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DirectPrep® 96 Miniprep Handbook

For rapid, high-throughput purification of plasmid DNA from high-copy vectors

Sample to Insight

QIAGEN

Contents

Kit Contents 3

Storage 4

Intended Use 4

Safety Information 4

Quality Control 5

Introduction 5

Principle and procedure 5

Equipment and Reagents to Be Supplied by User 7

Important Notes 7

Recommendations for culture media 7

Assembly of the vacuum manifold 8

Protocol: Growth of Cultures in a 96-Well Block 10

Protocol: Purification of Plasmid DNA using the DirectPrep 96 Miniprep Kit 11

Troubleshooting Guide 15

Ordering Information 19

Kit Contents

DirectPrep 96 Miniprep Kit	**(4)**
Catalog no.	**27361**
Number of preps	**4 x 96**
DirectPrep 96 Plates	4
Buffer P1	2 x 50 ml
Buffer P2	4 x 20 ml
Buffer DP3	70 ml
Buffer PE (concentrate)	3 x 55 ml
Buffer EB	2 x 55 ml
RNase A*	2 x 5 mg
S-Blocks	4
AirPore Tape Sheets	4
Tape Pads 1	1
Elution Microtubes RS	4 x 96
Caps for Strips	55 x 8
Quick-Start Protocol	1

* Provided as a 10 mg/ml solution.

Storage

All kit components, buffers, and RNase A stock solution can be stored at room temperature (15–25°C). Under these conditions, if no expiration date is mentioned on the kit label, kits can be stored for up to 6 months without showing any reduction in performance and quality. After addition of RNase A, Buffer P1 is stable for 6 months when stored at 2–8°C. RNase A stock solution can be stored for two years at room temperature.

Intended Use

The DirectPrep 96 Miniprep Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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

Safety Information

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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the DirectPrep 96 Miniprep Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The DirectPrep 96 method of plasmid DNA isolation uses a novel lysis chemistry that eliminates the need for a lysate-clearing step. The entire vacuum procedure is performed using one 96-well plate. This results in a protocol that is faster and easier to perform than most existing methods.

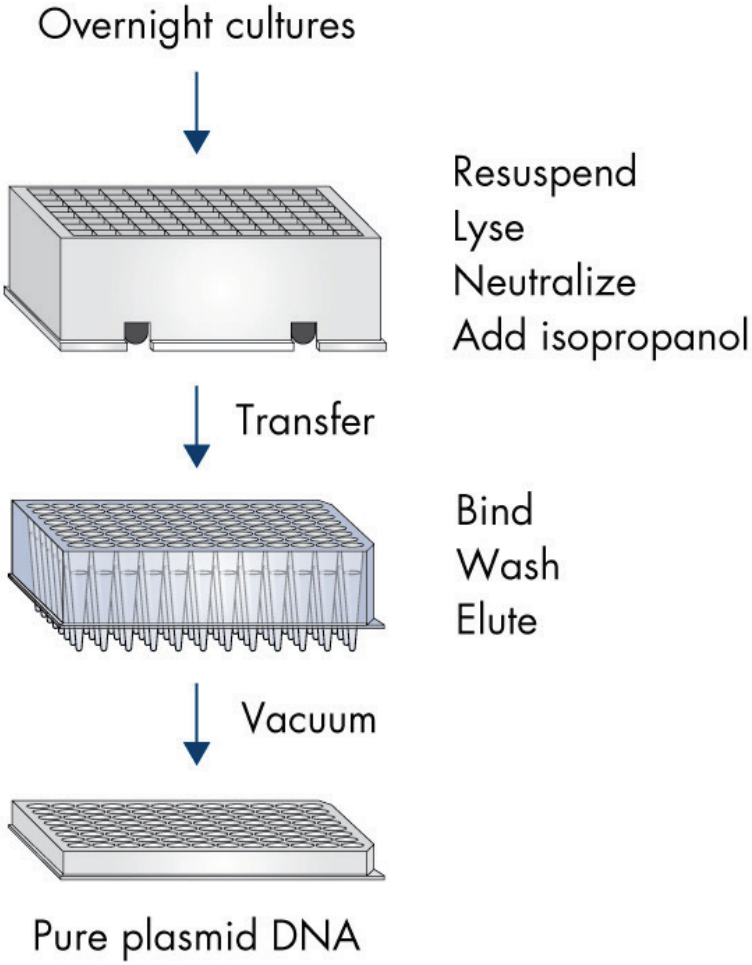
Principle and procedure

Pelleted bacterial cells are resuspended and then lysed. The lysate is neutralized using an optimized buffer, which leads to only a small amount of precipitated cellular components. Centrifugation or filtration of the lysate is not required. Isopropanol is added to optimize DNA-binding conditions on the DirectPrep 96 Plate. Plasmid DNA binds to the activated membrane, which is subsequently washed with an ethanol-containing buffer and then dried. Plasmid DNA is eluted using the elution buffer provided in the kit.

The DNA obtained is highly suited for automated fluorescent sequencing and other routine applications, such as restriction digestion and PCR.

Please note that the DirectPrep 96 procedure is suitable for use with high-copy plasmids only.

DirectPrep 96 Procedure



Equipment and Reagents to Be Supplied by User

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

- Luria Bertani (LB) media
- Centrifuge with a rotor for microplates (e.g., Centrifuge 4–16S or Centrifuge 4–16KS and Plate rotor 2 x 96; see Ordering Information, page 19)
- QIAvac Multiwell
- Ethanol
- Isopropanol

Important Notes

Recommendations for culture media

Luria Bertani broth is the recommended culture medium for use with the DirectPrep 96 Kit, because richer broths such as TB (Terrific Broth) or 2x YT lead to extremely high cell densities, which can overload the purification system or clog the DirectPrep 96 Plate membrane. It should be noted that cultures grown in rich media may yield 2–5 times the number of cells compared to cultures grown in LB. Therefore, it is not recommended to use these media in the DirectPrep 96 System. If these media are used, recommended culture volumes must be reduced to match the capacity of the DirectPrep 96 Plate membrane. When using LB medium, 1.25 ml is the optimal culture volume.

Please note that a number of slightly different LB culture broths, containing different concentrations of NaCl, are in common use. Although different LB broths produce similar cell densities after overnight culture, plasmid yields can vary significantly. We recommend

growing cultures in an LB medium containing 10 g NaCl, 10 g tryptone and 5 g yeast extract per liter to obtain the highest plasmid yields with the DirectPrep 96 System.

Assembly of the vacuum manifold

To assemble the QIAvac Multiwell vacuum manifold, begin by putting the waste tray on top of the base. Next, place the top plate on top of the base, thus completely enclosing the waste tray. Finally, put the DirectPrep 96 Plate onto the top plate. Ensure that the DirectPrep 96 Plate is positioned securely. Figure 1 illustrates assembly of the QIAvac Multiwell vacuum manifold.

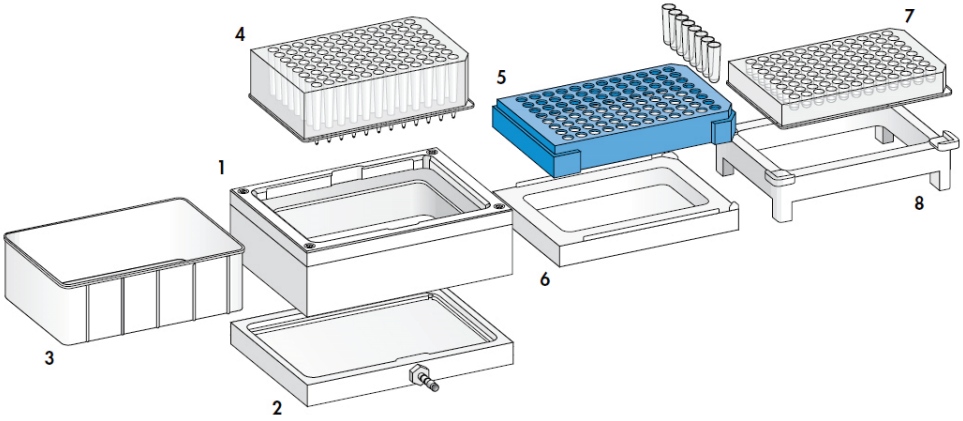


Figure 1. Components of the QIAvac Multiwell vacuum manifold.

1. QIAvac Multiwell top plate
2. QIAvac Multiwell base, which holds the waste tray and the elution microtube adapter or microplate adapter
3. Waste tray
4. DirectPrep 96 Plate
5. Elution microtube rack with elution microtubes
6. Elution microtube adapter
7. Microplate (96-well)
8. Microplate adapter

Working with a vacuum manifold

- Wear safety glasses when working near a manifold under pressure.
- For safety reasons, do not use 96-well plates that have been damaged in any way.
- Switch off the vacuum between steps to ensure that a consistent and even vacuum is applied during manipulations.

It is important to regulate the vacuum (negative pressure) using a vacuum regulator with pressure gauge. For DirectPrep procedures, vacuum pressures should be between –650 and –800 mbar. For elution, vacuum pressure should be between –550 and –650 mbar. The vacuum pressure is the pressure differential between the inside of the manifold and the atmosphere (standard atmospheric pressure: 1013 mbar or 760 mm Hg) and can be measured using a Vacuum Regulator (cat. no. 19530). Vacuum recommendations are given in negative units to indicate the required reduction in pressure with respect to the atmosphere. Table 1 provides pressure conversions to other units.

Table 1. Pressure conversion

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

Protocol: Growth of Cultures in a 96-Well Block

1. Fill each well of a 96-well S-Block with 1.25 ml of LB medium containing the appropriate selective agent. Inoculate each well from a single bacterial colony. Incubate the cultures for 16–24 h at 37°C with shaking at 220–300 rpm.

   The wells in the block may be protected against spilling over by covering the block with an AirPore microporous tape sheet, which promotes gas exchange during culturing. AirPore tape sheets are provided with the DirectPrep 96 Miniprep Kit.

2. Harvest the bacterial cells in the block by centrifugation for 5 min at 1500 x *g* in a centrifuge with a rotor for microplates. The block should be covered with adhesive tape during centrifugation. Remove medium by inverting the block.

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

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

Protocol: Purification of Plasmid DNA using the DirectPrep 96 Miniprep Kit

Things to do before starting

- Add the provided RNase A solution to Buffer P1, mix, and then store at 2–8°C.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- Check Buffer P2 before use for salt precipitation. Redissolve any precipitate by warming to 37°C. Do not shake Buffer P2 vigorously.
- Close the bottle containing Buffer P2 immediately after use to avoid acidification of Buffer P2 from CO_2 in the air.
- Buffer P2 contains irritants. Wear gloves when handling this buffer.
- Assemble the vacuum manifold as described on page 8.

Procedure

1. Add 100 µl Buffer EB to each well of the DirectPrep 96 Plate. Unused wells of the DirectPrep 96 Plate should be sealed with tape. Apply vacuum until buffer has passed through.
2. Resuspend bacterial pellets in the wells of an S-Block in 150 µl Buffer P1.

   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellets.
3. Add 150 µl Buffer P2 to each sample. Seal block with a tape sheet and gently invert the block 6 times to mix. Incubate for 3 min at room temperature (15–25°C).

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

4. Remove the tape from the block and add 150 µl Buffer DP3 to each well. For a tight seal, completely dry the block with a paper towel, and then seal tightly using a new tape sheet. Seal the tape sheet firmly to the block either manually or using a sealing roller (e.g., MSR-0001 Sealing Roller, cat. no. MSR-0001, from Bio-Rad, **www3.bio-rad.com***). Mix thoroughly by inverting the block 6 times.

   Ensure that the block is tightly sealed to avoid cross-contamination of wells.

   Note: Precipitation of cellular components will occur after the addition of Buffer DP3. After the addition of isopropanol (next step), the amount of precipitate will decrease. The remaining precipitate will not interfere with the vacuum procedure.

5. Remove the tape from the block and add 300 µl isopropanol to each well. Completely dry the block with a paper towel, and tightly seal the block with a new tape sheet. Seal the tape sheet firmly to the block either manually or using a sealing roller. Mix by inverting the block 1–2 times. Further inversions are not required and may cause leakage of the isopropanol.

   Ensure that the block is tightly sealed to avoid cross-contamination of wells. If the block is not tightly sealed, the isopropanol can cause the tape to detach from the block.

6. Remove the tape from the block. Pipet the lysates from step 5 into the wells of the DirectPrep 96 Plate. Apply vacuum until all samples have passed through.

7. Switch off the vacuum and ventilate the vacuum manifold slowly. Add 0.75 ml Buffer PE to each well and apply vacuum until buffer has passed through.

8. Repeat step 7.

9. After Buffer PE has been drawn through all wells, apply maximum vacuum for an additional 10 min to dry the membrane.

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

10. Switch off the vacuum and ventilate the vacuum manifold slowly. Remove the DirectPrep 96 Plate together with the top plate from the base. Vigorously tap the top plate on a stack of absorbent paper, and blot the nozzles of the DirectPrep 96 Plate with clean absorbent paper until no droplets remain.

    This step removes residual Buffer PE, which may be present around the outlet nozzles of the DirectPrep 96 Plate. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

11. For elution, QIAGEN recommends using Elution Microtubes RS, provided in DirectPrep Miniprep Kits.* For elution in Elution Microtubes RS, replace the waste tray with the elution microtube adapter. Place the racked elution microtubes directly onto the adapter. Place the top plate with the DirectPrep 96 Plate back on the base, making sure that the DirectPrep 96 Plate is positioned securely.

12. To elute DNA, pipet 75 µl Buffer EB onto the center of each well of the DirectPrep 96 Plate, incubate for 1 min, and apply vacuum (–550 to –650 mbar) for 1 min. Switch off vacuum and ventilate the vacuum manifold slowly.

    Ensure that the elution buffer is dispensed directly onto the center of the DirectPrep membrane for optimal elution of DNA. Average eluate volume is 50 µl from an elution buffer volume of 75 µl.

    For increased DNA yield, use a higher volume of elution buffer (e.g., 100 µl). For increased DNA concentration, use a lower elution-buffer volume (not less than 45 µl).

    If water is used for elution, make sure that the pH is between 7.0 and 8.5. Elution efficiency is dependent on pH, and the maximum elution efficiency is achieved within this range. A pH of <7.0 can decrease yield. DNA can also be eluted in TE

* For elution in microplates, replace the waste tray with the microplate adapter. Place a 96-well microplate directly onto the adapter. Place the top plate with the DirectPrep 96 Plate back on the base, making sure that the DirectPrep 96 Plate is positioned securely.

buffer (10 mM Tris·Cl; 1 mM EDTA, pH 8.0), but EDTA may inhibit subsequent enzymatic reactions.

Note: Store DNA at –20°C when eluted with water, as DNA may degrade in the absence of a buffering agent.

The yield and quality of plasmid DNA obtained should be analyzed by agarose gel electrophoresis, followed by ethidium bromide staining. For agarose gel quantification, compare 2 µl aliquots of sample to known concentrations (e.g., 50 ng, 100 ng, 150 ng) of a supercoiled plasmid DNA standard.

Troubleshooting Guide

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

	Comments and suggestions
Vacuum-related problems	
Clogging of the DirectPrep 96 Plate	Cell density too high. Use LB as culture medium or reduce culture volume.
Splashes on the microplate after elution	Vacuum during elution too high. Check the vacuum with a vacuum regulator or pressure gauge. Use elution microtubes for elution.
Low or no yield	
General	Low yields may be caused by a number of factors. To find the source of the problem, analyze fractions saved from each step in the procedure on an agarose gel. The lysate and the flow-through fraction can be precipitated by adding 0.7 volumes isopropanol and centrifuging at maximum speed (>10,000 x *g* or ~13,000 rpm) for 30 minutes. The entire wash flow-through can be precipitated by adding 0.1 volumes of 3 M sodium acetate, pH 5.0, and 0.7 volumes of isopropanol.

		Comments and suggestions
No DNA in the lysate		
a)	Plasmid did not propagate	Check that the conditions for optimal growth were met.
b)	Buffer P2 precipitated	Redissolve by warming to 37°C.
c)	Cell resuspension incomplete	Pelleted cells should be completely resuspended in Buffer P1. Do not add Buffer P2 until an even suspension is obtained.
DNA is found in the flow-through fraction of the cleared lysate		
	Binding incomplete	Ensure that addition of Buffer DP3 and isopropanol were performed correctly.
DNA is found in the wash flow-through fraction		
	Ethanol omitted	Repeat procedure with correctly prepared wash buffer (Buffer PE).
Little or no DNA in the eluate		
	Elution buffer incorrect	DNA is eluted only in the presence of low-salt buffer, e.g., Buffer EB (10 mM Tris·Cl, pH 8.5) or water. Elution efficiency is dependent on pH. The maximum efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH value is within this range.
Low DNA quality		
DNA does not perform well		
a)	Eluate salt concentration too high	Ensure that two wash steps are carried out prior to elution.
b)	Eluate contains residual ethanol	Ensure that steps 9 and 10 of the DirectPrep 96 protocol are performed.

		Comments and suggestions
RNA in the eluate		
	RNase A digestion omitted	Ensure that RNase A is added to Buffer P1 before use.
Genomic DNA in the eluate		
a)	Buffers P2 and DP3, or isopropanol added incorrectly	The lysate must be handled gently after addition of Buffers P2 and DP3 and isopropanol, to prevent shearing of genomic DNA.
b)	Lysis too long	Lysis in step 3 of the DirectPrep 96 protocol must not exceed 5 minutes.
c)	Culture overgrown	Overgrown cultures contain lysed cells and degraded DNA. Do not grow cultures for longer than 16–24 hours.
Sequencing-related problems		
General		
a)	Complete sequencing failure	Check the DNA yield on an agarose gel. Check the sequencing reaction setup, including the running conditions and correct concentration. Try using serial dilutions to optimize template concentration.
b)	Low signal	Increase the amount of template DNA used. Check plasmid DNA concentration on an agarose gel and/or perform serial dilutions to optimize template concentration. Increase the number of cycles for the sequencing reaction.
c)	Short read-length	Check the template concentration on an agarose gel; ensure that the Buffer PE wash step is performed correctly to avoid salt contamination; increase the number of cycles for the sequencing reaction; or increase the amount of template DNA used.

	Comments and suggestions
Fluorescent DNA sequencing	
Short read-length	Check that the ethanol wash step and drying step are performed correctly to avoid problems of salt and ethanol contamination. If using the ABI PRISM® 3700 sequencer, ensure that the dye terminators are removed from sequencing reactions, for example, using DyeEx® technology (96-well or spin column formats). If sequencing reactions are not cleaned up before loading, electroinjection into capillaries is inefficient, and short sequence reads are obtained due to salt contamination.

Ordering Information

Product	Contents	Cat. no.
DirectPrep 96 MiniPrep Kit (4)	For 4 x 96 plasmid minipreps: 4 DirectPrep 96 Plates, Buffers, S-Blocks, AirPore Tape Sheets, Tape Pads, Elution Microtubes RS, Caps	27361
DirectPrep 96 BioRobot® Kit (4)	For 4 x 96 plasmid minipreps: 4 DirectPrep 96 Plates, Buffers, S-Blocks, 96-Well Microplates FB, AirPore Tape Sheets, Tape Pads	962341
QIAGEN Plasmid *Plus* 96 Miniprep and BioRobot Kits — for purification of transfection-grade plasmid DNA in 96-well format		
QIAGEN Plasmid *Plus* 96 Miniprep Kit (4)	For 4 x 96 plasmid minipreps: TurboFilter® 96 Plates, Plasmid *Plus* 96 Plates, Buffers, Reagents, S-Blocks and Elution Microtubes; requires use of QIAvac 96 and Elution Microtube Adapter,* or a centrifugation system suitable for 96-well blocks	16181
QIAGEN Plasmid *Plus* 96 BioRobot Kit (4)	For 4 x 96 plasmid minipreps: TurboFilter 96 Plates and Plasmid *Plus* 96 Plates, Buffers, Reagents, S-Blocks and Elution Microtubes; for use with the BioRobot Universal System	960241

* Available from QIAGEN Technical Services.

Product	Contents	Cat. no.
QIAprep 96® *Plus* Miniprep and BioRobot Kits — for purification of plasmid DNA in 96-well format		
QIAprep 96 *Plus* Miniprep Kit (4)*	For 4 x 96 plasmid minipreps: TurboFilter 96 Plates, Plasmid Plus 96 Plates, Buffers, Reagents, S-Blocks, and Elution Microtubes	27291
QIAprep 96 *Plus* BioRobot Kit (4)†	For 4 x 96 plasmid minipreps: TurboFilter 96 Plates, Plasmid Plus 96 Plates, Buffers, Reagents, S-Blocks and Elution Microtubes	962241
QIAprep 96 Turbo Kits — for purification of molecular biology grade plasmid DNA using QIAprep 96 TurboFilter 96 plates		
QIAprep 96 Turbo Miniprep Kit (4)	For 4 x 96 high-purity plasmid minipreps, 4 each: TurboFilter 96 and QIAprep 96 Plates; S-Blocks, Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	27191
QIAprep 96 Turbo BioRobot Kit (4)	For 4 x 96 high-purity plasmid minipreps, 4 each: TurboFilter 96 and QIAprep 96 Plates; S-Blocks, Reagents, Buffers, Collection Microtubes (1.2 ml) and Caps, 96-Well Microplates RB and Lids, Tape Pads	962141
Accessories		
QIAvac Multiwell	Vacuum manifold for processing 96-well purification plates of SBS standard	9014579
Vacuum Regulator	For use with QIAvac manifolds	19530
AirPore Tape Sheets (50)	Microporous tape sheets for covering 96-well blocks: 50 sheets per pack	19571

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

† For use with the BioRobot Universal System.

Product	Contents	Cat. no.
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570
S-Blocks (24)	96-well blocks with 2.2 ml wells, 24 per case	19585
Plate rotor 2 x 96*	Rotor for 2 QIAGEN 96-well plates, for use with QIAGEN Centrifuges	81031
Centrifuges		
Centrifuge 4–16S	Universal laboratory centrifuge with brushless motor	81500†; 81510‡; 81525§; 81520**
Centrifuge 4–16KS	Refrigerated universal laboratory centrifuge with brushless motor	81600†; 81610‡; 81625§; 81620**

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

* A wide range of both fixed-angle and swing-out rotors is available from SIGMA® Laborzentrifugen GmbH for centrifuging 5 ml, 15 ml and 50 ml tubes, as well as 1.5 ml and 2 ml microcentrifuge tubes.
† Japan.
‡ North America.
§ UK.
** Rest of the world.

Notes

Handbook Revision History

Document	Changes	Date
HB-1161-004	Replaced Flat-Bottom Blocks with S-Blocks	December 2018

Limited License Agreement for DirectPrep 96 Miniprep Kit

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

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