March 2012

HiSpeed® Plasmid Mega/Giga EndoFree® Purification Handbook

HiSpeed Plasmid Mega and Giga EF Kits

QIAvac HiSpeed LS

For preparation of advanced transfection-grade plasmid DNA

QIAGEN®

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QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
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Kit Contents

HiSpeed Plasmid EF Kit Catalog no.	Mega (5) Inquire	Giga (5) Inquire
QIAfilter Mega-Giga Cartridges*	5	5
HiSpeed Tips	5 HiSpeed Mega-Tips	5 HiSpeed Giga-Tips
QIAconcentrator	5 QIAconcentrator Mega Modules	5 QIAconcentrator Giga Modules
250 ml Collection Vessels	5	5
50 ml Collection Tubes	10	10
Connection Adapters	6	6
Tube Extenders	5	5
Buffer P1	1 x 280 ml	1 x 700 ml
Buffer P2	1 x 280 ml	1 x 700 ml
Buffer P3	1 x 280 ml	1 x 700 ml
Buffer FWB2	2 x 140 ml	2 x 140 ml
Buffer ER	1 x 80 ml	1 x 200 ml
Buffer QBT	1 x 200 ml	1 x 400 ml
Buffer QC	1 x 1000 ml	2 x 1000 ml
Buffer QN	1 x 200 ml	1 x 510 ml
Buffer TE	1 x 110 ml	1 x 110 ml
Endotoxin-Free Water	1 x 17 ml	1 x 17 ml
RNase A (100 mg/ml)	1 x 28 mg	1 x 70 mg
LyseBlue®	1 x 280 µl	1 x 700 µl
Quick-Start Protocol Card	1	1

* QIAfilter Mega-Giga Cartridges are designed for use with a 1 liter, 45 mm-neck glass bottle (e.g., Schott, cat. no. 21810154; or Corning, cat. no. 1395-1L). **Note**: Bottle is not included.

QIAvac HiSpeed LS Catalog no.	(1) 19505
Basic Carrier with front and park positions	1
Column Holder	1
Elution Rack	1
VacValve Tool	1
Blind Plugs	5
Quick-Start Protocol Card	1

Storage

QIAGEN-tips and QIAfilter Cartridges should be stored dry and at room temperature (15–25°C). They can be stored for at least 2 years without showing any reduction in performance, capacity, or quality of separation.

HiSpeed Plasmid Mega/Giga EF Kits should be stored at room temperature (15–25°C). After adding RNase A, Buffer P1 should be stored at 2–8°C and is stable for 6 months. Other buffers and RNase A stock solution can be stored for 2 years at room temperature (15–25°C).

The QIAvac HiSpeed LS should be stored dry and clean at room temperature (15–25°C).

Intended Use

HiSpeed Plasmid Mega/Giga EF Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

The QIAvac HiSpeed LS is designed for vacuum processing of QIAGEN-tips in parallel.

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

Always use caution and wear safety glasses when working near a vacuum manifold under pressure.

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/Support/MSDS.aspx 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 Plasmid Mega/Giga EF 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, and QN

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

Buffer ER

Contains isopropanol, polyethylene glycol octylphenyl ether: flammable, irritant. Risk and safety phrases:* R10-41, S13-26-36-46

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 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.

Quality Control

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

Introduction

The QIAvac HiSpeed LS is designed for fast and efficient vacuum processing of up to 6 HiSpeed Mega/Giga-tips in parallel. Product and wash solutions are drawn through the tip resin by vacuum instead of gravity flow, providing greater speed and reduced hands-on time in purification procedures. The HiSpeed Mega-Tip, or HiSpeed Giga-Tip, is connected with a Connection Adapter to the QIAvac HiSpeed LS manifold.

HiSpeed Plasmid Mega/Giga EF Kits are based on the remarkable selectivity of patented QIAGEN® resin, allowing purification of ultrapure supercoiled plasmid DNA with high yields.

Anion-exchange–based QIAGEN-tips yield transfection-grade DNA that is highly suited for use in a broad variety of demanding applications such as transfection, plasmid-mediated gene silencing, and is also suitable for gene therapy research. QIAGEN offers the most comprehensive portfolio of tailored plasmid purification kits for any scale, throughput, or downstream application. 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 Effectene® transfection reagent. This reagent, combined with the high-quality plasmid DNA obtained from QIAGEN's EndoFree Plasmid Kits, HiSpeed Plasmid Kits, and QIAGEN Plasmid *Plus* Kits, provides optimal transfection results.

Principle and procedure

QIAGEN's HiSpeed plasmid purification protocols are based on a modified alkaline lysis procedure followed by vacuum-driven purification of plasmid DNA using the 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 and concentrated with QIAconcentrator Mega/Giga Modules. HiSpeed Plasmid Mega/Giga EF Kits allow very fast large-scale plasmid preparations using a specially developed vacuum system without time consuming gravity flow procedures or centrifugation procedures following isopropanol precipitation.

The QIAconcentrator revolutionizes the isopropanol precipitation step, making it fast, easy, and risk-free. Plasmid DNA eluted from the HiSpeed Mega/Giga-tip is mixed with isopropanol and applied to the QIAconcentrator using the QIAvac HiSpeed LS provided. The precipitated DNA is trapped in the QIAconcentrator as a thin layer that allows thorough drying and removal of alcohol by centrifugation. The DNA is then simply eluted from the QIAconcentrator with, for example, Buffer TE into a fresh tube also by centrifugation.

HiSpeed Mega/Giga-tips are highly suitable for rapid and easy preparation of multiple samples, while QIAfilter cartridges provided in HiSpeed Plasmid Mega/Giga EF Kits enable quick and efficient clearing of bacterial lysates without centrifugation. QIAfilter Mega-Giga Cartridges are special filter units that operate with any vacuum source to clear bacterial lysates from up to 2.5 liters of bacterial culture. QIAfilter Mega-Giga Cartridges completely remove SDS precipitates and ensure 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 Mega/Giga-tip.

QIAGEN HiSpeed Plasmid Mega/Giga EF Kits allow very fast large-scale plasmid preparations using the vacuum system without time consuming gravity-flow procedures or centrifugation, pellet drying, and resuspension procedures after isopropanol precipitation. The highly concentrated DNA is ready for immediate use.

LyseBlue

LyseBlue is an optional color indicator that provides visual identification of optimum buffer mixing. This prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. This makes LyseBlue highly suitable for use by researchers who are inexperienced with plasmid preparation, 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 to 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 according to the usual protocol. 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), 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.

Determination of yield

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

Agarose gel analysis

We recommend removing and saving an aliquot of the cleared lysate. If the plasmid DNA is of low yield or poor quality, the lysate sample and an aliquot of eluate can be analyzed by agarose gel electrophoresis to determine the stage of the purification procedure where the problem occurred.

Assembling the QIAvac HiSpeed LS

Components

The QIAvac HiSpeed LS is comprised of three components: the Basic Carrier (see Figure 1), the Elution Rack (see Figure 1), and the Column Holder (see Figure 2).

Figure 1. Basic Carrier with Elution Rack in parking position.

Figure 2. Column Holder.

Assembly of the QIAvac HiSpeed LS

1. **Position the Basic Carrier on a flat, planar surface. Place the Column Holder onto the Basic Carrier and fix it with the Snap-on Connection by rotating clockwise (see Figure 3).**

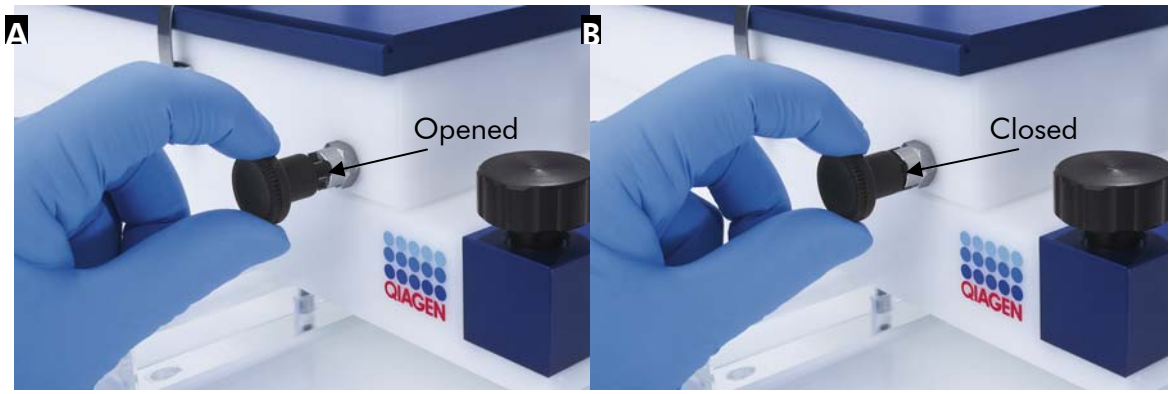

Figure 3. Fixing the Column Holder to the Basic Carrier. A Opened Snap-on Connection (left side). B Closed Snap-on Connection (right side).

2. **Press slightly onto the Column Holder to make sure that the connection is fixed. Place the Elution Rack in the parking position at the back of the Basic Carrier.**

Figure 4. Assembled QIAvac HiSpeed LS.

3. **Connect the QIAvac HiSpeed LS with a suitable waste container and vacuum source to collect the flow-through from the HiSpeed Plasmid Mega/Giga purification.**

   For connection, use suitable tubing that fits onto the tubing connection on the left side of the Basic Carrier. The outlet on the left side of the Basic Carrier is connected directly with the waste container. The vacuum source is separately connected to the waste container. See recommendations regarding the waste system in Appendix A.

   Note: Make sure that liquid cannot be sucked into the vacuum pump.

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.

- 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)
- Vacuum pump (e.g., Vacuum Pump, cat. no. 84020)
- 1 liter 45 mm-neck vacuum-resistant glass bottle (e.g., Schott, cat. no. 2181054)
- A suitable waste container system to be used in combination with the QIAvac HiSpeed LS to collect the waste volumes during purification (see Appendix A)
- Centrifuge with swing out rotor for 50 ml collection tubes
- 96–100% ethanol
- Isopropanol

Protocol: Plasmid DNA Purification from Bacterial Cells Using HiSpeed Mega/Giga EF Kits and the QIAvac HiSpeed LS

This protocol is designed for the preparation of up to 2.5 mg high-copy plasmid DNA using the HiSpeed Plasmid Mega EF Kit with a maximum culture volume (i.e., LB medium) of 500 ml, or up to 10 mg plasmid DNA using the HiSpeed Plasmid Giga EF Kit with a maximum culture volume (i.e., LB medium) of 2500 ml. It is not recommended to increase the culture volume given in the HiSpeed Plasmid Giga EF protocol.

Note: The HiSpeed Plasmid Giga EF Kit is not recommended for low-copy plasmids or cosmids.

QIAfilter Mega-Giga Cartridges are used instead of conventional centrifugation to clear bacterial lysates. 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.

Important points before starting

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.

- When working with chemicals always wear a suitable lab coat, disposable gloves and protective goggles.
- Wear protective goggles when working with the system under vacuum.
- Use endotoxin-free or pyrogen-free plastic pipet tips and tubes for elution and subsequent steps. Endotoxin-free or pyrogen-free plasticware can be obtained from many common vendors. Please check with your current supplier to obtain recommendations. Alternatively, glass tubes may be used if they are baked overnight at 180°C to destroy endotoxins.
- The QIAfilter Mega-Giga Cartridge operates with any vacuum source (e.g., a house vacuum, vacuum pump, or water aspirator) that generates vacuum between –200 and –600 millibars (mbar) (–150 and –450 mm Hg). The vacuum pressure is measured as differential pressure between the inside of the bottle and the atmosphere (1013 mbar or 760 mm Hg). Vacuum recommendations are given in negative units to indicate the required reduction in pressure with respect to the atmosphere.
- To avoid the possibility of implosion, do not use plastic/glass bottles, or any other vessels, that are not designed for use with a vacuum. Do not use

plastic/glass bottles or any other vessels that are cracked or scratched. Wear safety glasses when working near a bottle under vacuum.

- Regularly check the liquid level of the waste container and empty the container before it is completely filled.
- Centrifugation: the drying and centrifugation should be performed at 4500 x *g* to 5000 x *g* in a centrifuge with a swing out rotor.
- The QIAvac HiSpeed LS manifolds are not resistant to ethanol, methanol, or other organic solvents. Do not bring solvents into contact with QIAvac HiSpeed LS. Do not use cleaning materials that contain abrasives.
- 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.
- To prepare endotoxin-free 70% ethanol, add 40 ml of 96–100% ethanol to the endotoxin-free water supplied with the kit.
- Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.
- Close the bottle containing Buffer P2 immediately after use to avoid acidification of Buffer P2 from CO_2 in the air.
- Pre-chill Buffer P3 at 4°C.
- **Important: Make sure that the HiSpeed Mega/Giga-tips do not run dry during purification. This will lead to significantly decreased flow rates until the air is flushed out of the resin.**
- **Optional**: Add the provided LyseBlue reagent to Buffer P1 and mix before use.
- ▲ denotes values for the HiSpeed Plasmid Mega EF Kit; ● denotes values for the HiSpeed Plasmid Giga EF Kit.

Table 1. Maximum recommended culture volumes

Plasmids	HiSpeed Mega-Tip	HiSpeed Giga-Tip
High-copy plasmids*	500 ml LB culture 1.5 g pellet wet weight†	2.5 liters LB culture 7.5 g pellet wet weight†

* For high-copy plasmids, expected yields are 1.5–2.5 mg for the HiSpeed Plasmid Mega EF Kit and 7.5–10 mg for the HiSpeed Plasmid Giga EF Kit.

† On average, a healthy 1 liter shaker culture yields a pellet with a wet weight of approximately 3 g. When working with fermentation cultures, however, the pellet wet weight may be significantly higher. Therefore, when using fermentation cultures please refer to the pellet wet weight instead of the recommended culture volumes.

Bacterial culture and preparation of cleared lysates

1. **Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 5–10 ml LB medium containing the appropriate selective antibiotic. Incubate for approximately 8 hours at 37°C with vigorous shaking (300 rpm).**

   Use a 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 ▲ 500 ml or ● 2.5 liters medium with ▲ 500–1000 µl or ● 2.5–5 ml of starter culture. Grow at 37°C for 12–16 hours 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/ml. This typically corresponds to a pellet wet weight of approximately 3 g per liter of medium.

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

   To stop the protocol and continue later, freeze the cell pellets at –20°C.

4. **Screw the QIAfilter Mega-Giga Cartridge onto a 45 mm-neck glass bottle and connect it to a vacuum source.**

   Do not overtighten the QIAfilter Mega-Giga Cartridge on the bottle neck, because the QIAfilter cartridge plastic may crack.

5. **Resuspend the bacterial pellet in ▲ 50 ml or ●125 ml of Buffer P1.**

   For efficient lysis it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. We recommend a 500 ml bottle for mega preps and a 1000 ml bottle for giga preps. Ensure that the 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.

6. **Add ▲ 50 ml or ● 125 ml of Buffer P2, mix thoroughly by vigorously inverting 4–6 times, and incubate at room temperature for 5 minutes.**

   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 minutes. After use, the bottle containing Buffer P2 should be closed immediately to avoid acidification of Buffer P2 from CO_2 in the air. If LyseBlue has been added to Buffer P1, the cell suspension will turn blue after addition of Buffer P2.

7. **Add ▲ 50 ml or ● 125 ml chilled Buffer P3 and mix thoroughly by vigorously inverting 4–6 times. Mix well until white, fluffy material has formed and the lysate is no longer viscous.**

   Precipitation is enhanced by using chilled Buffer P3. After addition of Buffer P3, a fluffy, white precipitate containing genomic DNA, proteins, cell debris, and SDS becomes visible. The lysate should be mixed well to reduce the viscosity and prevent clogging of the QIAfilter cartridge. If LyseBlue reagent has been used, the suspension should be mixed until there are no traces of blue color and the suspension is colorless.

8. **Pour the lysate into the QIAfilter Mega-Giga Cartridge and incubate at room temperature for 10 minutes.**

   Important: This 10-minute incubation at room temperature is essential for optimal performance of the QIAfilter Mega-Giga 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.

9. **During the incubation period, prepare the assembled QIAvac HiSpeed LS (see "Assembling the QIAvac HiSpeed LS" on page 11, and Figure 4) for the purification procedure. Open the lid of the Column Holder by releasing both Cramps (see Figure 5 and Figure 6).**

Figure 5. Cramp that is released to open Column Holder.

Figure 6. Opened Column Holder.

10. **Exchange the Blind Plugs with the number of Connection Adapters required for the purification.**

    Any positions that are not needed are closed by Blind Plugs.

Figure 7. Connection Adapter showing VacValve inserted as upper part.

11. **First place the clear part of a Connection Adapter into empty plug positions.**

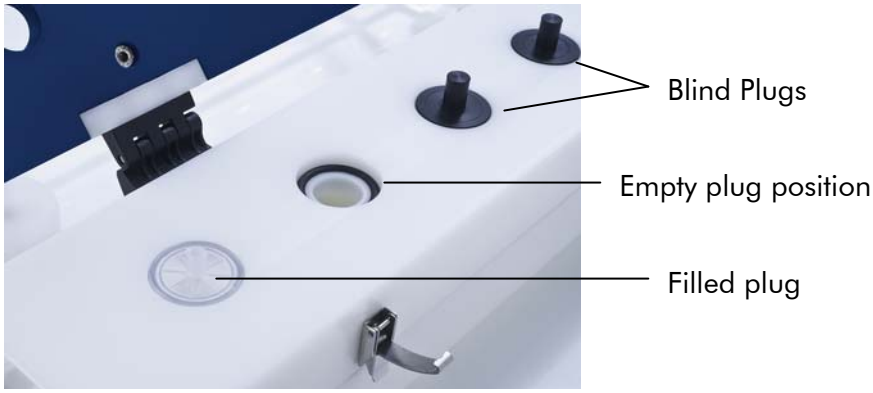

Figure 8. Opened Column Holder showing inside and plug position filled with lower, clear part of Connection Adapter or Blind Plugs.

12. **Close the lid of the Column Holder by fixing the Cramps. After the lid is closed, plug the VacValves into the Connection Adapters (see Figure 9).**

Figure 9. Closed Column Holder with closed VacValve inserted into the lower part of the Connection Adapter.

13. **After the 10-minute incubation of the lysate in the QIAfilter Mega-Giga Cartridge, switch on the vacuum source.**
14. **After all liquid has been pulled through into the bottle, switch off the vacuum source. Leave the QIAfilter Cartridge attached to the bottle.**
15. **Add 50 ml (both▲ Mega and ● Giga) Buffer FWB2 to the QIAfilter Mega-Giga Cartridge and gently stir the precipitate using a sterile spatula.**
16. **Switch on the vacuum source until the liquid has been pulled through completely.**

    Take care not to disperse the precipitate. Dispersal may result in carryover of cell debris and SDS that will affect flow and binding characteristics of the HiSpeed Mega/Giga-tip.

    Note: The filtered lysate in the bottle contains the plasmid DNA.
17. **Discard the QIAfilter Mega-Giga Cartridge.**

Loading cleared lysate

1. **Add ▲12.5 ml or ● 30 ml Buffer ER to the filtered lysate. Mix by inverting the bottle approximately 10 times.**

   After addition of Buffer ER the lysate appears turbid.
2. **Insert the required number of HiSpeed Mega-tips or Giga-tips into the luer slots of the VacValves on the inlet side of the Connection Adapters (see Figure 10).**

Figure 10. HiSpeed Giga-tip inserted into the luer slot of the VacValve.

3. **Make sure that the VacValves are closed before equilibration.**

Figure 11. Close VacValves with the use of the supplied VacValve Tool.

4. **Start the vacuum pump and adjust the vacuum to –100 mbar with the vacuum regulator.**
5. **Equilibrate a ▲ HiSpeed Mega-tip by applying ▲ 35 ml Buffer QBT or a ● HiSpeed Giga-tip by applying ● 75 ml Buffer QBT.**
6. **When the vacuum is stable, open the VacValves by turning the tap in a vertical position.**

   Note: Opening of the VacValves has to be done in a stepwise fashion as Buffer QBT flows through in less than a minute, and it is important to avoid air being drawn into the resin.
7. **Close the VacValve when the QBT level nearly reaches the upper frit of the tip.**

   Important: Make sure that the HiSpeed Mega/Giga-tips do not run dry during equilibration.
8. **Apply the cleared lysate to the ▲ HiSpeed Mega-tip or ● HiSpeed Giga-tip.**

Note: Before loading of the cleared lysate, make sure that the VacValves are closed.

9. **Open the VacValve and draw the lysate through the tip resin by vacuum.**

   Before opening the VacValve, adjust the vacuum up to –300 mbar using the vacuum regulator.

10. **Refill the cleared lysate when the level is near the upper frit until the total volume of lysate has been loaded into the Hi Speed Mega/Giga-tip (refilling is not necessary in mega scale).**

    Note: Make sure that the HiSpeed Mega/Giga-tips do not run dry during product loading.

11. **After complete loading of the cleared lysate, and shortly before the liquid level reaches the upper frit, close the VacValve by turning the tap to a horizontal position.**

Washing of HiSpeed Mega/Giga-tip

1. **Wash the ▲ HiSpeed Mega-tip with 150 ml Buffer QC or ● HiSpeed Giga-tip with 300 ml Buffer QC.**

   Note: Before loading of Buffer QC, make sure that the VacValves are closed.

2. **Open the VacValve and draw Buffer QC through the HiSpeed Mega/Giga-tip resin by vacuum.**

   Before opening the VacValve, adjust the vacuum up to –500 mbar using the vacuum regulator.

3. **Refill with Buffer QC until the total volume of 300ml Buffer QC has been loaded into the HiSpeed Mega/Giga-tip (refilling is not necessary in mega scale).**

   Important: Make sure that the HiSpeed Mega/Giga-tips do not run dry during the wash step.

4. **After complete loading of Buffer QC, and shortly before the liquid level reaches the upper frit, close the VacValve by turning the tap to a horizontal position.**
5. **Switch off the vacuum pump and release the vacuum through the vacuum regulator.**

Elution of plasmid DNA from HiSpeed Mega/Giga-tip

1. **To rebuild the QIAvac HiSpeed LS for elution mode, insert the required number of collection vessels into the corresponding holder of the Elution Rack (see Figure 12).**

Figure 12. Collection vessels inserted into the corresponding holders of the Elution Rack in parking position.

2. **Open the Snap-on Connection between the Basic Carrier and Column Holder and place the Column Holder onto the Elution Rack. Fix both Snap-on Connections between Column Holder and Elution Rack (see Figure 13).**

Figure 13. Column Holder placed onto the Elution Rack in parking position.

3. **Remove the connected Column Holder and Elution Rack from the parking position and place them onto the front position of the Basic Carrier. Connect the Basic Carrier to the Elution Rack with both Snap-on Connections (see Figure 14).**

Figure 14. Connected Column Holder and Elution Rack placed onto the front position of the Basic Carrier.

4. **Elute plasmid DNA from the HiSpeed Mega/Giga-tips with ▲ 35 ml or ● 100 ml Buffer QN.**

   Note: Before loading of Buffer QN, make sure that the VacValves are closed.

5. **Start the vacuum pump and adjust the vacuum to –200 mbar with the vacuum regulator. When the vacuum is stable, open the VacValves by turning the tap in a vertical position.**

   Note: At this step, it is not necessary to close the valve when Buffer QN level reaches the resin.

6. **When air bubbles can be seen coming out of the outlet of the HiSpeed Mega/Giga-tips, close the valve by turning the tap in a horizontal position.**
7. **After complete elution of plasmid DNA from all HiSpeed Mega/Giga-tips, switch off the vacuum pump and release the vacuum through the vacuum regulator.**
8. **Open the Snap-on Connection between the Basic Carrier and the Elution Rack. Place the Elution Rack with the still-connected Column Holder into the parking position.**
9. **Open the Snap-on Connection between the Column Holder and the Elution Rack then place the Column Holder onto the front position of the Basic Carrier.**
10. **Connect both components with the Snap-on Connections (see Figure 15).**

Figure 15. Column Holder re-placed onto the Elution Rack in parking position.

11. Take out collection vessels for further processing (see Figure 16).

Figure 16. QIAvac HiSpeed LS with collection vessels removed.

Precipitation and concentration of plasmid DNA

1. **Precipitate plasmid DNA by adding ▲ 28 ml or ● 77 ml room-temperature isopropanol to the eluted plasmid DNA.**

   Close the collection vessels and mix the solutions carefully.

2. **To bind plasmid DNA to the QIAconcentrator Mega/Giga Module, disconnect the HiSpeed Mega/Giga-tips from the blue VacValves and discard the tips.**
3. **Remove the upper Cross-Beam from the Column Holder (see Figure 17).**

Figure 17. Removing the upper Cross-Beam from the Column Holder.

4. **Attach the Tube Extender to the QIAconcentrator by plugging them together (see Figure 18).**

Figure 18. QIAconcentrator attached to Tube Extender

5. **Connect the outlet of the QIAconcentrator-Tube Extender assembly with the inlet of the blue VacValve (see Figure 19).**

   Note: Before commencing the binding step, ensure that the VacValves are closed.

Figure 19. Set up of QIAconcentrator-Tube Extender assembly in the QIAvac HiSpeed LS.

6. **Start the vacuum pump and adjust the vacuum to –200 mbar.**
7. **When the vacuum is stable, transfer the eluate/isopropanol mixture from the elution vessel into the empty Tube Extender.**
8. **Open the VacValves by turning the tap in a vertical position.**

   The solution is drawn through the QIAconcentrator by the vacuum.
9. **Close the valve by turning the tap in a horizontal position after the solution is completely drawn through the QIAconcentrator.**
10. **To wash the DNA, add 10 ml 70% ethanol to the QIAconcentrator.**

    Note: Before commencing the washing step, ensure that the VacValves are closed.

11. **Start the vacuum pump and adjust the vacuum to –200 mbar with the vacuum regulator on the vacuum system. When the vacuum is stable, open the VacValves by turning the tap in a vertical position.**
12. **To dry plasmid DNA and elute it from a QIAconcentrator Mega/Giga Module, disconnect the Tube Extender from the QIAconcentrator and discard it.**
13. **Remove and discard the Connection Adapter with VacValves. Insert the Blind Plugs.**
14. **Transfer the QIAconcentrator into the supplied 50 ml collection tube (see Figure 20). Centrifuge at 4500–5000 x *g* for 5 minutes at room temperature to dry the membrane.**

Figure 20. Transfer of the QIAconcentrator into the supplied 50 ml collection tube.

15. **Place the QIAconcentrator in a new 50 ml collection tube. To elute the DNA, add ▲ 1 ml Elution Buffer (e.g. endotoxin-free Buffer TE) or ● 5 ml Elution Buffer into the QIAconcentrator, close the tube, and let it stand for at least 1 minute. After incubation, centrifuge at 4500–5000 x *g* for 3 minutes at room temperature.**

    To increase the yield, repeat the elution step with ▲ **0.5 ml** Elution Buffer or ● **3 ml** Elution Buffer. After at least 1 minute of incubation, repeat the centrifugation at 4500–5000 x *g* for 3 minutes at room temperature.

    To increase the yield and achieve as high a DNA concentration as possible, the second elution can be performed with the eluate from the first elution step. Add the complete volume of eluate into the QIAconcentrator, incubate for at least 1 minute, and repeat the centrifugation at 4500–5000 x *g* for 3 minutes at room temperature.

    Endotoxin-free water or endotoxin-free buffers commonly used to dissolve DNA may also be used for elution.

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

Note: When DNA is eluted with water, store at –20°C as DNA may degrade in the absence of buffering and chelating agents.

16. **Replace the removed Cross-Beam back onto the Column Holder (see Figure 21).**

Figure 21. Replacing the upper Cross-Beam onto the Column Holder.

Note: For cleaning of the QIAvac HiSpeed LS, see Appendix C. For handling and maintenance of the QIAvac HiSpeed LS, see Appendix D.

Troubleshooting Guide

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

	Comments and suggestions
Low, or no DNA yield	
a) Plasmid did not propagate	Please read "Growth of bacterial cultures" on our Web page at 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 freeing the plasmid DNA 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 9 and background on our Web page at www.qiagen.com/goto/plasmidinfo).
d) Lysate incorrectly prepared	Check Buffer P2 for SDS precipitation resulting from low prepared storage temperatures and dissolve the SDS by warming. The bottle containing Buffer P2 should always be closed immediately after use.

	Comments and suggestions
No liquid flow	
a) Insufficient vacuum	Check recommendations given under troubleshooting section “Low, or no vacuum”.
b) VacValves are closed	Check that VacValves are in a vertical position.
Low, or no vacuum	
a) Column Holder not placed properly onto the Basic Carrier	Press slightly on the Column Holder, until a soft click is heard.
b) VacValves are closed	Open the VacValves by turning the blue handles using the provided VacValve Tool.
c) General vacuum problems	Make sure that the vacuum source is running. If yes, check that the vacuum regulator is closed.
	Check that all components are correctly assembled.
	Check that all tubings to the waste container are correctly connected and the lid of the waste container is tightened.
	Check that all gaskets of the QIAvac HiSpeed LS are present and in the right position.
	Check that all positions of the Column Holder are either closed by Blind Plugs or the Connection Adapters are assembled.
	Check that all VacValves are closed.
	Check that the vacuum regulator is closed.
QIAfilter Mega-Giga 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 P3	Mix well until a fluffy white material has formed and the lysate is no longer viscous. Use LyseBlue to visualize efficiency of mixing
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 that may clog the QIAfilter Mega-Giga Cartridge.

	Comments and suggestions
d) QIAfilter Mega-Giga Cartridge was not loaded immediately after addition of buffer P3	After addition of Buffer P3, the lysate should be poured into the QIAfilter Mega-Giga Cartridge immediately. Decanting after incubation may disrupt the precipitate into tiny particles that may clog the QIAfilter Mega-Giga Cartridge.
e) QIAfilter Mega-Giga Cartridge was agitated during incubation	Pour the lysate into the QIAfilter Mega-Giga Cartridge immediately after addition of Buffer P3 and do not agitate during the 10 minute incubation. Agitation causes the precipitate to be disrupted into tiny particles, instead of forming a layer.
f) 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 minute incubation, carefully run a sterile pipet tip around the cartridge wall to dislodge the precipitate before continuing with the filtration.
g) Vacuum pressure was to weak	Ensure that the vacuum generates a vacuum pressure of –200 to –600 millibar (–150 to –450 mmHg).
h) HiSpeed Mega/Giga-tip was overloaded	Check the culture volume and yield against the capacity of the HiSpeed Mega/Giga-tip, as detailed at the beginning of the protocol. Reduce the culture volume accordingly, or select a larger QIAGEN-tip if a higher yield is desired. For very low-copy plasmid and cosmid preps requiring very large culture volumes, please see www.qiagen.com/goto/plasmidinfo.
i) SDS (or other ionic detergent) was in lysate	Chill Buffer P3 before use. If the lysate is cleared by centrifugation, load onto HiSpeed Mega/Giga-tip promptly after lysate 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.

	Comments and suggestions
j) Column flow was uneven	Store HiSpeed Mega/Giga-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.
No DNA in eluate	
a) No DNA in the lysate	Check recommendations given under troubleshooting section "Low, or no DNA yield".
b) Elution buffer was incorrect	Check pH and salt concentration of Buffer QN. Recover DNA by eluting with fresh buffer.
c) DNA passed through in the flow through or wash fractions	Check recommendations given under troubleshooting sections "Low, or no DNA yield" or "Low, or no vacuum".
Handling problems during vacuum-based purification	
a) Flow rates decline during purification with HiSpeed Mega/Giga-tips	Valves were closed too late at previous step and resin in column had run dry. Purification can be continued with this column but total purification time will be prolonged. Flow rates increase again when air is flushed out of the resin. Close valves on time when liquid level reaches the surface of the upper frit.
b) Flow rates decline during concentration on QIAconcentrator	QIAconcentrator is blocked due to high amounts of DNA. Increase vacuum strength to increase flow rates.
c) Vacuum strength declines during concentration on QIAconcentrator	Close connected valves of all QIAconcentrator units where liquid has been completely drawn through.
No, or less, DNA in eluate from QIAconcentrator	
a) Eluate from HiSpeed Mega/Giga-tip and isopropanol were not sufficiently mixed	Mix both solutions by carefully inverting the collection vessel.

	Comments and suggestions
b) Elution of QIAconcentrator was not sufficient	Repeat the elution with fresh elution buffer, or with the eluate from first elution step to achieve a high DNA concentration. Several elution steps will increase the yield.

Appendix A: Waste Container Requirements

Depending on the number and scale of plasmid purifications, different waste volumes will be generated. Data in Table 2 may help to determine an appropriate waste container. Up to 6 HiSpeed Mega/Giga-tips can be processed in parallel on the QIAvac HiSpeed LS.

Table 2. Waste volumes

Step	HiSpeed Plasmid Mega Kit		HiSpeed Plasmid Giga Kit	
	1 prep	**5 preps**	**1 prep**	**5 preps**
Equilibration	35 ml	175 ml	75 ml	375 ml
Lysis	135 ml	675 ml	330 ml	1650 ml
Wash	150 ml	750 ml	300 ml	1500 ml
Isopropanol precipitation wash	65 ml	325 ml	185 ml	925 ml
Ethanol wash	10 ml	60 ml	10 ml	60 ml
Total	**395 ml**	**1985 ml**	**900 ml**	**4510 ml**

Table 3. Volume capacities

Setup	Assembly	Volume capacity
A	QIAvac HiSpeed LS + 2 x 5 liter containers	5 liter
B	QIAvac HiSpeed LS + 10 liter container	8 liter
C	QIAvac HiSpeed LS + 20 liter container	18 liter

To protect the vacuum source, it is recommended to use a waste system of two containers. The container connected directly to the Basic Carrier will be filled with liquid waste, while the other container is an overflow trap for vacuum source protection.

Setup A can also be used with a 10 liter container for liquid waste collection and a 5 liter container for an overflow trap.

Ordering information for waste system accessories

The following components may be used:

- 5 liter waste container (e.g., Nalgene Heavy Duty Vacuum Bottle [PP], cat. no. 2126-5000)
- 10 liter waste container (e.g., Nalgene Heavy Duty Vacuum Carboy [PP], cat. no. 2226-0020)
- 20 liter waste container (e.g., Nalgene Heavy Duty Vacuum Carboy [PP], cat. no. 2226-0050)
- Quick filling/venting closure (e.g., Nalgene, cat. no. 2158-0021)
- Vacuum tubing (e.g., Nalgene 180 clear plastic vacuum tubing, cat. no. 8000-0065 [50 ft. per case])
- Vacuum tubing (e.g., Nalgene 180 clear plastic vacuum tubing, cat. no. 8000-1065 [10 ft. per case])

Appendix B: Vacuum Regulator

The Vacuum Regulator measures the pressure difference between the inside and outside of a vacuum system in millibars. See Table 4 for conversion from millibars to other units of pressure. Use of the Vacuum Regulator makes it easy to monitor the pressure generated by the vacuum source, ensuring that it is sufficient for the appropriate QIAGEN purification chemistry.

Figure 22. Vacuum Regulator.

Table 4. Conversion from millibars

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

Appendix C: Cleaning of QIAvac HiSpeed LS

The QIAvac HiSpeed LS should be cleaned regularly to maintain optimum performance. The QIAvac HiSpeed LS must also be decontaminated before removal from the laboratory.

- Do not use cleaning materials that contain abrasives.
- The acrylic parts of the QIAvac HiSpeed LS are not resistant to ethanol, methanol or other organic solvents. Do not bring solvents into contact with the acrylic parts. If solvents are spilled on the unit, rinse thoroughly with distilled water. Do not incubate acrylic components in alcohol-containing reagents. The acrylic parts of the QIAvac HiSpeed LS should be cleaned with water or laboratory detergent after use. Ethanol should not be used.
- The white plastic parts of the QIAvac HiSpeed LS should also be cleaned with standard laboratory detergents. Disinfection of the base unit where the liquid waste is collected and removed should be done with standard laboratory disinfectant.
- Avoid bringing acrylic parts of the QIAvac HiSpeed LS in contact with disinfectant.

Appendix D: Handling and Storage of the QIAvac HiSpeed LS

These guidelines should be followed when working with the QIAvac HiSpeed LS.

- Always use caution and wear safety glasses when working near a vacuum manifold under pressure.
- Always place the QIAvac HiSpeed LS on a secure bench top or work area. If dropped, the QIAvac HiSpeed LS manifold may crack.
- Always store the QIAvac HiSpeed LS clean and dry.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
HiSpeed Plasmid Mega EF Kit (5)	For 5 preps: 5 HiSpeed Mega-Tips, QIAfilter Mega/Giga Cartridges, 5 QIAconcentrators, Tube Extenders, Collection Vessels, Connection Adapters, Buffers	Inquire
HiSpeed Plasmid Giga EF Kit (5)	For 5 preps: 5 HiSpeed Giga-Tips, QIAfilter Mega/Giga Cartridges, 5 QIAconcentrators, Tube Extenders, Collection Vessels, Connection Adapters, Buffers	Inquire
Accessories		
QIAvac HiSpeed LS	Vacuum device for parallel processing of up to 6 HiSpeed Mega or Giga Columns	19505
Vacuum Pump (230V [50Hz])	Universal vacuum pump	84020
Spare parts		
VacValve Tool (1)		Inquire
Blind Plug (1)		Inquire
Lock Bolt (1)		Inquire
Snap-on Connector (1)		Inquire
Cramp (1)		Inquire
Gasket Kit (1)		Inquire
Gauge (1)		Inquire
Tube clip (1)		Inquire

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Notes

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