Fourth Edition April 2010

QIAamp® 96 DNA Blood Handbook

For high-throughput purification of DNA from

- whole blood
- plasma
- serum
- body fluids
- lymphocytes
- bone marrow
- cultured cells

QIAGEN®

Sample & Assay Technologies

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
- microRNA research and RNAi
- Automation of sample and assay technologies

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Contents

Kit Contents

QIAamp 96 DNA Blood Kit	**(4)**	**(12)**
Cat. no.	**51161**	**51162**
Number of preps	**4 x 96**	**12 x 96**
QIAamp 96 Plates	4	12
S-Blocks*	2	2
Collection Microtubes (racked)	4 x 96	12 x 96
Elution Microtubes CL (racked)	4 x 96	12 x 96
Caps for Collection Microtubes	2 x 55	2 x 120, 1 x 55
Caps for Elution Microtubes	1 x 50	3 x 50
AirPore Tape	25 sheets	50 sheets
QIAGEN® Protease	2 vials†	5 vials‡
Protease Solvent§	2 x 4.4 ml	5 x 5.5 ml
Buffer AL¶	2 x 54 ml	2 x 126 ml
Buffer AW1 (concentrate)¶	1 x 95 ml	3 x 95 ml
Buffer AW2 (concentrate)§	1 x 66 ml	3 x 66 ml
Buffer AE§	1 x 110 ml	5 x 60 ml
96-Well Plate Register	4	12
Handbook	1	1

* Reusable; see "Reuse of S-Blocks" on page 16 for cleaning instructions.

† Resuspension volume 4.4 ml.

‡ Resuspension volume 5.5 ml.

§ Contains sodium azide as a preservative.

¶ Contains chaotropic salt. Take appropriate safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 7 for safety information.

Storage

QIAamp 96 plates and all buffers and reagents can be stored dry at the temperature indicated on the kit label. The expiration date for the kit is printed on the kit label and is valid only when the kit is stored at the indicated temperature.

QIAGEN Protease is provided lyophilized. Reconstituted QIAGEN Protease is stable for 2 months when stored at 2–8°C, but only until the kit expiration date. Keeping the QIAGEN Protease stock solution at room temperature for prolonged periods of time should be avoided. Storage at –15 to –30°C will prolong its life, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and freezing at –15 to –30°C is recommended.

Product Use Limitations

The QIAamp 96 DNA Blood 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.

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 or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

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 back cover).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QIAamp 96 DNA Blood Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are 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 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 see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAamp 96 DNA Blood Kits is tested against predetermined specifications to ensure consistent product quality.

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.

CAUTION: DO NOT add bleach or acidic solutions directly to waste containing Buffers AW1 and AL.

Buffers AL and AW1 contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

The following risk and safety phrases apply to components of the QIAamp 96 DNA Blood Kit:

Buffers AW1 and AL

Contain guanidine hydrochloride: harmful, irritant. Risk and safety phrases:* Xn, R22-36/38, S13-26-36-46

QIAGEN Protease

Contains subtilisin: sensitizer, irritant. Risk and safety phrases:* Xn, R37/38-41-42, S22-24-26-36/37/39-46

24-hour-emergency information

Emergency medical information can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R22: Harmful if swallowed; R36/38: Irritating to eyes and skin; R37/38: Irritating to respiratory system and skin; R41: Risk of serious damage to eyes; R42: May cause sensitization by inhalation; S13: Keep away from food, drink and animal feedingstuffs; S22: Do not breathe dust; 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; S36/37/39: Wear suitable protective clothing, gloves and eye/face protection; S46: If swallowed, seek medical advice immediately and show container or label.

Introduction

The QIAamp 96 DNA Blood Kit uses well-established technology for purification of DNA from a wide range of clinical starting materials. The kit combines the selective binding properties of a silica-gel membrane with a high-throughput 96-well format and allows simultaneous processing of up to 192 samples. Sample types include fresh or frozen whole blood, plasma, serum, bone marrow, other body fluids, lymphocytes, and cultured cells that have a normal set of chromosomes. Whole blood treated with citrate, heparin, or EDTA, or white blood cells treated with Ficoll can be used.

The purification protocol requires no phenol/chloroform extraction or alcohol precipitation, and involves minimal handling. DNA is eluted in Buffer AE or water, ready for direct use in downstream applications such as Southern blotting or PCR, or for storage at –20°C. The purified DNA is free of protein, nucleases, and other contaminants or inhibitors and ranges in size up to 50 kb, with fragments of approximately 20–30 kb predominating. DNA of this length denatures completely during thermal cycling and is amplified very efficiently.

General considerations for purification of nucleic acids

Purification of high-molecular-weight DNA

To purify high-molecular-weight DNA, larger than the 50 kb achieved with QIAamp Kits, we recommend using QIAGEN Genomic-Tips or Gentra® Puregene® Kits. QIAGEN Genomic-Tips are gravity-flow, anion-exchange tips that enable purification of DNA of up to 150 kb from a wide range of sample types. The tips are available separately or, with QIAGEN Protease and buffers, as part of Blood & Cell Culture DNA Kits.

Gentra Puregene Kits use a modified salting-out precipitation method for purification of archive-quality DNA of 100–200 kb. The procedure is scalable for large or small sample volumes, and kits are available for a wide range of sample types. An ongoing study of archived DNA has shown that purified DNA can be stored for at least 14 years without degradation.

Purification from large-volume samples

QIAamp DNA Blood Midi and Maxi Kits are available for low-throughput purification of DNA from up to 2 ml and 10 ml of blood, respectively (see page 29 for ordering information). These kits use the same silica-membrane technology as the QIAamp 96 DNA Blood Kit.

The FlexiGene® DNA Kit provides scalable purification of genomic DNA from whole blood, buffy coat, or cultured cells in a single tube. The simple, rapid procedure yields pure DNA of up to 150 kb, ready to use in downstream applications such as PCR or Southern blotting.

Gentra Puregene Kits provide a scalable procedure for large or small sample volumes. The kits use a modified salting-out precipitation method for purification of archive-quality DNA, and kits are available for a wide range of sample types.

Samples with low DNA content

Use carrier DNA (e.g., poly dA, poly dT, poly dA:dT) if the sample contains less than 5 ng DNA. We recommend the addition of 1 µl of an aqueous solution containing 5–10 µg carrier DNA to 200 µl Buffer AL. Ensure that carrier DNA does not interfere with the downstream application.

Automated high-throughput sample processing

For automated nucleic acid purification from large numbers of blood samples, we recommend kits designed for automated processing with the BioRobot® MDx or the QIAsymphony® SP. The QIAamp DNA Blood BioRobot MDx Kit uses the same silica-membrane technology as the QIAamp 96 DNA Blood Kit, with fully automated processing on the BioRobot MDx (see page 29 for ordering information). QIAsymphony DNA Mini and Midi Kits provide fully automated purification of DNA from 1–96 blood or buffy coat samples on the QIAsymphony SP (see page 29 for ordering information). Sample volumes of up to 200 µl can be processed with the QIAsymphony DNA Mini Kit and up to 1000 µl with the QIAsymphony DNA Midi Kit.

For purification of viral nucleic acids, the QIAamp Virus BioRobot MDx Kit provides QIAamp silica-membrane technology with fully automated processing on the BioRobot MDx (see page 29 for ordering information).

Sample storage

Whole blood samples treated with EDTA, ACD, or heparin can be used, and may be either fresh or frozen. Frozen samples should be equilibrated to room temperature (15–25°C) before beginning the procedure. Yield and quality of the purified DNA depend on the storage conditions of the blood. Fresher blood samples may yield better results.

For short-term storage of up to 10 days, collect blood in tubes containing EDTA as and anticoagulant, and store at 2–8°C. However, for applications requiring maximum fragment size, such as Southern blotting, we recommend storage at 2–8°C for up to 3 days only, as low levels of DNA degradation will occur after this time.

For long-term storage (over 10 days), collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required), and store at –70°C.

The QIAamp procedure

Sample volumes

Yields may vary from sample to sample depending on factors such as the health of the donor, patient medication, or sample storage conditions. The procedure is optimized for use with 200 µl samples of human whole blood but samples up to 400 µl can also be used. Small samples should be adjusted to 200 µl with PBS before starting the protocol.

For sample volumes larger than 200 µl, the amount of lysis buffer and other reagents added to the sample before loading must be increased proportionally. To ensure optimal mixing during lysis, we recommend dividing each sample for lysis between two collection microtubes and then loading the combined lysate into one well of the QIAamp 96 plate. To avoid the S-Block overflowing, lysate flow-through should be discarded before addition of the first wash buffer, Buffer AW1.

Note: Additional QIAamp buffers and QIAGEN Protease can be purchased to supplement the QIAamp 96 DNA Blood Kit when using samples larger than 200 µl (see ordering information, page 30).

Sample lysis with QIAGEN Protease

Intensive research has shown that QIAGEN Protease is the optimal enzyme for use with the lysis buffer provided in the QIAamp 96 DNA Blood Kit. QIAGEN Protease is completely free of DNase and RNase activities. For optimal results, samples must be equilibrated to room temperature before lysis.

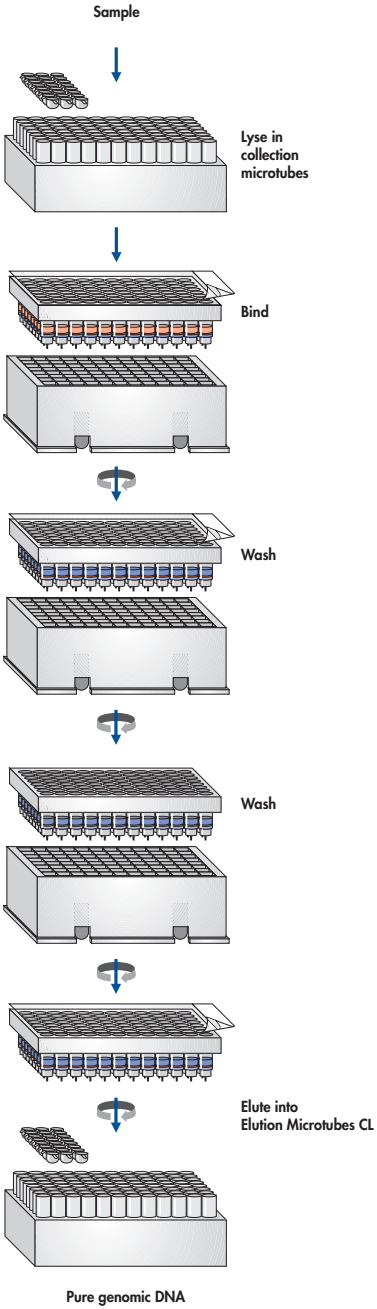
When using the QIAamp 96 DNA Blood Kit for a sample that requires a modified protocol, please contact QIAGEN Technical Services for advice about whether your lysis conditions are compatible with QIAGEN Protease. When >8 mM EDTA is used together with >0.5% SDS, QIAGEN Protease activity decreases. For samples that require an SDS-containing lysis buffer or that contain high levels of EDTA, use of the QIAamp DNA Mini Kit is recommended. The QIAamp DNA Mini Kit contains Proteinase K, which performs well in combination with both SDS-containing and SDS-free buffers.

Purification on the QIAamp membrane

Following sample lysis, buffering conditions are adjusted by adding ethanol to optimize the binding of DNA to the QIAamp membrane before the sample is loaded onto the QIAamp 96 plate. DNA is adsorbed onto the QIAamp membrane in a brief centrifugation step. If the initial sample volume is larger than 300 µl, additional centrifugation steps are used to load the entire lysate onto the QIAamp 96 plate. The optimized salt and pH conditions of the lysate ensure that contaminants such as protein, which can inhibit PCR or other downstream reactions, are not retained on the QIAamp membrane.

DNA bound to the membrane is washed in two centrifugation steps. Optimal wash conditions are achieved through the use of two wash buffers, Buffers AW1 and AW2, ensuring complete removal of residual contaminants without affecting DNA binding.

QIAamp 96 DNA Blood Procedure



Copurification of RNA

When using QIAamp 96 plates, DNA and RNA are purified in parallel if both are present in the sample. If RNA-free genomic DNA is required, addition of 20 µl RNase A stock solution (20 mg/ml) to the sample prior to addition of Buffer AL is recommended.

Elution of pure nucleic acids

Purified, concentrated DNA is eluted from the QIAamp 96 plate in either low-salt Buffer AE or water. If water is used, ensure that the pH of the water is at least 7.0 since acidic conditions may reduce DNA yield. Elution buffer should be equilibrated to room temperature before it is applied to the QIAamp 96 plate. Yields are increased if the QIAamp 96 plate is incubated with the elution buffer at room temperature for 5 minutes before centrifugation.

If the purified DNA will be stored, elution in Buffer AE (10 mM Tris·Cl; 0.5 mM EDTA; pH 9.0)* and storage at –20°C is recommended. DNA stored in water is subject to degradation by acid hydrolysis.

Genomic DNA can be conveniently stored for years and transported at room temperature in QIAsafe™ DNA Tubes and 96-Well Plates (see page 30 for ordering information).

Elution mode for maximum yield or concentration

Yields depend on the sample type and the number of cells in the sample. Typically, a 200 µl sample of whole blood from a healthy individual yields 3–12 µg of DNA. (If higher yields are required, use QIAamp DNA Blood Midi or Maxi Kits with 1–2 ml or 5–10 ml blood, respectively.) For most whole blood samples, a single elution with 200 µl elution buffer is sufficient. However, if the initial sample volume is greater than 200 µl, elution in 2 x 200 µl elution buffer is recommended.

Samples with elevated white blood cell (WBC) counts, (1–1.5 x 10^7 cells/ml) yield 13–20 µg DNA. If such a sample is loaded onto a QIAamp membrane, approximately 80% of the DNA is eluted in the first 200 µl elution buffer and up to 20% more in the next 200 µl. In samples with WBC counts exceeding 1.5 x 10^7 cells/ml, up to 60% of the DNA is eluted in the first 200 µl, and up to 70% of the remaining material in each subsequent 200 µl (see Table 1). Elution into fresh elution microtubes is recommended to prevent dilution of the first eluate. Eluting in 4 x 100 µl instead of 2 x 200 µl does not increase elution efficiency. In all cases a single elution with 200 µl elution buffer will provide sufficient DNA to perform multiple amplification reactions.

* Contains sodium azide as a preservative.

For some downstream applications, concentrated DNA may be required. Elution with volumes of less than 200 µl increases the final DNA concentration in the eluate significantly but slightly reduces overall DNA yield (see Table 2). For samples containing less than 3 µg DNA, elution in 100 µl is recommended. For samples containing less than 1 µg DNA, a single elution in 50 µl Buffer AE or water is recommended.

Table 1. Yields of nucleic acids in successive elutions of 200 µl Buffer AE using QIAamp Kits

		DNA yield (µg)			
Sample	**Amount**	**Elution 1**	**Elution 2**	**Elution 3**	**Total**
Whole blood	200 µl	3–8	1–2	0–2	4–12
Lymphocytes	5 x 10^6	25–35	10–15	5–10	40–60

Table 2. Effect of elution volume on DNA yield and concentration

Elution volume (µl)	Yield (µg)*	DNA concentration (ng/µl)*
200	6.80	34.0
150	6.51	43.4
100	6.25	62.5
50	5.84	116.8

* Average DNA yield and concentration from 20 preparations after purification using QIAamp Kits.

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.

- Centrifuge 4-16 or 4-16K with Plate Rotor 2 x 96 (see pages 30 and 31 for ordering information)
- Incubator or oven
- Multichannel pipet with tips
- Reagent reservoirs for multichannel pipet
- Ethanol (96–100%)*
- RNase A (optional)
- Phosphate-buffered saline (PBS) may be required for some samples

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Important Notes

Preparation of reagents and equipment

All reagent volumes supplied in the QIAamp 96 DNA Blood Kit are sufficient for purification of DNA in 4 or 12 runs of 96 samples.

QIAGEN Protease stock solution (store at 2–8°C or –15 to –30°C)

Resuspend the vials of lyophilized QIAGEN Protease in the protease solvent* provided with the kit.

Dissolved QIAGEN Protease is stable for up to 2 months when stored at 2–8°C, but only until the kit expiration date. Storage at –15 to –20°C is recommended to prolong the life of QIAGEN Protease, but repeated freezing and thawing should be avoided. Storage of aliquots of QIAGEN Protease is recommended.

Note: Do not add QIAGEN Protease directly to Buffer AL.

Buffer AL† (store at room temperature, 15–25°C)

Mix Buffer AL by shaking the bottle before use. Buffer AL is stable until the kit expiration date when stored at room temperature.

Note: Do not add QIAGEN Protease directly to Buffer AL.

Buffer AW1† (store at room temperature, 15–25°C)

Buffer AW1 is supplied as a concentrate. Before using for the first time, add 125 ml ethanol (96–100%) to a bottle containing 95 ml Buffer AW1 concentrate, as described on the bottle.

Note: Always mix reconstituted Buffer AW1 by shaking the bottle before use.

Buffer AW2 (store at room temperature, 15–25°C)

Buffer AW2 is supplied as a concentrate. Before using for the first time, add 160 ml ethanol (96–100%) to a bottle containing 66 ml of Buffer AW2 concentrate, as described on the bottle.

Note: Always mix reconstituted Buffer AW2 by shaking the bottle before use.

* Contains sodium azide as a preservative.

† Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 7 for safety information.

Reuse of S-Blocks

Two S-Blocks are supplied with each kit as supports for the QIAamp 96 plate during centrifugation. They collect flow-through from the QIAamp 96 plate and are intended for repeated use. To avoid cross-contamination, after use rinse thoroughly in tap water, incubate for 1 minute at room temperature in 0.4 M HCl,* empty, and wash thoroughly with distilled water. Used S-Blocks can also be autoclaved after washing. Additional S-Blocks can be ordered separately (see ordering information, page 30).

General comments

Adhesive tape

AirPore Tape is used to prevent cross-contamination during all centrifugation steps except step 17. It is also used when reusing plates containing unused wells. Label used wells of the QIAamp 96 plate with a waterproof marker pen. Cover unused wells with tape and store the QIAamp 96 plate in the blister pack in which it was supplied. Before starting the next run, remove the tape and cover the previously used wells with fresh tape.

Mixing

Optimal lysis and binding conditions are important to obtain maximum DNA yields. Efficient lysis and adjustment of binding conditions is achieved by ensuring that samples are thoroughly mixed after addition of Buffer AL and QIAGEN Protease. After capping the collection microtubes, cover the rack with the plastic cover supplied, hold the racked collection microtubes with both hands, and shake vigorously up and down for at least 15 seconds. Shaking the racked collection microtubes with only one hand will result in less efficient mixing in the tubes on the side of the racked collection microtubes held in the hand, with lower yields being obtained from those tubes.

Caps

Two types of cap are supplied in this kit. Caps for collection microtubes are used to seal collection microtubes. Caps for elution microtubes are used to close the blue elution microtubes. As the cap types have different diameters, it is important to use the correct caps at each stage. Use of caps that are too large will impair sealing, while use of caps that are too small will result in sample leakage and may lead to sample evaporation during storage.

* 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.

Multichannel pipets

For efficient processing, we recommend the use of an electric multichannel pipet with a minimum capacity of 650 µl per pipet tip, such as the Matrix Impact® cordless electronic multichannel pipet or the Matrix Multi-8 Electrapette®. These have a unique expandable tip-spacing system, allowing the user to transfer liquid directly from test tubes to microtiter plates. Extended tips (Matrix, cat. no. 8255) are suitable for use with these pipets. For ordering information, please contact Matrix Technologies Corporation, USA, or its subsidiaries or distributors in other countries.

Centrifugation

Centrifugation of QIAamp 96 plates is performed at 6000 rpm (5788 x *g*). The speed limit of the centrifuge is programmed so that the required *g*-force will not be exceeded. All centrifugation steps are carried out at room temperature. Use an AirPore tape sheet to seal the QIAamp 96 plate during all centrifugation steps except step 17.

Note: When using the refrigerated Centrifuge 4-16K, set the temperature to 40°C for all centrifugation steps.

Abbreviated instructions for using the Centrifuge 4-16

1. **Switch on the centrifuge by pressing the main switch on the back.**
2. **Select the rotor selection list in the display field by turning the knob. Press the knob and turn it again to select the rotor/bucket combination "09100/09158" for the Plate Rotor 2 x 96. Confirm entry by pressing the knob.**

   Entering the rotor number automatically sets the time and speed limits for centrifugation for that rotor, eliminating the danger of the centrifuge running too fast.
3. **Select "Speed" by turning the knob. Press the knob and turn it again to set the speed to "6000". Confirm entry by pressing the knob.**

   The corresponding relative centrifugal force (RCF) is calculated from the rotor number and speed and appears automatically in the RCF field. It is also possible to enter the RCF value "5788 x *g*" manually in the RCF field after selecting "RCF" in the same way.
4. **Select "Time" by turning the knob. Press once and turn the knob again to set the time required. Confirm entry by pressing the knob.**
5. **Open the lid, place the 96-well plates with metal carriers in the buckets, then close the lid.**

   The start and lid keys light up.

6. **Push "Start" to start the centrifuge.**

   When the centrifuge is running the lid key will not be lit. Each run can be interrupted by pushing "Stop".

7. **At the end of the run, the lid key will light up. Open the centrifuge lid by pressing the lid key. Remove the plates.**

   All preset parameters remain in memory after a run has finished.

Warning: Do not centrifuge the plate carriers without the QIAamp 96 plates and S-Blocks. If unsupported, the carriers will collapse under high *g*-forces. Remove the carriers during test runs. Standard microtiter plates may be centrifuged in the same carriers if the *g*-force does not exceed 500 x *g*.

Protocol: Purification of DNA from Whole Blood, Plasma, Serum, or Body Fluids

Important point before starting

- Use of a multichannel pipet is recommended.

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Mix samples by inverting tubes, avoiding foaming.
- Preheat an incubator or oven to 70°C.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to the instructions on page 15.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 70°C.

Procedure

1. **Pipet 20 µl QIAGEN Protease stock solution into the bottom of the collection microtubes.**

   If possible, use a multichannel pipet with a small tip volume so that the QIAGEN Protease is dispensed accurately into the bottoms of the tubes. If it is necessary to use a multichannel pipet with a tip volume >200 µl, ensure that the tips touch near the bottoms of the tubes so that all the QIAGEN Protease is expelled and the rims of the tubes remain dry.

2. **Add samples to the collection microtubes by touching the insides of the tubes without wetting the rims. Use either 200 µl whole blood, plasma, serum, or body fluids per tube, or up to 5 x 10^6 lymphocytes or cultured cells in 200 µl PBS per tube. Use the Plate Register provided to record the locations of the samples.**

   Mark the collection microtubes so that samples can be easily identified throughout the protocol.

   RNA and DNA will be purified in parallel if both are present in the sample. RNA may inhibit some downstream enzymatic reactions. If RNA-free genomic DNA is required, 20 µl of an RNase A stock solution (20 mg/ml) should be added to the sample prior to the addition of Buffer AL in step 3.

   Note: To avoid cross-contamination when sealing the collection microtubes with caps, do not touch the rims of the tubes with the pipet tips.

   For samples smaller than 200 µl, add the appropriate volume of PBS to bring them to 200 µl.

   For sample volumes larger than 200 µl, split each sample into 200 µl aliquots and use two tubes of the collection microtubes for lysis. Load the combined lysates into one well of the QIAamp 96 plate in two consecutive steps at step 13.

3. **Add 200 µl Buffer AL to each sample, taking care not to wet the rims of the collection microtubes. Seal the tubes using the caps for collection microtubes provided.**

   Note: Use only the caps for collection microtubes, since using AirPore tape or caps for elution microtubes at this stage of the procedure will lead to cross-contamination. Ensure that the tubes are properly sealed to avoid leaks during shaking.

4. **Cover the rack with the plastic cover supplied, and mix thoroughly by shaking vigorously for 15 s.**

   For efficient lysis, it is essential that the samples and Buffer AL are mixed immediately and thoroughly to yield a homogeneous solution. Hold the racked collection microtubes with both hands and shake up and down vigorously.

   Note: Simply inverting the racked collection microtubes several times is not sufficient to initiate efficient lysis. Similarly, vortexing or placing the plate on a shaker is not adequate.

5. **Centrifuge briefly at 3000 rpm to collect any solution from the caps.**

   Allow centrifuge to reach 3000 rpm, then stop the centrifuge.

   Note: When processing 96 samples using a single rack of collection microtubes, the centrifuge must be accurately balanced.

6. **Incubate at 70°C for at least 10 min in an incubator or oven.**

   Longer incubation times have no effect on the quality of the purified DNA. However, extended incubation at high temperatures may lead to DNA degradation.

   Note: Placing a weight on top of the collection microtubes will prevent the lids popping off during incubation.

7. **Centrifuge briefly at 3000 rpm to collect any lysate from the caps.**

   Allow centrifuge to reach 3000 rpm, then stop the centrifuge.

8. **Remove the caps and add 200 µl ethanol (96–100%) to each tube.**

   If the sample volume is greater than 200 µl, increase the amount of ethanol proportionally (e.g., a 400 µl sample will require 400 µl alcohol).

9. **Seal the tubes using new caps for collection microtubes. Shake vigorously for 15 s.**

10. **Centrifuge briefly at 3000 rpm to collect any solution from the caps.**

    Allow centrifuge speed to reach 3000 rpm, then stop the centrifuge.

11. **Place QIAamp 96 plate on top of an S-Block. Mark the plate for later identification.**

12. **Carefully apply the mixture from step 8 (620 µl per collection microtube) to the QIAamp 96 plate.**

    Take care not to wet the rims of the wells to avoid aerosol formation during centrifugation.

    Note: Lowering pipet tips to the bottoms of the collection microtubes may cause sample overflow and cross-contamination. Therefore, remove one set of caps at a time, and begin drawing up the samples as soon as the pipet tips contact the liquid. Repeat until all the samples have been transferred to the QIAamp 96 plate.

13. **Seal the QIAamp 96 plate with an AirPore Tape sheet. Load the S-Block and QIAamp 96 plate onto the carrier, then place it in the rotor bucket. Centrifuge at 6000 rpm for 4 min.**

    Up to 2 x 200 µl blood samples can be loaded onto each well of the QIAamp 96 plate. When using such large sample volumes, it is necessary to empty the S-Block before the first wash step (step 14).

14. **Remove the tape. Carefully add 500 µl Buffer AW1 to each well.**

    It is not necessary to increase the volume of Buffer AW1 if the original sample volume was larger than 200 µl. However, the S-Block must be emptied before centrifugation.

15. **Seal the QIAamp 96 plate with a new AirPore Tape sheet. Centrifuge at 6000 rpm for 2 min.**

16. **Remove the tape. Carefully add 500 µl Buffer AW2 to each well.**

17. **Centrifuge at 6000 rpm for 15 min.**

    The heat generated during centrifugation ensures evaporation of residual ethanol in the sample (from Buffer AW2) that might otherwise inhibit downstream reactions.

18. **Place the QIAamp 96 plate on top of a rack of elution microtubes (provided).**

19. **To elute DNA, add 200 µl Buffer AE or distilled water, equilibrated to room temperature, to each well using a multichannel pipet. Seal the QIAamp 96 plate with a new AirPore tape sheet and incubate for 1 min at room temperature. Centrifuge at 6000 rpm for 4 min. Seal the wells of the microtubes for storage using the caps for elution microtubes provided.**

    DNA yields can be increased by incubating the QIAamp 96 plate loaded with Buffer AE for 5 min at room temperature before centrifugation.

    A second elution step with a further 200 µl Buffer AE increases yields by up to 20%.

    Elution with volumes of less than 200 µl significantly increases the final DNA concentration, but slightly reduces the overall yield. For samples containing less than 1 µg DNA, elution in 50 µl Buffer AE or water is recommended. Eluting with 2 x 100 µl instead of 1 x 200 µl does not increase elution efficiency.

    For long-term storage of DNA, eluting in Buffer AE and storing at –15 to –30°C is recommended, since DNA stored in water is subject to acid hydrolysis.

    Genomic DNA can be conveniently stored for years and transported at room temperature in QIAsafe DNA Tubes and 96-Well Plates (see page 30 for ordering information).

    A 200 µl sample of whole human blood (approximately 5 x 10^6 leukocytes/ml) typically yields 4–6 µg DNA in 200 µl Buffer AE (30 ng/µl), with an A_{260}/A_{280} ratio of 1.65–1.75.

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
General handling	
a) Blocked wells in the QIAamp 96 plate	(1) Blood was insufficiently mixed after phlebotomy, resulting in coagulation. Remove clots and repeat. (2) Precipitates have formed in blood that has been stored either frozen or at room temperature for extended periods. Use fresh blood instead. (3) White blood cell count too high or buffy coat used. Dilute sample at least 1:1 with PBS and repeat the purification procedure. (4) Buffer AE used for lysis instead of Buffer AL. Repeat the purification procedure using Buffer AL. (5) Insufficient lysis due to inadequate mixing. Mix thoroughly and repeat the purification procedure.
b) Variable elution volumes	Adhesive tape used instead of AirPore Tape to seal plates. Be sure to use AirPore Tape in all protocol steps where tape is required.
Colored residues remain on the QIAamp 96 plate after washing	
a) Inefficient cell lysis due to insufficient mixing of the sample with Buffer AL	Repeat the purification procedure with new samples. Mix the samples and Buffer AL immediately and thoroughly.
b) Inefficient lysis due to reduced QIAGEN Protease activity	Repeat the purification procedure with new samples. First dispense QIAGEN Protease, then add samples. Ensure that QIAGEN Protease and sample are mixed before addition of Buffer AL.
c) Buffer AW1 or AW2 prepared incorrectly	Ensure that Buffer AW1 or AW2 concentrates were diluted with the correct amounts of ethanol. Repeat the purification procedure with new samples.

	Comments and suggestions
d) Animal blood used	Hemoglobin can be difficult to remove from the blood of some animal species (e.g., monkey and mouse), and may interfere with downstream applications. Optimize the procedure by reducing the starting volume of blood used. Performing extra washes with Buffer AW1 may also be helpful (see ordering information, page 30).
Little or no DNA in the eluate	
a) No ethanol added to the lysate before loading onto the QIAamp 96 plate	Repeat the purification procedure with new samples.
b) Inefficient cell lysis due to insufficient mixing of the sample with Buffer AL	Repeat the purification procedure with new samples. Mix the samples and Buffer AL immediately and thoroughly.
c) Inefficient cell lysis or protein degradation due to insufficient incubation time or temperature	Repeat the purification procedure with new samples.
d) Inefficient lysis due to reduced QIAGEN Protease activity	Repeat the purification procedure with new samples. First dispense QIAGEN Protease, then add samples. Ensure that QIAGEN Protease and sample are mixed before addition of Buffer AL.
e) QIAamp 96 plate not incubated at room temperature for 1 min prior to elution	After addition of Buffer AE or water, the QIAamp plate must be incubated at room temperature for 1 min before elution.
f) DNA not eluted efficiently	To increase elution efficiency, pipet Buffer AE or water onto the QIAamp 96 plate and incubate at 70°C for 5 minutes before elution.
g) pH of water incorrect	Low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0 or use Buffer AE for elution.
Low concentration of DNA in the eluate	
a) DNA eluted with more than 200 µl Buffer AE or water	Elution with volumes greater than 200 µl decreases the final DNA concentration in the eluate. For samples containing less than 1 µg of DNA, elution in 50 µl Buffer AE or water is recommended.

	Comments and suggestions
b) Gradient in yield across the QIAamp 96 plate	In steps 4 and 9, holding the QIAamp 96 plate with only one hand will lead to higher yields on the side of the plate where the movement was greater. Hold the QIAamp 96 plate with both hands and mix samples by shaking the plate up and down: do not use a rotating action.
A_{260}/A_{280} ratio for purified nucleic acids is low	
a) Inefficient cell lysis due to insufficient mixing of the sample with Buffer AL	Repeat the purification procedure with new samples. Mix the samples and Buffer AL immediately and thoroughly.
b) Inefficient cell lysis or protein degradation due to insufficient incubation time or temperature	Repeat purification procedure with new samples.
c) Inefficient lysis due to reduced QIAGEN Protease activity	Repeat the purification procedure with new samples. First dispense QIAGEN Protease, then add samples. Ensure that QIAGEN Protease and sample are mixed before addition of Buffer AL.
d) Buffer AW1 or AW2 prepared incorrectly	Ensure that Buffer AW1 and AW2 concentrates were diluted with the correct amount of ethanol. Repeat the purification procedure with new samples.
e) Animal blood used	Hemoglobin can be difficult to remove from the blood of some animal species (e.g., monkey and mouse), and may interfere with downstream applications. Optimize the procedure by reducing the starting volume of blood used. Performing extra washes with Buffer AW1 may also be helpful (see ordering information, page 30).
DNA does not perform well in downstream enzymatic reactions	
a) Not enough DNA in sample	Check "Little or no DNA in the eluate" for possible reasons. Increase the amount of eluate added to the reaction, if possible. If necessary, concentrate the eluted DNA under vacuum, or increase the amount of sample used and repeat the purification procedure. If the amount of purified DNA is still expected to be low, reduce the elution

	Comments and suggestions
	volume to 50 µl. Lowering the elution volume slightly reduces overall DNA yield, but results in a higher concentration of nucleic acids (see Table 2, page 13).
b) Purified DNA contaminated with inhibitory substances	Check "A_{260}/A_{280} for purified nucleic acids is low" for possible reasons. Increase the eluate volume to 200 µl if necessary, and repeat the purification procedure.
c) Too much DNA in the PCR	Repeat the PCR, using less DNA template.
d) Animal blood used	Hemoglobin can be difficult to remove from the blood of some animal species (e.g., monkey and mouse), and may interfere with downstream applications. Optimize the procedure by reducing the starting volume of blood used. Performing extra washes with Buffer AW1 may also be helpful (see ordering information, page 30).
e) Residual Buffer AW2 in the eluate	Adjust volume of eluate to 200 µl if necessary, and repeat the purification procedure by adding 200 µl Buffer AL and 200 µl ethanol. Continue with step 9. At step 17, centrifuge at 6000 rpm for 3 minutes. After centrifugation, remove the AirPore tape and incubate the QIAamp 96 plate in an oven at 70°C for 10 minutes.
f) Buffer AW1 used instead of Buffer AW2 in step 16	Adjust volume of eluate to 200 µl if necessary, and repeat the purification procedure by adding 200 µl Buffer AL and 200 µl ethanol. Continue with step 9. At step 17, centrifuge at 6000 rpm for 3 minutes. After centrifugation, remove the AirPore tape and incubate the QIAamp 96 plate in an oven at 70°C for 10 minutes.
g) DNA contaminated with RNA	Repeat the purification procedure with new samples and include an RNase A treatment at step 2.

Appendix A: Determination of Concentration, Yield, Purity, and Length of DNA

Determination of concentration, yield, and purity

DNA yields are determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an A_{260}/A_{280} ratio of 1.7–1.9.

Absorbance readings at 260 nm should lie between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 and 280 nm, or scan absorbance from 220–320 nm (a scan will show if there are other factors affecting absorbance at 260 nm). Both DNA and RNA are measured with a spectrophotometer. To measure only DNA, a fluorometer must be used.

Elution Buffer AE contains the preservative sodium azide, which shows absorbance at 260 nm. Therefore, when quantifying DNA in the eluate by absorbance measurement at 260 nm, when determining DNA purity in the eluate by absorbance measurements at 260 nm and 280 nm, or when scanning absorbance in the range between 220 nm and 250 nm, ensure that the blank contains the same concentration of sodium azide as the eluate. For example, if preparing eluate for absorbance measurements by diluting 50 µl eluate with 100 µl water, then prepare the blank by diluting 50 µl Buffer AE with 100 µl water. Use fresh, distilled water for the dilutions.

Determination of DNA length

The length of genomic DNA can be determined by pulsed-field gel electrophoresis (PFGE) through an agarose gel. The DNA should be concentrated by alcohol* precipitation and reconstituted by gentle agitation in approximately 30 µl TE buffer, pH 8.0,* for at least 30 minutes at 60°C. Avoid drying the DNA pellet for more than 10 minutes at room temperature since over-dried genomic DNA is very difficult to redissolve. Load 3–5 µg DNA per well. Standard PFGE conditions are as follows:

- 1% agarose gel in 0.5x TBE electrophoresis buffer*
- Switch intervals: 5–40 s
- Run time: 17 h
- Voltage: 170 V

* 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.

Appendix B: Protocol for Viral DNA

Protocol for viral DNA

Integrated viral DNA

Integrated viral or proviral DNA is prepared using the same procedures as for genomic DNA (see standard protocols).

Viral DNA from fluids or suspensions

Viral DNA or RNA from extracellular viruses can be prepared using the QIAamp 96 DNA Blood Kit. Cell-free samples such as plasma or serum (other than urine) must be used, since cellular DNA copurifies with viral DNA. We recommend adding 1 µl of an aqueous solution containing 5–10 µg of carrier DNA (e.g., poly dA, poly dT, poly dA:dT) to 200 µl Buffer AL. For optimal binding of viral nucleic acids, increase the volume of ethanol added at step 8 to give a final concentration of 35% (e.g., for a 200 µl plasma or serum sample, add 230 µl of ethanol). Elution in a small volume (e.g., 50 µl) of nuclease-free water is recommended.

Automated viral DNA and RNA purification

For automated, simultaneous purification of viral RNA and DNA, we recommend using the QIAamp Virus BioRobot MDx Kit on the BioRobot MDx. All components of this kit are guaranteed to be RNase-free.

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.
QIAamp 96 DNA Blood Kit (4)*	4 QIAamp 96 Plates, QIAGEN Protease, Reagents, Buffers, Lysis Blocks, Tape Pads, and Collection Vessels	51161
QIAamp 96 DNA Blood Kit (12)*	12 QIAamp 96 Plates, QIAGEN Protease, Reagents, Buffers, Lysis Blocks, Tape Pads, and Collection Vessels	51162
Related products		
QIAamp DNA Blood Mini Kit (50)	50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104
QIAamp DNA Blood Midi Kit (20)	20 QIAamp Midi Spin Columns, QIAGENProtease, Buffers, Collection Tubes (15 ml)	51183
QIAamp DNA Blood Maxi Kit (10)	10 QIAamp Maxi Spin Columns, QIAGEN Protease, Buffers, Collection Tubes (50 ml)	51192
QIAamp DNA Blood BioRobot MDx Kit (12)	For 12 x 96 DNA preps on the BioRobot MDx: 12 QIAmp 96 Plates, Buffers,† QIAGEN Protease, Elution Microtubes CL, Caps, S-Blocks, Tape Pad	965152
QIAsymphony DNA Mini Kit (192)	For 192 preps of 200 µl each on the QIAsymphony SP: Includes 2 reagent cartridges and enzyme racks and accessories	931236
QIAsymphony DNA Midi Kit (96)	For 96 preps of 1000 µl each on the QIAsymphony SP: Includes 2 reagent cartridges and enzyme racks and accessories	931255
QIAamp Virus BioRobot MDx Kit (12)	For 12 x 96 preps: 12 QIAamp 96 Plates, RNase-Free Buffers,† QIAGEN Protease, Elution Microtubes CL, Caps, S-Blocks, Carrier RNA	965652

* Requires use of the QIAGEN 96-Well-Plate Centrifugation System.

† Wash buffers are labeled with bar codes, and expiration date is stated on the Q-Card in the kit.

Ordering Information

Product	Contents	Cat. no.
Accessories		
Buffer AW1	242 ml Wash Buffer (1) Concentrate for 1000 spin, 250 midi, or 100 maxi preps	19081
Buffer AW2	324 ml Wash Buffer (2) Concentrate	19072
Buffer AL	216 ml Buffer AL for 1000 preps	19075
S-Blocks (24)	96-well blocks with 2.2 ml wells,	19585
Collection Microtubes	Nonsterile polypropylene tubes (1.2 ml), 960 in racks of 96	19560
Collection Microtube Caps (120 x 8)	Nonsterile polypropylene caps for collection microtubes (1.2 ml), 960 in strips of 8	19566
Tape Pads (5)	For sealing multi-well plates and blocks: 25 sheets per pad, 5 pads per pack	19570
AirPore Tape Sheets (50)	Microporous tape sheets for covering 96-well blocks: 50 sheets per pack	19571
QIAGEN Protease (7.5 AU)	7.5 Anson units (lyophilized)	19155
QIAGEN Protease (30 AU)	4 x 7.5 Anson units (lyophilized)	19157
QIAsafe DNA Tubes (50)	50 QIAsafe DNA Tubes in moisture-barrier foil packages	159104
QIAsafe DNA 96-Well Plates (10)	10 QIAsafe DNA 96-Well Plates in moisture-barrier foil packages, 10 QIAsafe Seals	159112
QIAGEN 96-Well Centrifugation System		
Centrifuge 4-16	Universal laboratory centrifuge with brushless motor (100 V, 50/60 Hz)	81300*
Centrifuge 4-16	Universal laboratory centrifuge with brushless motor (120 V, 60 Hz)	81310†
Centrifuge 4-16	Universal laboratory centrifuge with brushless motor (220 V, 50 Hz)	81320‡

* For Japan.

† For US.

‡ For rest of world.

Ordering Information

Product	Contents	Cat. no.
Centrifuge 4-16K	Universal refrigerated laboratory centrifuge with brushless motor (100 V, 50/60 Hz)	81400*
Centrifuge 4-16K	Universal refrigerated laboratory centrifuge with brushless motor (120 V, 60 Hz)	81410†
Centrifuge 4-16K	Universal refrigerated laboratory centrifuge with brushless motor (220 V, 50 Hz)	81420‡
Plate Rotor 2 x 96	Rotor for 2 QIAGEN 96 plates, for use with QIAGEN Centrifuges¶	81031¶

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.

*For Japan.

† For US.

‡ For rest of world.

¶ The Plate Rotor 2 x 96 is available exclusively from QIAGEN and its distributors. Under the current liability and warranty conditions, the rotor may only be used in Centrifuges 4-16 and 4-16K from QIAGEN and freely programmable models of centrifuges 4-15, 4K15, 6-10, 6K10, 6-15, and 6K15 from Sigma Laborzentrifugen GmbH.

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Australia ▪ Orders 1-800-243-800 ▪ Fax 03-9840-9888 ▪ Technical 1-800-243-066

Austria ▪ Orders 0800-28-10-10 ▪ Fax 0800/28-10-19 ▪ Technical 0800-28-10-11

Belgium ▪ Orders 0800-79612 ▪ Fax 0800-79611 ▪ Technical 0800-79556

Brazil ▪ Orders 0800-557779 ▪ Fax 55-11-5079-4001 ▪ Technical 0800-557779

Canada ▪ Orders 800-572-9613 ▪ Fax 800-713-5951 ▪ Technical 800-DNA-PREP (800-362-7737)

China ▪ Orders 86-21-3865-3865 ▪ Fax 86-21-3865-3965 ▪ Technical 800-988-0325

Denmark ▪ Orders 80-885945 ▪ Fax 80-885944 ▪ Technical 80-885942

Finland ▪ Orders 0800-914416 ▪ Fax 0800-914415 ▪ Technical 0800-914413

France ▪ Orders 01-60-920-926 ▪ Fax 01-60-920-925 ▪ Technical 01-60-920-930 ▪ Offers 01-60-920-928

Germany ▪ Orders 02103-29-12000 ▪ Fax 02103-29-22000 ▪ Technical 02103-29-12400

Hong Kong ▪ Orders 800 933 965 ▪ Fax 800 930 439 ▪ Technical 800 930 425

Ireland ▪ Orders 1800 555 049 ▪ Fax 1800 555 048 ▪ Technical 1800 555 061

Italy ▪ Orders 800-789-544 ▪ Fax 02-334304-826 ▪ Technical 800-787980

Japan ▪ Telephone 03-6890-7300 ▪ Fax 03-5547-0818 ▪ Technical 03-6890-7300

Korea (South) ▪ Orders 080-000-7146 ▪ Fax 02-2626-5703 ▪ Technical 080-000-7145

Luxembourg ▪ Orders 8002-2076 ▪ Fax 8002-2073 ▪ Technical 8002-2067

Mexico ▪ Orders 01-800-7742-639 ▪ Fax 01-800-1122-330 ▪ Technical 01-800-7742-639

The Netherlands ▪ Orders 0800-0229592 ▪ Fax 0800-0229593 ▪ Technical 0800-0229602

Norway ▪ Orders 800-18859 ▪ Fax 800-18817 ▪ Technical 800-18712

Singapore ▪ Orders 1800-742-4362 ▪ Fax 65-6854-8184 ▪ Technical 1800-742-4368

Spain ▪ Orders 91-630-7050 ▪ Fax 91-630-5145 ▪ Technical 91-630-7050

Sweden ▪ Orders 020-790282 ▪ Fax 020-790582 ▪ Technical 020-798328

Switzerland ▪ Orders 055-254-22-11 ▪ Fax 055-254-22-13 ▪ Technical 055-254-22-12

UK ▪ Orders 01293-422-911 ▪ Fax 01293-422-922 ▪ Technical 01293-422-999

USA ▪ Orders 800-426-8157 ▪ Fax 800-718-2056 ▪ Technical 800-DNA-PREP (800-362-7737)

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