HiSpeed™ Plasmid Purification Handbook

For

HiSpeed Plasmid Midi Kit

HiSpeed Plasmid Maxi Kit

December 2001

QIAGEN Worldwide

QIAGEN Companies

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QIAGEN Distributors

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Contents

Kit Contents

Catalog No.	HiSpeed Plasmid Midi Kit (25) 12643	HiSpeed Plasmid Maxi Kit (10) 12662	HiSpeed Plasmid Maxi Kit (25) 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™ Modules	25 QIAprecipitator Midi Modules	10 QIAprecipitator Maxi Modules	25 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	1x 170 ml	1x 420 ml
Buffer TE	1 x 30 ml	1 x 30 ml	1 x 30 ml
RNase A*	1 x 22 mg	1 x 11 mg	1 x 28 mg
Handbook	1	1	1

** Provided as a 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 2–8°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 inside front cover).

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 Services or your local distributor (see inside front 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 the 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

Buffer QBT, Buffer QC, and Buffer QF

Contains 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

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* *R10: Flammable; R36/38: Irritating to eyes and skin; R42/43: May cause sensitization by inhalation and skin contact; S13: Keep away from food, drink, and animal feedingstuffs; S23: Do not breathe spray; 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, that satisfies all your needs.

QIAGEN also offers PolyFect®, Effectene®, and SuperFect® Transfection Reagents for high-efficency transfection of plasmid DNA. Combined with the high-quality plasmid DNA obtained from HiSpeed or EndoFree® Plasmid Kits, these reagents provide efficient transfection and low cytotoxicity with a broad spectrum of cell types (for ordering information, see page 42).

The QIAGEN Principle

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, page10). 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 10).

** Potassium Dodecyl Sulfate*

Figure 1. *HiSpeed Midi and Maxi Tips.*

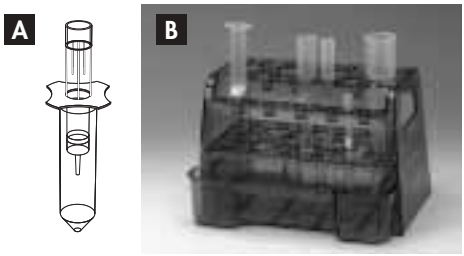
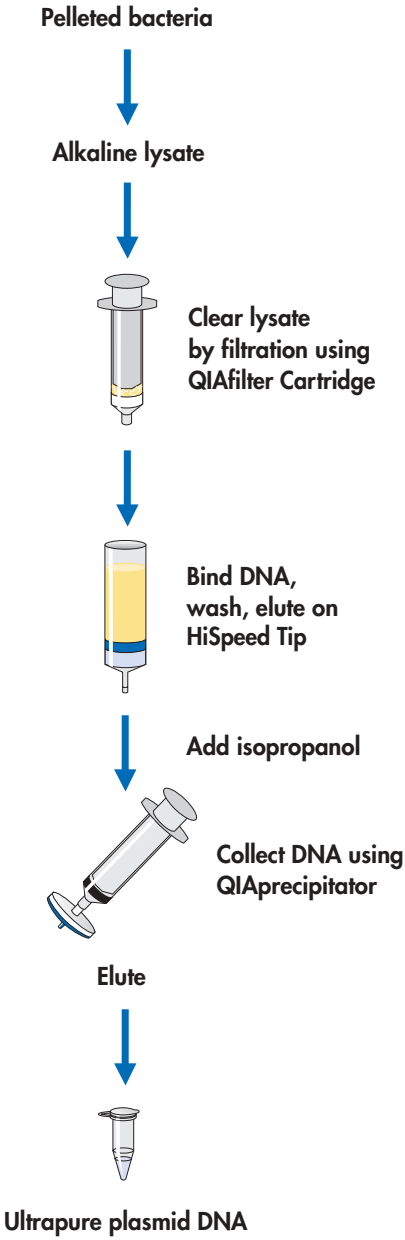


Figure 2. *Setup of HiSpeed Tips* **A** *with tip holder or* **B** *with the QIArack.*

Figure 3. *The syringe format QIAfilter in use.*

Figure 4. *The QIAprecipitator in use.*

HiSpeed Procedure



Brief Considerations for Plasmid/Cosmid Purification Procedures

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 pay particular attention to the "General Considerations for Optimal Results" section on pages 28–35, and be sure to follow the detailed protocol.

Plasmid size

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

Plasmid/cosmid copy number

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 limiting and may lead to lower yields. Furthermore, since the QIAprecipitator requires a minimum volume of 500 µl for elution, the final DNA concentration may be considerably lower than for high-copy plasmids.

Culture media

QIAGEN plasmid purification protocols are optimized for use with cultures grown in standard Luria Bertani medium to a cell density of approximately 3–4 x 10^9 cells/ml, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium (see page 33). 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 1) to obtain the highest plasmid yields.

Rich media are not recommended for plasmid preparation with the HiSpeed Kit. If rich media must be used, growth time must be optimized, and culture volumes reduced (see pages 31–32).

Table 1. Composition of Luria Bertani medium

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

Please refer to Appendix A on page 37 for preparation of LB medium.

Culture volume

Do not exceed the maximum recommended culture volumes given at the beginning of the protocol. Using larger culture volumes can affect the efficiency of alkaline lysis, leading to reduced yield and purity of the preparation.

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 3, page 29), 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 spinning in a centrifuge under vacuum or ethanol precipitation.

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, page 10). 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, page 10).

Analytical gel analysis

The success of the plasmid purification procedure can be monitored on an analytical gel (see Figure 5, page 19). 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 20).

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°C*, after which the protocol can be continued. These stopping points are indicated by the symbol ⊗.

** Longer storage is not recommended.*

Protocol for 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

- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (spin down 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 temperature and, if necessary, dissolve by warming to 37°C.
- Pre-chill Buffer P3 to 4°C.
- **Optional:** remove samples at the steps indicated with the symbol ☞ in order to monitor the procedure on an analytical gel.

Protocol

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 ~8 h at 37°C with vigorous shaking (~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 (~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 3–4 x 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.**

   6000 x *g* corresponds to 6000 rpm in Sorvall GSA or GS3 or Beckman JA-10 rotors. 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. 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 gently but thoroughly by inverting 4–6 times, and incubate at room temperature 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 CO_2 in the air.

   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 but gently by 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.

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 µl or 120 µl 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 µl or 120 µl 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 µl or 240 µl 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 µl or 60 µl 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 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.

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, 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. Do not use excessive force, bending, or twisting to attach the QIAprecipitator! 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 µl 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 and quantitative analysis on an agarose gel.

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 2), and analyze by agarose gel electrophoresis.

Preparation of samples

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

Table 2. Sample volumes required for agarose gel analysis

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

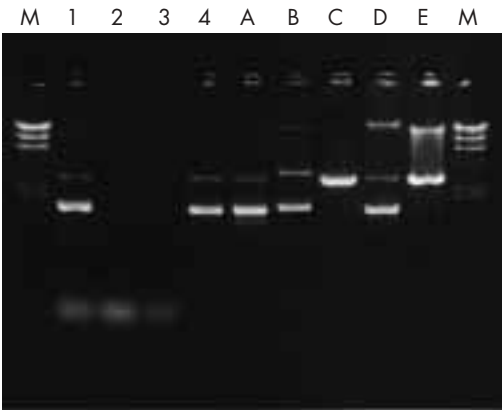


Figure 5. *Agarose gel analysis of the plasmid purification procedure.*

Agarose gel analysis

Run 2 µl of each sample on a 1% agarose gel for analysis of the fractions at each stage of the plasmid purification procedure. Figure 5, page 19 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 page 22. If the problem remains unresolved, or if you have any further questions, please call QIAGEN Technical Services (see inside front cover).

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

2: Flow-through fraction containing only degraded RNA. Plasmid DNA is bound to the QIAGEN Resin (Sample 2).

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

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

M: Lambda DNA digested with *Hin*dIII.

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

A: 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.

B: 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 C).

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

D: Sample contaminated with bacterial genomic 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.

E: *Eco*RI digestion of a sample contaminated with bacterial genomic DNA that gives a smear above the plasmid DNA.

Reliability of DNA Quantification by Spectrophotometry

The concentration of nucleic acids in solution can be readily calculated from the absorbance at 260 nm. Figure 6 shows the standard deviation of A_{260} measurements for serial dilutions of DNA. Although A_{260} values between 0.1 and 1.0 are very reproducible, A_{260} readings <0.1 and >1.0 lead to considerably lower reproducibility. Furthermore, readings above 3.0 are incorrect, which can potentially lead to underestimation of the DNA quantity. Therefore, for reliable spectrophotometric DNA quantification, A_{260} readings should lie between 0.1 and 1.0. When working with small amounts of DNA, quantitation by agarose gel electrophoresis may be more reliable.

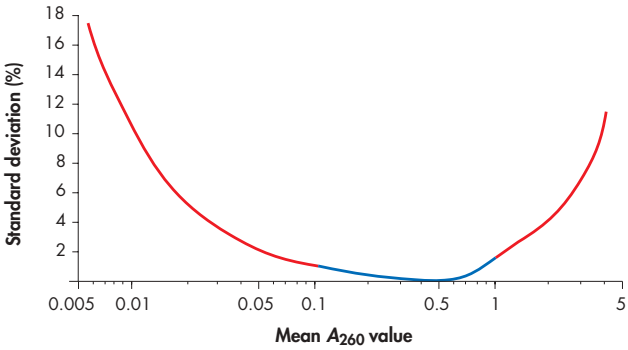


Figure 6. *Reliability of A_{260} readings. A_{260} readings were taken for serial dilutions of plasmid DNA. For each sample the percentage standard deviation was plotted against the mean A_{260} value from 4 measurements. The blue region of the curve represents the range of A_{260} readings that are most reproducible (0.1–1.0). The red regions represent A_{260} readings with high standard deviations.*

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 the QIAGEN-tip, culture volume, and culture media will ensure consistent and optimal results.

The following troubleshooting guide, as well as "General Considerations for Optimal Results" provided on pages 28–35 of this manual, may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol(s) in this handbook or molecular biology applications (see inside front 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" (page 28), 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.
c) Insufficient lysis for low-copy plasmids	For low-copy–plasmid preparations, doubling the volumes of Buffers P1, P2, and P3 may help to increase plasmid yield and quality (see Figure 8, page 32).

	Comments and suggestions
d) Lysate incorrectly prepared	Check Buffer P2 for SDS precipitation resulting from low storage temperatures and, if neccessary, 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 37.
DNA in flow-though 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.
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 37.
d) Column flow was uneven	Store HiSpeed Tips at room temperature. 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.

No DNA in eluate (sample 4)

a) No DNA in the lysate	See page 22.
b) Elution Buffer QF was incorrect	Check pH and salt concentration of Elution 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 must be handled gently 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. 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 increase the volumes of Buffers P1, P2, and P3.
f) Plasmid DNA is nicked/sheared/degraded	DNA was poorly buffered. Redissolve DNA in TE, pH 8.0, to inhibit nuclease activity and maintain stable pH during storage.
g) Endonuclease-containing host	Refer to page 30 of this handbook, and consider changing *E. coli* host strain.

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 using the 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 19). 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 19). 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 HiSpeed Tip

a) 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

	Comments and suggestions
QIAfilter Cartridge 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 P3	Mix well until a fluffy white material has formed and the lysate is no longer viscous.
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 in the QIAfilter Cartridge. Precipitate floatation 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	
a) Precipitate was forced through the QIAfilter Cartridge	Filter until all of the lysate has passed through the QIAfilter Cartridge, but do not apply extreme force. Approximately 15 ml (Midi) or 25 ml (Maxi) of the lysate is typically recovered.

QIAprecipitator Modules

	Comments and suggestions
DNA does not perform well	
a) 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.
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) QIAprecipitator attached with excessive force	Do not apply excessive force, bending, or twisting when attaching the QIAprecipitator to the syringe.
b) 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.

General Considerations for Optimal Results

The HiSpeed plasmid purification procedure is an optimized protocol based on the alkaline lysis method of Birnboim and Doly (2). The procedure has been condensed to three steps and, in combination with purification on QIAGEN Resin, allows selective preparation of ultrapure plasmid DNA without the use of phenol, chloroform, ethidium bromide, or cesium chloride. It can be used for the preparation of plasmid and cosmid DNA.

1. Growth of bacterial cultures

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

Plasmid copy number

Plasmids vary widely in their copy number (see Table 3, page 29). Copy number depends on the origin of replication (pMB1 or pSC101, for example), which determines whether plasmids are under relaxed or stringent control, as well as the size of the plasmid and its associated insert. Some plasmids, such as the pUC series and derivatives, have mutations that allow them to reach very high copy numbers within the bacterial cell. Plasmids based on pBR322 and many cosmids are generally maintained at lower copy numbers. Very large plasmids are often maintained at very low copy numbers per cell.

Note: the copy number of plasmids and cosmids can be substantially influenced by the cloned insert. For example, a high-copy pUC plasmid may behave like a medium- or low-copy plasmid when containing certain inserts (e.g., very large DNA fragments), resulting in lower DNA yields than expected.

Table 3. 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.*

Cosmid copy number

HiSpeed Plasmid Kits are also suitable for purification of cosmid DNA. Due to their relatively large size and slow replication time, cosmids are generally present in low or very low copy numbers in bacterial cells (Table 3). Like plasmids, cosmids vary in copy number, depending on the origin of replication they contain, their size, and the size of insert. Cosmids should be treated as low-copy plasmids.

For purification of P1 and BAC DNA, QIAGEN Plasmid Kits or the Large-Construct Kit are recommended. Please contact QIAGEN Technical Services or your local distributor for more information (see inside front cover).

Host strains

Most *E. coli* strains can be used successfully to isolate plasmid DNA, although the strain used to propagate a plasmid can have a substantial influence on the quality of the purified DNA. Host strains such as DH1, DH5α™, and C600 yield high-quality DNA with QIAGEN protocols. The slower growing strain XL1-Blue also yields DNA of very high quality that works extremely well for sequencing. 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 (3). In addition, some strains such as JM101, JM110, and HB101 have high levels of endonuclease activity, and yield DNA of lower quality than that prepared from strains such as XL1-Blue, DH1, DH5α, and C600. The methylation and growth characteristics of the host strain can also affect plasmid isolation. If after performing a QIAGEN plasmid preparation, 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 used for cleared lysate preparation, or using the same amount of culture volume but doubling the volumes of Buffers P1, P2, and P3 in order to improve the ratio of biomass to lysis buffers for optimized lysis conditions.

Inoculation

Bacterial cultures for plasmid preparation should always be grown from a single colony picked from a freshly streaked selective plate. Subculturing directly from glycerol stocks, agar stabs, and liquid cultures is poor microbiological practice and may lead to loss of the plasmid. Inoculation from plates that have been stored for a long time may also lead to loss or mutation of the plasmid.

The desired clone should be streaked from a glycerol stock onto a freshly prepared agar plate containing the appropriate selective agent such that single colonies can be isolated. This procedure should then be repeated to ensure that a single colony of an antibiotic-resistant clone can be picked. A single colony should be inoculated into 2–10 ml LB medium (see page 12) containing the appropriate selective agent and grown for ~8 hours (logarithmic phase). Using a vessel with a volume of at least four times greater than the volume of medium, the starter culture should then be diluted 1/500 to 1/1000 into a larger volume of selective medium, and grown with vigorous shaking (~300 rpm) to saturation (12–16 hours). It is often convenient to grow the starter culture during the day and the larger culture overnight for harvesting the following morning.

Antibiotics

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

The stability of the selective agent should also be taken into account. Resistance to ampicillin, for example, is mediated by β-lactamase which is encoded by the plasmid-linked *bla* gene and which hydrolyzes ampicillin. Levels of ampicillin in the culture medium are thus continually depleted. This phenomenon is clearly demonstrated on ampicillin plates, where "satellite colonies" appear as the ampicillin is hydrolyzed in the vicinity of a growing colony. It is important to inoculate cultures from freshly prepared plates to ensure that the antibiotic is effective. Ampicillin is also very sensitive to temperature, and should be stored frozen in single-use aliquots. Table 4 gives the concentrations of commonly used antibiotics.

Table 4. Concentrations of commonly used antibiotics

Antibiotic	Stock solutions		Working concentration
	Concentration	Storage	(dilution)
Ampicillin (sodium salt)	50 mg/ml in H_2O	–20°C	100 µg/ml (1/500)
Chloramphenicol	34 mg/ml in ethanol	–20°C	170 µg/ml (1/200)
Kanamycin	10 mg/ml in H_2O	–20°C	50 µg/ml (1/200)
Streptomycin	10 mg/ml in H_2O	–20°C	50 µg/ml (1/200)
Tetracycline HCl	5 mg/ml in ethanol	–20°C	50 µg/ml (1/100)

Culture media

The protocol for HiSpeed Midi and Maxi Kits is optimized for use with cultures grown in standard Luria Bertani (LB) medium (see page 12), grown to a cell density of approximately 3–4 x 10^9 cells per ml (see below). We advise harvesting cultures after approximately 12–16 hours of growth, which typically is the transition from logarithmic into stationary growth phase. At this time, the ratio of plasmid DNA to RNA is higher than during the logarithmic phase. Also, the DNA is not yet degraded due to overaging of the culture, as in the later stationary phase. Please note the maximum recommended culture volumes given at the beginning of the protocol.

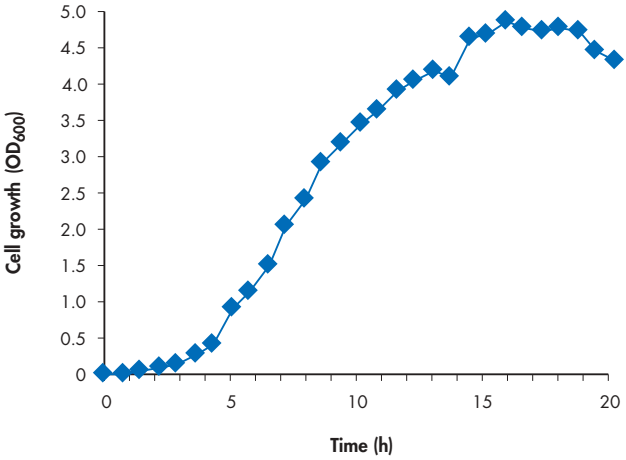


Figure 7. *Growth curve of* E. coli *in LB medium. Host strain: DH5α; plasmid: pUC21. High OD_{600} readings were calculated by diluting the sample to enable photometric measurement in the linear range between 0.1–0.5 OD_{600}.*

Several bacterial strains can grow to very high cell densities. It is best to assess the cell density of the culture, and reduce the culture volumes accordingly or increase the volumes of Buffers P1, P2, and P3 if necessary. A high ratio of biomass to lysis buffers will result in poor lysis conditions and subsequently low DNA yield and purity.

If working with low-copy vectors, it may be beneficial to increase the lysis buffer volumes in order to increase the efficiency of alkaline lysis, and thereby the DNA yield (Figure 8). In case additional Buffers P1, P2, and P3 are needed, their compositions are provided on page 36. Alternatively, the buffers may be purchased separately (see page 43).

It is not recommended to use super-rich growth media such as TB (terrific broth) or 2x YT for most commonly used high-copy plasmids. Although TB or 2x YT have the obvious advantage of producing more bacteria (2–5 times), this does not necessarily lead to greater yields or higher-quality DNA.

If rich media must be used, the culture volume should be reduced to match the recommended cell biomass, which in turn should correspond to the capacity of the HiSpeed Tip used. If the culture volume used is too high, alkaline lysis will be inefficient, resulting in lower yield than expected. Furthermore, the excessive viscosity of the lysate will require vigorous mixing, resulting in shearing of bacterial genomic DNA and subsequent contamination of the plasmid DNA.

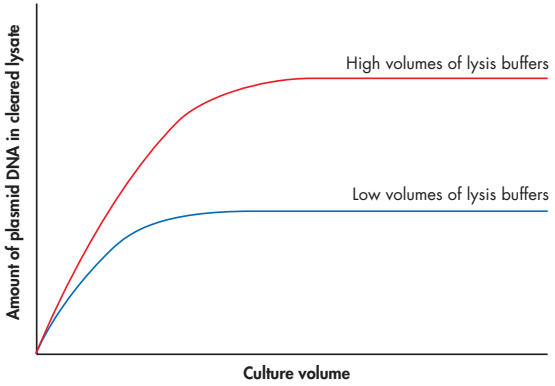


Figure 8. *The effect of lysis buffer volumes on the amount of DNA in the cleared lysate.*

Measuring cell density

Photometric measurements of cell density can vary between different spectrophotometers. The optical density reading of a bacterial culture is a measure of the light scattering, which varies depending on the distance between the sample and the detector.

Calibration of each individual spectrophotometer is required to facilitate accurate conversion of OD_{600} 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 OD_{600} values.

Pellet wet weight

If spectrophotometric measurement of the cell density or calibration of the photometer is not possible, another way of estimating the amount of cell harvest is by assessment of the pellet wet weight. Typically, a 1 liter, overnight shaker-culture of *E. coli* with a cell density of 3–4 x 10^9/ml corresponds to a pellet wet weight of approximately 3 g/liter.

Chloramphenicol amplification

The copy numbers of the current generation of plasmids are so high that selective amplification in the presence of chloramphenicol is not necessary to achieve high yields. However, when low-copy-number plasmids containing the pMB1 or ColE1 origin of replication are prepared, the yield can be improved by adding chloramphenicol (170 mg/liter) to amplify the copy number (1). Cultures of bacteria containing low-copy-number plasmids amplified in the presence of chloramphenicol should be treated as if they contain high-copy–number plasmids when choosing the appropriate culture volumes for the HiSpeed Tip to be used.

2. Key steps in the plasmid purification protocol

After lysis of bacteria under alkaline conditions, the lysate is applied under defined salt conditions to the HiSpeed Tip. Plasmid DNA is selectively bound and purified from RNA, proteins, and other cellular contaminants.

Preparation of the cell lysate

DNA yield depends on the quality of the cell lysate used. Preparation of a cleared cell lysate is therefore a critical step in the QIAGEN purification procedure, which has been carefully designed to provide ideal lysis conditions.

After harvesting and resuspension, the bacterial cells are lysed in NaOH–SDS (Buffer P2) in the presence of RNase A (2, 4). SDS solubilizes the phospholipid and protein components of the cell membrane, leading to lysis and release of the cell contents. NaOH denatures the chromosomal and plasmid DNAs, as well as proteins. The optimized lysis time allows maximum release of plasmid DNA from the cell without release of cell-wall–bound chromosomal DNA, while minimizing the exposure of the plasmid to denaturing conditions. Long exposure to alkaline conditions may cause the plasmid to become irreversibly denatured. This denatured form of the plasmid runs faster on agarose gels and is resistant to restriction enzyme digestion (see Figure 5, page 19).

The lysate is neutralized by the addition of acidic potassium acetate (Buffer P3). The high salt concentration causes KDS* to precipitate, and the denatured proteins, genomic DNA, and cellular debris become trapped in salt–detergent complexes. Plasmid DNA, being smaller and covalently closed, renatures correctly and remains in solution. Since any SDS remaining in the lysate will inhibit binding of DNA to QIAGEN Resin HS, the solution must be thoroughly but gently mixed to ensure complete precipitation of the detergent.

Separation of plasmid from genomic DNA is based on coprecipitation of the cell-wall–bound genomic DNA with the insoluble complexes containing salt, detergent, and protein. Plasmid DNA remains in the clear supernatant. Vigorous treatment during the lysis procedure will shear the bacterial chromosome, leaving free genomic DNA fragments in the supernatant. Since these fragments are chemically indistinguishable from plasmid DNA under the conditions used, the two species will not be separated on QIAGEN Resin and will elute under the same salt conditions.

RNase A, which is added at the beginning of the procedure, digests the liberated RNA efficiently during the alkaline lysis. The resulting RNA fragments do not bind to QIAGEN Resin under the salt and pH conditions present in the lysate.

The precipitated debris is removed by use of a QIAfilter Cartridge, producing a cleared lysate for loading onto the HiSpeed Tip. It is important that the lysate is clear at this stage to ensure good flow rates and, ultimately, to obtain protein-free plasmid DNA preparations.

** Potassium Dodecyl Sulfate*

Clearing of bacterial lysates using QIAfilter Cartridges

QIAfilter Cartridges are special filtration units designed to replace the centrifugation step after alkaline lysis of bacterial cells. After cultures are pelleted, bacterial cells are lysed in NaOH–SDS, neutralized by the addition of acidic potassium acetate, and incubated directly in the QIAfilter Cartridge. The lysate is cleared in a matter of seconds by passing the liquid through the filter. Insoluble complexes containing chromosomal DNA, salt, detergent, and proteins, which form during the neutralization step are completely removed. QIAfilter Cartridges clear bacterial lysates more efficiently than conventional centrifugation. In addition, small-sized KDS precipitates which cannot be separated by centrifugation are completely removed by the QIAfilter process.

DNA binding and washing on the HiSpeed Tip

The cleared lysate is loaded onto a pre-equilibrated HiSpeed Tip by gravity flow. The salt and pH conditions of the lysate and the superior selectivity of the QIAGEN Resin HS ensure that only plasmid DNA binds, while degraded RNA, cellular proteins, and metabolites are not retained and appear in the flow-through fraction.

The HiSpeed Tip is then washed with medium-salt buffer (Buffer QC) which completely removes any remaining contaminants, such as traces of RNA and protein (e.g., RNase A), without affecting the binding of the plasmid DNA (see Figure 10, page 39). Buffer QC also disrupts nonspecific interactions, and allows removal of nucleic acid-binding proteins without the use of phenol. The low concentration of alcohol in the wash buffer eliminates nonspecific hydrophobic interactions, further enhancing the purity of the bound DNA. The plasmid DNA is then efficiently eluted from the HiSpeed Tip with high-salt buffer (Buffer QF). For further information about QIAGEN Anion-Exchange Resin, see Appendix B, pages 38–40.

Desalting and concentration

The eluted plasmid DNA is mixed with isopropanol and applied to the QIAprecipitator Module using the syringe provided in the kit. The module traps the precipitated DNA while the isopropanol mixture flows through. An additional ethanol wash step is recommended, to maximize DNA purity. The precipitated DNA is trapped in the QIAprecipitator as a thin layer, which allows thorough drying and removal of ethanol by simply pushing air through the QIAprecipitator with a syringe. The DNA is then eluted from the QIAprecipitator into a microcentrifuge tube with Buffer TE provided in the kit. Alternatively, any common buffer or water can be used. DNA should be stored at –20°C when eluted with water, as DNA may degrade in the absence of a buffering and a chelating agent.

The DNA is ready for use in transfection, sequencing, labeling, cloning, or any other experimental procedure.

Appendix A

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)	Room temp.
Buffer P3 (Neutralization Buffer)	3.0 M potassium acetate, pH 5.5	Room temp. or 2–8°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)	Room temp.
Buffer QC (Wash Buffer)	1.0 M NaCl; 50 mM MOPS, pH 7.0;15% isopropanol (v/v)	Room temp.
Buffer QF (Elution Buffer)	1.25 M NaCl; 50 mM Tris·Cl, pH 8.5;15% isopropanol (v/v)	Room temp.
Buffer TE	10 mM Tris·Cl, pH 8.0; 1 mM EDTA	Room temp.

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-HCl reagent, the quantities used should be recalculated.

P1: Dissolve 6.06 g Tris base, 3.72 g $Na_2EDTA{\cdot}2H_2O$ in 800 ml dH_2O. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with dH_2O. Add 100 mg RNase A per liter of P1.

P2: Dissolve 8.0 g NaOH pellets in 950 ml dH_2O, 50 ml 20% SDS solution. The final volume should be 1 liter.

P3: Dissolve 294.5 g potassium acetate in 500 ml dH_2O. Adjust the pH to 5.5 with glacial acetic acid (~110 ml). Adjust the volume to 1 liter with dH_2O.

QBT: Dissolve 43.83 g NaCl, 10.46 g MOPS (free acid) in 800 ml dH_2O. Adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol and 15 ml 10% Triton X-100 solution. Adjust the volume to 1 liter with dH_2O.

QC: Dissolve 58.44 g NaCl and 10.46 g MOPS (free acid) in 800 ml dH_2O. Adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with dH_2O.

QF: Dissolve 73.05 g NaCl and 6.06 g Tris base in 800 ml dH_2O and adjust the pH to 8.5 with HCl. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with dH_2O.

Preparation of LB medium

Dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 800 ml dH_2O. Adjust the pH to 7.0 with 1 M NaOH. Adjust the volume to 1 liter with dH_2O. Sterilize by autoclaving.

Appendix B

General information about QIAGEN Anion-Exchange Resin and Resin HS

QIAGEN-tips and HiSpeed Tips contain unique, patented anion-exchange resins which eliminate the need for expensive equipment and reagents such as ultracentrifuges, HPLC/FPLC®, or CsCl. Toxic and mutagenic substances such as phenol, chloroform, and ethidium bromide are also not required.

Plasmid purification on QIAGEN Resins is based on the interaction between negatively charged phosphates of the DNA backbone and positively charged DEAE groups on the surface of the resins (Figure 9). The salt concentration and pH conditions of the buffers used determine whether DNA is bound or eluted from the column. The key advantage of QIAGEN Anion-Exchange Resins arises from their exceptionally high charge density. The resins consist of silica beads with a defined particle size, a large pore size, and a hydrophilic surface coating. The large surface area allows dense coupling of the DEAE groups. Plasmid DNA remains tightly bound to the DEAE groups over a wide range of salt concentrations (Figure 10). Impurities such as RNA, proteins, carbohydrates, and small metabolites are washed from QIAGEN Resins with medium-salt buffers, while plasmid DNA remains bound until eluted with a high-salt buffer. The separation range of QIAGEN Resins is extremely broad, extending from 0.1 M to 1.6 M salt (Figure 10), and DNA can be efficiently separated from RNA and other impurities. In contrast, conventional anion-exchangers, based on cellulose, dextran, or agarose, have separation ranges only up to 0.4 M salt, so that binding and elution of all substances is limited to a narrow range of salt concentrations. This means that the elution peaks of proteins, RNA, and DNA overlap extensively with one another, and a satisfactory separation cannot be achieved. Thus, the separation and purification qualities of QIAGEN Resins as well as their ease of use, surpass those of conventional anion-exchange resins.

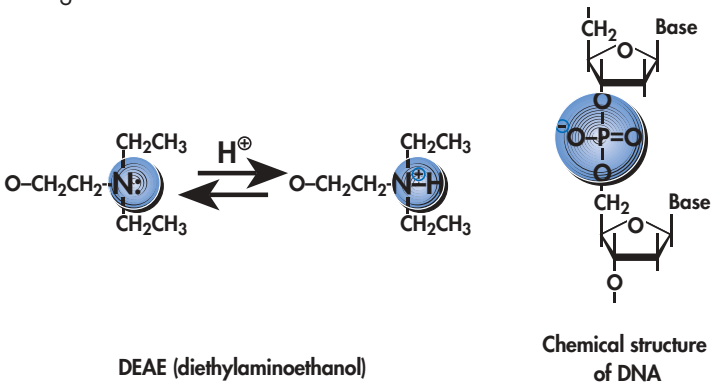


Figure 9. *Chemical structure of positively charged DEAE groups of QIAGEN Resins, and negatively charged groups of the DNA backbone which interact with the resins.*

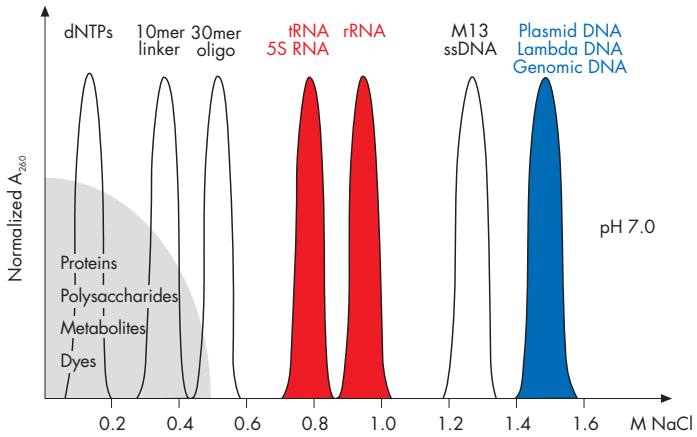


Figure 10. *Separation of nucleic acids at neutral pH on QIAGEN Anion-Exchange Resin.*

Purity and biological activity

Nucleic acids prepared on QIAGEN Resin are of equivalent or superior purity to nucleic acids prepared by two rounds of purification on CsCl gradients. DNA prepared using QIAGEN-tips and HiSpeed Tips has been thoroughly tested. Subsequent procedures such as transfection, transformation, sequencing, cloning, and in vitro transcription proceed with optimal efficiency.

Capacity and recovery

The HiSpeed Tip has a binding capacity of 200 µg (Midi) or 750 µg (Maxi) plasmid DNA. Large nucleic acids such as cosmids are bound at a slightly lower capacity than plasmid DNA. This relationship between the binding capacity of the QIAGEN Resin and the size of the nucleic acids being prepared must be taken into account when calculating expected yields.

Buffers

The binding, washing, and elution conditions for QIAGEN Resin are strongly influenced by pH. Figure 11, page 40 shows the influence of pH on the salt concentration required for elution of various types of nucleic acids. Deviations from the appropriate pH values of the buffers at a given salt concentration may result in losses of the desired nucleic acid.

Buffers, such as MOPS, sodium phosphate, Tris·Cl, and sodium acetate can be used at the indicated pH. MOPS (3-[N-morpholino]propanesulfonic acid, pK_a 7.2) is frequently the buffer of choice in QIAGEN protocols, since it has a higher buffering capacity at pH 7.0 than sodium phosphate, Tris·Cl, or sodium acetate buffers.

SDS and other anionic detergents interfere with the binding of nucleic acids to QIAGEN Resin by competing for binding to the anion-exchange groups. If SDS is used during sample preparation, it must be removed through steps such as potassium acetate precipitation or alcohol precipitation prior to column application. SDS removal steps are incorporated into the QIAGEN protocols described in this manual.

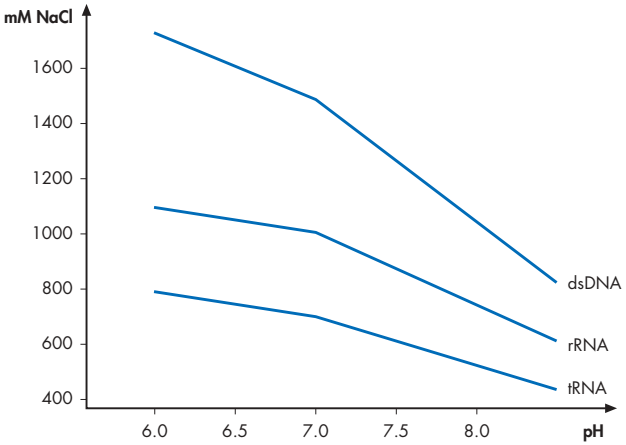


Figure 11. *Elution points of different nucleic acids from QIAGEN Resin as a function of pH and NaCl concentration.*

References

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3. *Ausubel, F.M. et al., eds. (1991)* Current protocols in molecular biology, *New York, Wiley Interscience.*
4. *Birnboim, H.C. (1983) A rapid alkaline extraction method for the isolation of plasmid DNA. Methods Enzymol.* ***100****, 243–255.*

Ordering Information

Product	Contents	Cat. No.
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
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 in12-well plates	301425
SuperFect Transfection Reagent (1.2 ml)	For 40 transfections in 60 mm dishes or 160 transfections in 12-well plates	301305

**ATP solution required for the buffer not provided.*

Ordering Information

Product	Contents	Cat. No.
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 manifold	Vacuum manifold for processing spin columns or QIAprecipitators	19403
QIAVac 6S manifold	Vacuum manifold for processing 8-well strips or QIAprecipitators	19503
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_2O, 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.*

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