)	100 QIAGEN-tip 100, Reagents, Buffers	12145
QIAGEN Plasmid Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, Buffers	12162
QIAGEN Plasmid Maxi Kit (25)	25 QIAGEN-tip 500, Reagents, Buffers	12163
QIAGEN Plasmid Maxi Kit (100)	100 QIAGEN-tip 500, Reagents, Buffers	12165
QIAGEN Plasmid Mega Kit (5)	5 QIAGEN-tip 2500, Reagents, Buffers	12181
QIAGEN Plasmid Mega Kit (25)	25 QIAGEN-tip 2500, Reagents, Buffers	12183
QIAGEN Plasmid Giga Kit (5)	5 QIAGEN-tip 10000, Reagents, Buffers	12191
QIAfilter Plasmid Kits		
QIAfilter Plasmid Midi Kit (25)	25 QIAGEN-tip 100, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12243
QIAfilter Plasmid Midi Kit (100)	100 QIAGEN-tip 100, Reagents, Buffers, 100 QIAfilter Midi Cartridges	12245

Ordering Information

Product	Contents	Cat. no.
QIAfilter Plasmid Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, Buffers, 10 QIAfilter Maxi Cartridges	12262
QIAfilter Plasmid Maxi Kit (25)	25 QIAGEN-tip 500, Reagents, Buffers, 25 QIAfilter Maxi Cartridges	12263
QIAfilter Plasmid Mega Kit (5)	5 QIAGEN-tip 2500, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12281
QIAfilter Plasmid Giga Kit (5)	5 QIAGEN-tip 10000, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12291
EndoFree Plasmid Kits		
EndoFree Plasmid Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, 10 QIAfilter Maxi Cartridges, Endotoxin-free Buffers	12362
EndoFree Plasmid Mega Kit (5)	5 QIAGEN-tip 2500, Reagents, 5 QIAfilter Mega-Giga Cartridges, Endotoxin-free Buffers	12381
EndoFree Plasmid Giga Kit (5)	5 QIAGEN-tip 10000, Reagents, 5 QIAfilter Mega-Giga Cartridges, Endotoxin-free Buffers	12391
QIAprep Spin Kits		
QIAprep Spin Miniprep Kit (50)	50 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27104
QIAprep Spin Miniprep Kit (250)	250 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27106
CompactPrep Plasmid Kits*		
CompactPrep Plasmid Midi Kit (25)*	25 CompactPrep Midi Columns, Extender Tubes, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12743
CompactPrep Plasmid Midi Kit (100)*	100 CompactPrep Midi Columns, Extender Tubes, Reagents, Buffers, 100 QIAfilter Midi Cartridges	12745
CompactPrep Plasmid Maxi Kit (25)*	25 CompactPrep Maxi Columns, Extender Tubes, Reagents, Buffers, 25 QIAfilter Maxi Cartridges	12763

* CompactPrep Kits require use of a vacuum device for operation (e.g., QIAvac 24 Plus, cat. no. 19413).

Ordering Information

Product	Contents	Cat. no.
CompactPrep Plasmid Maxi Kit (100)*	100 CompactPrep Maxi Columns, Extender tubes, Reagents, Buffers, 100 QIAfilter Maxi Cartridges	12765
CompactPrep Plasmid Midi Core Kit (100)*	100 CompactPrep Midi Columns, Extender tubes, Reagents, Buffers	12845
CompactPrep Plasmid Maxi Core Kit (100)*	100 CompactPrep Maxi Columns, Extender tubes, Reagents, Buffers	12865
Large-Construct Kit		
QIAGEN Large-Construct Kit	10 QIAGEN-tip 500, ATP-Dependent Exonuclease [†] , Reagents, Buffers	12462
Transfection reagents		
PolyFect Transfection Reagent (1 ml)	For 25–65 transfections in 60 mm dishes or 50–100 transfections in 6-well plates	301105
Effectene Transfection Reagent (1 ml)	For 40 transfections in 60 mm dishes or 160 transfections in 12-well plates	301425
SuperFect Transfection Reagent (1.2 ml)	For 40 transfections in 60 mm dishes or 160 transfections in 12-well plates	301305
Accessories		
QIArack	1 rack for 12 x QIAGEN-tip 20, 8 x QIAGEN-tip 100, 6 x QIAGEN-tip 500 or 6 x HiSpeed Midi Tips, 4 x QIAGEN-tip 2500 or 4 x HiSpeed Maxi Tips, and 10 QIAfilter Midi or Maxi Cartridges	19015
QIAvac 24 Plus	Vacuum manifold for processing 1–24 spin columns: QIAvac 24 Plus Vacuum manifold, Luer Plugs, Quick Couplings	19413
QIAvac 6S manifold	Vacuum manifold for processing 8-well strips or QIAprecipitators	19503

* CompactPrep Kits require use of a vacuum device for operation (e.g., QIAvac 24 Plus, cat. no. 19413).

[†] ATP solution required for the buffer not provided.

Ordering Information

Product	Contents	Cat. no.
VacConnectors (500)	500 disposable connectors for use with luer connectors	19407
Luer Adapter Set*	For processing 1–24 QIAGEN spin columns on QIAvac 6S: 6 adapters with 4 luer connectors each, 24 plugs	19541
QIAfilter Midi Cartridges (25)	25 QIAfilter Midi Cartridges	19743
QIAfilter Maxi Cartridges (25)	25 QIAfilter Maxi Cartridges	19763
RNase A	2.5 ml (100 mg/ml; 7000 units/ml solution)	19101
Plasmid Buffer Set	Buffers P1, P2, P3, QBT, QC, QF, RNase A; for 100 plasmid mini-, 25 midi-, or 10 maxipreps	19046
EndoFree Plasmid Buffer Set	Buffers P1, P2, P3, QBT, QC, QN, ER, TE, Endotoxin-free H ₂ O, RNase A; for 10 plasmid mega- or 5 gigapreps (endotoxin-free)	19048
Buffer P1	500 ml Resuspension Buffer (RNase A not included)	19051
Buffer P2	500 ml Lysis Buffer	19052
Buffer P3	500 ml Neutralization Buffer	19053
Buffer QBT	1000 ml Equilibration Buffer	19054
Buffer QC	1000 ml Wash Buffer	19055
Buffer QF	1000 ml Elution Buffer	19056

* Compatible only with QIAvac Top Plates containing flip-up lid.

Notes:

Notes:

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