May 2025

QIAwave® DNA Blood & Tissue Handbook

For purification of total DNA from

- Animal blood
- Animal tissue
- Rodent tails
- Ear punches
- Cultured cells
- Bacteria
- Insects

Sample to Insight

Table of Contents

Kit Contents

QAwave DNA Blood & Tissue Kit Cat. no. Number of prep	(50) 69554 50	(250) 69556 250
DNeasy® Mini Spin Columns (colorless)	50	250
Waste Tubes (2 mL)	50	250
Buffer ATL	14 mL	50 mL
Buffer AL*	12 mL	2 x 33 mL
Buffer AW1/C (concentrate)*†	15 mL	98 mL
Buffer AW2/C (concentrate)†	1.5 mL	6 mL
Buffer AE/C (concentrate)†	2 mL	10 mL
Proteinase K	1.25 mL	6 mL

* Contains a guanidine salt. Not compatible with disinfecting agents containing bleach. See page 6 for Safety Information.

† Buffer AW1, Buffer AW2, and Buffer AE are supplied as concentrates. Mix with ultrapure water and/or ethanol (96–100%) according to protocol to obtain a working solution.

Storage

DNeasy Mini Spin Columns and all buffers should be stored at room temperature (15–25°C) and are stable for 1 year after delivery, if not otherwise stated on the label.

The QIAwave DNA Blood & Tissue Kit contains a ready-to-use Proteinase K solution, which is supplied in a specially formulated storage buffer. Proteinase K is stable for at least 1 year after delivery when stored at room temperature. For storage longer than 1 year or if ambient temperatures often exceed 25°C, we suggest storing Proteinase K at 2–8°C.

Intended Use

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

QIAcube® Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

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.

CAUTION

Do not add bleach or acidic solutions directly to the sample preparation waste.

Buffers AL and AW1 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a 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.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAwave DNA Blood & Tissue Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAwave DNA Blood & Tissue Kit is designed for rapid purification of total DNA (e.g., genomic, mitochondrial, and pathogen) from a variety of sample sources including fresh or frozen animal tissues and cells, blood, or bacteria. QIAwave-purified DNA is free of contaminants and enzyme inhibitors and is highly suited for PCR, qPCR, dPCR, NGS, Southern blotting, RAPD, AFLP, and RFLP applications.

Purification requires no phenol or chloroform extraction, or alcohol precipitation, and involves minimal handling. This makes the QIAwave DNA Blood & Tissue Kit highly suited for simultaneous processing of multiple samples.

The buffer system is optimized to allow direct cell lysis followed by selective binding of DNA to the DNeasy membrane. After lysis, the QIAwave DNA Blood & Tissue spin column procedure can be completed in as little as 20 minutes.

Simple centrifugation processing completely removes contaminants and enzyme inhibitors, such as proteins and divalent cations, and allows simultaneous processing of multiple samples in parallel. In addition, the QIAwave DNA Blood & Tissue procedure is suitable for a wide range of sample sizes.

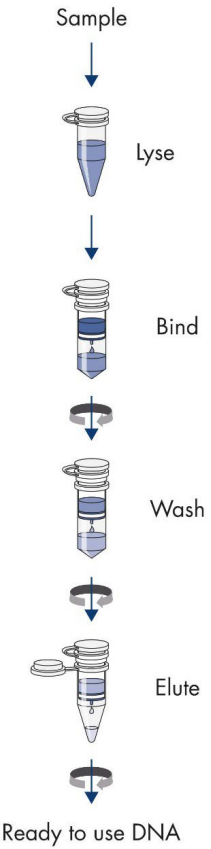
Purified DNA is eluted in low-salt buffer or water, ready for use in downstream applications. QIAwave-purified DNA typically has an A_{260}/A_{280} ratio between 1.7 and 1.9, and is up to 50 kb in size, with fragments of 30 kb predominating. The QIAwave procedure also efficiently recovers DNA fragments as small as 100 bp.

Principle and procedure

The QIAwave DNA Blood & Tissue procedure is simple (see the flowchart on the next page). Samples are first lysed using K*. Buffering conditions are adjusted to provide optimal DNA-binding conditions and the lysate is loaded onto the DNeasy Mini Spin Column. During centrifugation, DNA is selectively bound to the DNeasy membrane as contaminants pass through. Remaining contaminants and enzyme inhibitors are removed in two efficient wash steps and DNA is then eluted in water or buffer, ready for use. QIAwave-purified DNA has A_{260}/A_{280} ratios of 1.7–1.9, and absorbance scans show a symmetric peak at 260 nm confirming high purity.

The DNeasy membrane combines the binding properties of a silica-based membrane with simple spin column technology. DNA adsorbs to the DNeasy membrane in the presence of high concentrations of guanidine salt, which remove water from hydrated molecules in solution. Buffer conditions in the QIAwave DNA Blood & Tissue procedure are designed to enable specific adsorption of DNA to the silica membrane and optimal removal of contaminants and enzyme inhibitors.

* Lysis can be improved by cell disruption using a rotor–stator homogenizer, such as the TissueRuptor II, or a bead mill, such as the TissueLyser III or the TissueLyser II. A supplementary protocol allowing the simultaneous disruption of up to 48 tissue samples using the TissueLyser III and TissueLyser II is available from QIAGEN Technical Services.



QIAwave DNA Blood & Tissue Procedure

Description of protocols

Different protocols in this handbook provide detailed instructions to use the QIAwave DNA Blood & Tissue Kit for purification of total DNA.

- "Protocol: Purification of Total DNA from Animal Blood or Cells", on page 28, is for use with the QIAwave DNA Blood & Tissue Kit, for purification of DNA from animal blood (with nucleated or non-nucleated erythrocytes) or from cultured animal or human cells.
- "Protocol: Purification of Total DNA from Animal Tissues", on page 34, is for use with the QIAwave DNA Blood & Tissue Kit, for purification of DNA from animal tissues, including rodent tails.

Pretreatment and specialized protocols

There are several pretreatment protocols included in this handbook, which are optimized for specific sample types. These pretreatment protocols are used in conjunction with one of the DNA purification protocols described above.

The following pretreatment protocols are included in this handbook.

- "Protocol: Pretreatment for Gram-Negative Bacteria" on page 40
- "Protocol: Pretreatment for Gram-Positive Bacteria" on page 41

Additional optimized protocols for purification of DNA from yeast, hair, insects, crude lysates, bone, saliva, and other specialized sample types are available online at **qiagen.com/shop/Sample-Technologies/DNeasy-Blood-and-Tissue-Kit** or from QIAGEN Technical Services (**support.qiagen.com**). The QIAwave DNA Blood & Tissue Kit can be used with these protocols.

Automated purification of DNA on QIAcube instruments

Purification of DNA can be fully automated on QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the

manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using the QIAwave DNA Blood & Tissue Kit for purification of high-quality DNA.

QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **qiagen.com/qiacubeprotocols**

The QIAwave kits can be run on the QIAcube Connect using either the DNeasy Blood & Tissue Kit protocols or the dedicated QIAwave DNA Blood & Tissue Kit protocols. The protocol steps remain the same for both options. For added convenience, utilizing dedicated QIAwave protocols allows users to initiate run-setup by scanning the QR code on the kit label, with the kit name automatically appearing in the run reports. Please note that kit content is calculated for manual use. While automating on the QIAcube Connect, the processed sample number could be less than that stated in the kit handbook or on the kit label.

QIAcube Connect

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 (SDSs), available from the product supplier.

For all protocols

- Pipettes and pipette tips
- Vortexer
- Ultrapure water
- Glassware for reconstitution of buffers (see "Important Notes" on page 15 for more information)
- Ethanol (96–100%)*
- **Optional**: RNase A (100 mg/mL; cat. no. 19101)
- **Optional**: Waste Tubes (2 mL) (cat. no. 19211), in case user prefers to use a new Waste Tube for each washing step. We recommend to reuse the same Waste Tube throughout the procedure to reduce plastic consumption.
- Microcentrifuge tubes for elution (1.5 or 2 mL)
- Microcentrifuge with rotor for 1.5 and 2 mL tubes
- Thermomixer, shaking water bath or rocking platform for heating at 56°C

For blood and cultured cells

- PBS, pH 7.2 (50 mM potassium phosphate, 150 mM NaCl)

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

For pretreatment of Gram-positive bacteria

(page 41)

- Enzymatic lysis buffer:
- 20 mM Tris·Cl, pH 8.0
- 2 mM sodium EDTA
- 1.2% Triton® X-100
- Immediately before use, add lysozyme to 20 mg/mL

Important Notes

Working with QIAwave products

Preparation of functional buffers

Kit (cat. no.)	Final buffer	Buffer*	Ultrapure water	Ethanol (96–100%) (mL)	Final volume (mL)
69554	AW1	AW1/C	–	20	35
	AW2	AW2/C	15	40	56.5
	AE	AE/C	22	–	24
69556	AW1	AW1/C	–	130	228
	AW2	AW2/C	60	160	226
	AE	AE/C	110	–	120

* Use entire volume

Selected buffers are provided as concentrates in 15 mL bottles or 2 mL tubes to shrink bottles and reduce the amount of plastics used. Before using the kit for the first time, concentrates have to be reconstituted to acquire the functional buffer. This is done with either water or water and ethanol. To reconstitute, the entire volume of the buffer concentrate should be transferred from the 15 mL bottle and 2 mL tube into a suitably sized glass bottle, either by using a pipette or by pouring. Subsequently, the appropriate volume of water or water and ethanol should be added as indicated in the table above. Afterwards, the glass bottle should be capped tightly and the reconstituted buffer mixed thoroughly by inverting.

For detailed instructions see "Things to do before starting" above or watch our educational "how-to video" on **qiagen.com/qiawavebuffer**

Water quality used for preparation of functional buffers

We strongly recommend using high purity water for reconstitution. Ultrapure water (also known as type 1 water) with a resistivity of 18.2 MΩ-cm at 25°C, such as from a Milli-Q® system, works well. In case users do not have access to type 1 water, QIAGEN offers nuclease-free water (5 L, cat. no. 129117; 1000 mL, cat. no. 129115). Please note that these items need to be purchased separately. Usage of tap water should be avoided as this can have detrimental impact on the extraction of the target analyte.

Glassware

We suggest the use of glass bottles for the reconstitution of buffers. Glass bottles can be cleaned, sterilized, and reused more easily than plastic bottles, which will further reduce the plastic footprint of the kit.

Glassware should be treated before use to ensure that it is DNase free. Autoclaving is sufficient to inactivate DNases, but will not fully inactive RNases.

If DNase and RNase removal is desired, one of the following procedures should be applied:

- Glassware should be cleaned with a detergent, thoroughly rinsed, the opening covered with aluminum foil, and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Plastic lids can be treated with DEPC (diethyl pyrocarbonate). Incubate lids in 0.1% DEPC solution overnight at room temperature and then dry under the clean fume hood.
- Alternatively, glassware can be treated with DEPC (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 h) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Labeling of functional buffers in glass bottles

Reconstituted buffers from buffer concentrates can be labeled with the additional label supplied with the kit. Use the enclosed label and transfer it onto the glass bottle containing the functional buffer prepared before using the kit for the first time.

Waste Tubes

The newly introduced Waste Tube is made of recycled plastic recovered from post-consumer plastic waste and can differ in color from lot to lot due to slight differences in composition of the raw material. This, however, has no effect on its intended use to collect the flow-through from sample binding and membrane washing. After each of these steps, the flow-through is discarded and the Waste Tube is reused. The Waste Tube is only used for processing waste and never comes into direct contact with the analyte of interest.

For detailed instructions see "Things to do before starting" on page 29 or watch our educational "how-to video" **qiagen.com/qiawavewastetube**

Elution tubes

Elution tubes are not included in the kit. This allows the flexibility to use elution tubes of one's own choice and purchase them in, for example, eco-friendlier big packs.

Recycling information

Please visit **qiagen.com/recycling-card** to learn more about how to recycle kit components.

Sample collection and storage

Best results are obtained with fresh material or material that has been immediately frozen and stored at –90°C to –15°C. Repeated freezing and thawing of stored samples should be

avoided, since this leads to reduced DNA size. Use of poor-quality starting material will also lead to reduced length and yield of purified DNA.

After Proteinase K digestion, tissue samples can also be stored in Buffer ATL for up to 6 months at ambient temperature without any reduction in DNA quality.

For certain bacterial cultures that accumulate large amounts of metabolites and/or form very dense cell walls, it is preferable to harvest cells in the early log phase of growth. Fresh or frozen cell pellets can be used in the procedure.

Starting amounts of samples

The QIAwave DNA Blood & Tissue procedure give DNA yields that increase linearly over a wide range, for both very small and large sample sizes (e.g., from as little as 100 cells up to 5 x 10^6 cells).

Maximum amount of starting material

To obtain optimum DNA yield and quality, it is important not to overload the DNeasy Mini Spin Column, as this can lead to significantly lower yields than expected (see Figure 1 on the facing page). For samples with very high DNA contents (e.g., spleen, which has a high cell density, and cell lines with a high degree of ploidy), less than the recommended amount of sample listed in Table 1 (page 20) should be used. If your starting material is not shown in Table 3 (page 26) and you have no information regarding DNA content, we recommend beginning with half the maximum amount of starting material indicated in Table 1 (page 20). Depending on the yield obtained, the sample size can be increased in subsequent preparations.

Very small sample sizes

The QIAwave DNA Blood & Tissue procedure is also suitable for purifying DNA from very small amounts of starting material. If the sample has less than 5 ng DNA (<10,000 copies), 3–5 µg carrier DNA (a homopolymer, such as poly-dA, poly-dT, or gDNA) should be added to the starting material. Ensure that the carrier DNA does not interfere with your downstream application. To prevent any interference of the carrier with the downstream application, an RNA carrier can be used. This can be removed later by RNase digestion. DNA or RNA homopolymers can be purchased from various suppliers.

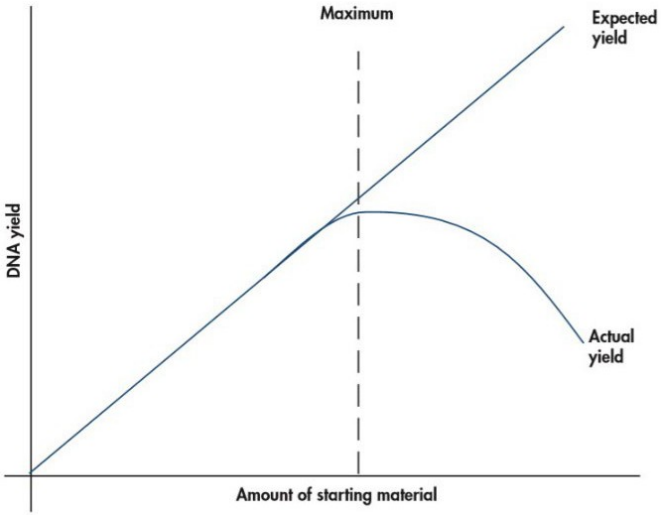


Figure 1. Schematic diagram of effect of sample size on DNA yield. If more than the maximum amount of starting material is used, DNA yield will be lower than expected.

Table 1. Maximum amounts of starting material

Sample	Amount
Animal tissue (see Table 3)	25 mg (spin-column protocols)
Mammalian blood (see Table 4)	100 µL
Bird or fish blood (with nucleated erythrocytes)	10 µL
Mouse tail	0.6–1.2 cm
Rat tail	0.6 cm
Cultured cells	5×10^6
Bacteria	2×10^9

Quantification of starting material

Weighing tissue or counting cells is the most accurate way to quantify starting material. However, the approximate guidelines given below can also be followed.

Animal tissue

A 2 mm cube (approx. this size: ■; volume, approx. 8 mm^3) of most animal tissues weighs approximately 10–15 mg.

Cells from cell culture

The number of HeLa cells obtained in various culture dishes after confluent growth is given in Table 2.

Table 2. Growth area and number of HeLa cells in various culture dishes

Cell culture vessel	Growth area* (cm^2)	Number of cells†
Multiwell plates		
96-well	0.32–0.6	4–5 x 10^4
48-well	1	1 x 10^5
24-well	2	2.5 x 10^5
12-well	4	5 x 10^5
6-well	9.5	1 x 10^6
Dishes		
35 mm	8	1 x 10^6
60 mm	21	2.5 x 10^6
100 mm	56	7 x 10^6
145–150 mm	145	2 x 10^7
Flasks		
40–50 mL	25	3 x 10^6
250–300 mL	75	1 x 10^7
650–750 mL	162–175	2 x 10^7

* Per well, if multiwell plates are used; varies slightly depending on the supplier.

† Cell numbers given are for HeLa cells (approx. length = 15 µm) assuming confluent growth. Cell numbers vary since animal cells can vary in length from 10 to 100 µm.

Bacteria

Bacterial growth is usually measured using a spectrophotometer. However, it is very difficult to give specific and reliable recommendations for the correlation between OD values and cell numbers in bacterial cultures. Cell density is influenced by a variety of factors (e.g., species, media, and shaker speed) and OD readings of cultures measure light scattering rather than absorption. Measurements of light scattering are highly dependent on the distance between

the sample and the detector, and therefore, readings vary between different types of spectrophotometer. In addition, different species show different OD values at defined wavelengths (e.g., 600 or 436 nm).

We therefore recommend calibrating the spectrophotometer used by comparing OD measurements at appropriate wavelengths with viable cell densities determined by plating experiments (e.g., see Ausubel, F.M. et al., eds. [1991] Current Protocols in Molecular Biology, New York: John Wiley & Sons, Inc.). OD readings should be between 0.05 and 0.3 to ensure significance. Samples with readings above 0.3 should be diluted so that the readings fall within this range and the dilution factor used in calculating the number of cells per milliliter.

The following calculation can be considered as a rough guide, which may be helpful. An *E. coli* culture of 1 x 10^9 cells per milliliter, diluted 1 in 4, gives OD_{600} values of 0.25 measured using a Beckman DU®-7400, or 0.125 using a Beckman DU-40 spectrophotometer. These correspond to calculated OD values of 1.0 or 0.5, respectively, for 1 x 10^9 cells per milliliter.

Preparation of Buffer AW1, Buffer AW2, and Buffer AE

Buffer AW1, Buffer AW2, and Buffer AE are supplied as concentrates. Before using for the first time, mix with ultrapure water and/or ethanol (96–100%) as indicated on the bottle and shake thoroughly. For detailed instructions, see Things to do before starting sections.

Buffer AW1 and Buffer AW2 are stable for at least 1 year after the addition of ethanol when stored closed at room temperature.

Buffer AL

Purification of DNA from animal blood, cultured cells, or Gram-positive bacteria

Buffer AL must be added to the sample and incubated at 56°C before ethanol is added. Ensure that ethanol has not been added to Buffer AL beforehand. Buffer AL can be purchased separately (see "Ordering Information" starting on page 49).

Purification of DNA from animal tissues

Buffer AL and ethanol (96–100%) are added in the same step. Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples.

Buffer AL is stable for 1 year after the addition of ethanol when stored closed at room temperature.

Proteinase K

The QIAwave DNA Blood & Tissue Kit contains ready-to-use Proteinase K supplied in a specially formulated storage buffer. The activity of Proteinase K is 600 mAU/mL solution (or 40 mAU/mg protein) and has been chosen to provide optimal results.

Also included in the kits is an optimized buffer for tissue lysis, Buffer ATL. To enable efficient lysis, it is advisable to cut animal tissue into small pieces. If desired, lysis time can be reduced to 20 minutes by grinding the sample in liquid nitrogen* before the addition of Buffer ATL and Proteinase K. Alternatively, tissue samples can be effectively disrupted before Proteinase K digestion using a rotor–stator homogenizer, such as the TissueRuptor® II, or a bead mill, such as the TissueLyser III or TissueLyser II. A supplementary protocol for simultaneous disruption of

*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.

up to 48 tissue samples using the TissueLyser II can be obtained by contacting QIAGEN Technical Services (**support.qiagen.com**).

Copurification of RNA

The QIAwave DNA Blood & Tissue Kit copurifies DNA and RNA when both are present in the sample. Transcriptionally active tissues, such as liver and kidney, contain high levels of RNA, which will be copurified. RNA may inhibit some downstream enzymatic reactions, although it does not affect PCR. If RNA-free genomic DNA is required, RNase A should be added to the sample before addition of Buffer AL to digest the RNA. DNeasy protocols describe the use of an RNase A stock solution of 100 mg/mL. However, the amounts of RNase can be adjusted with less concentrated stock solutions, but not more than 20 µL of RNase solution should be used. Refer to the protocols for details.

Elution of pure nucleic acids

Purified DNA is eluted from the DNeasy Mini Spin Column in either Buffer AE or water. For maximum DNA yield, elution is performed in two successive steps using 200 µL Buffer AE each. For more concentrated DNA, elution can be performed in two successive steps of 100 µL each. Keep in mind that elution volume and number of elution steps depends on the amount of DNA bound to the DNeasy membrane (see Figure 2).

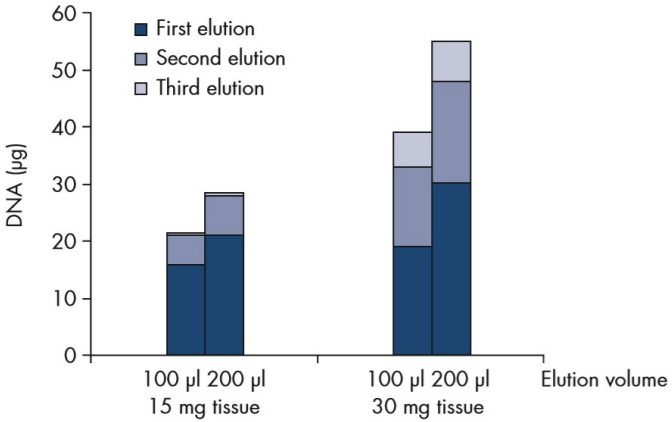


Figure 2. Yields of total nucleic acids in successive elutions of 100 or 200 µL.

For samples containing up to 10 µg DNA, a single elution step using 200 µL is sufficient. For samples containing more than 10 µg DNA, a second elution step with another 200 µL Buffer AE is recommended. Approximately 60–80% of the DNA will elute in the first elution. If less than 30 µg DNA is bound to the DNeasy membrane, elution in 3 x 200 µL may increase yield (Figure 2).

Elution in 100 µL increases the DNA concentration in the eluate but reduces overall DNA yield. To prevent dilution of the first eluate, the subsequent elution step can be performed using a fresh 1.5 mL microcentrifuge tube. More than 200 µL should not be eluted into a 1.5 mL microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation.

For very small samples (containing less than 1 µg DNA), only one elution in 50 µL of Buffer AE or water is recommended.

Buffer AE guarantees optimal recovery and stability of eluted DNA. However, if you wish to elute DNA with water, please ensure that the pH of the water is at least 7.0 (deionized water

from certain sources can be acidic). For long-term storage of DNA, elution in Buffer AE is strongly recommended because DNA stored in water is subject to acid hydrolysis. Buffer AE should be used at room temperature. Heating Buffer AE before elution is not necessary.

Expected yields

Yields of genomic DNA will vary from sample to sample, depending on the amount and type of material processed. In addition, the quality of starting material will affect DNA yield.

Tables 3 and 4 can be used to provide an estimate of expected yield.

Table 3. Typical DNA yields from animal tissues and cells

Source	Amount	DNA (µg)
Lymphocytes	5 x 10^6	15–25
HeLa cells	2 x 10^6	15–25
Liver	25 mg	10–30
Brain	25 mg	15–30
Lung	25 mg	5–10
Heart	25 mg	5–10
Kidney	25 mg	15–30
Spleen	10 mg	5–30
Mouse tail	1.2 cm (tip)	10–25
Rat tail	0.6 cm (tip)	20–40
Pig ear	25 mg	10–30
Horsehair	10 hairs	2–4
Fish fin	20 mg	10–20
Fish spawn (mackerel)	10 mg	5–10

Table 4. Typical DNA yields from animal blood

Source	Amount	DNA (µg)
Cattle	100	4–5
Horse	100	3–5
Pig	100	3–6
Sheep	100	3–6
Dog	100	4–5
Cat	100	3–6
Goat	50*	3
Chicken†	5	9–15

* Using more than 50 µL goat blood gave no significant increase in DNA yield.

† Bird blood contains nucleated erythrocytes, giving higher DNA yields than mammalian blood.

Purification of high-molecular-weight DNA

QIAGEN Genomic-tips and Blood & Cell Culture DNA Kits are recommended for large-scale purification of high-molecular-weight DNA (see "Ordering Information" starting on page 49). QIAGEN Genomic- tips are available for purification of up to 500 µg of genomic DNA ranging in size from 50 to 150 kb. They are highly suited for use in Southern blotting, library construction, genome mapping, and other demanding applications. QIAGEN also offers the MagAttract® HMW DNA Kit enables purification of high-molecular-weight (100–200 kb) DNA using a simple, magnetic bead-based protocol.

Please contact QIAGEN Technical Services at **support.qiagen.com** for more information.

Protocol: Purification of Total DNA from Animal Blood or Cells

This protocol is designed for purification of total DNA from animal blood (with nucleated or nonnucleated erythrocytes) or from cultured animal or human cells.

Important points before starting

- If using the QIAwave DNA Blood & Tissue Kit for the first time, read "Important Notes" on page 15.
- All centrifugation steps are carried out at room temperature in a microcentrifuge.
- Vortexing should be performed by pulse-vortexing for 5–10 s.
- PBS is required for use in step 1 (see page 13 for composition). Buffer ATL is not required in this protocol.
- **Optional**: RNase A may be used to digest RNA during the procedure. RNase A is not provided in the QIAwave DNA Blood & Tissue Kit (see "Copurification of RNA" on page 24).

Things to do before starting

Table 5. Preparation of Buffer concentrates

Kit (cat. no.)	Final buffer	Buffer*	Ultrapure water (mL)	Ethanol (96–100%) (mL)	Final volume (mL)
69554	AW1	AW1/C	–	20	35
	AW2	AW2/C	15	40	56.5
	AE	AE/C	22	–	24
69556	AW1	AW1/C	–	130	228
	AW2	AW2/C	60	160	226
	AE	AE/C	110	–	120

* Use entire volume.

- Buffer AL may form a precipitate upon storage. If necessary, warm to 56°C until the precipitate has fully dissolved.
- **Preparation of Buffer AW1 (kit cat. no. 69554)**: Transfer the entire volume of Buffer AW1/C from the 15 mL bottle into a glass bottle larger than 35 mL, either by using a pipette or by pouring. Add 20 mL ethanol (96–100%) to Buffer AW1 /C to obtain a final volume of 35 mL. Cap the glass bottle tightly and mix thoroughly by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer AW1 (kit cat. no. 69556)** : Transfer the entire volume of Buffer AW1/C from the 125 mL bottle into a glass bottle larger than 230 mL, either by using a pipette or by pouring. Add 130 mL ethanol (96–100%) to Buffer AW1/C to obtain a final volume of 228 mL. Cap the glass bottle tightly and mix thoroughly by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer AW2 (kit cat. no. 69554)**: Transfer the entire volume of Buffer AW2/C from the 2 mL tube into a glass bottle larger than 57 mL, either by using a pipette

or by pouring. Add 15 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 L, cat. no. 129117) and 40 mL ethanol (96–100%) to obtain a final volume of 56.5 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.

- **Preparation of Buffer AW2 (kit cat. no. 69556)**: Transfer the entire volume of Buffer AW2/C from the 15 mL bottle into a glass bottle larger than 230 mL, either by using a pipette or by pouring. Add 60 mL ultrapure water such as Nuclease-Free water (1000 mL, cat. no. 129115; 5 L, cat. no. 129117) and 160 mL ethanol (96–100%) to obtain a final volume of 226 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer AE (kit cat. no. 69554)**: Transfer the entire volume of Buffer AE/C from the 2 mL tube into a glass bottle larger than 24 mL, either by using a pipette or by pouring. Add 22 mL ultrapure water such as Nuclease- free Water (1000 mL, cat. no.129115; or 5 L, cat. no. 129117) to obtain a final volume of 24 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- **Preparation of Buffer AE (kit cat. no. 69556)**: Transfer the entire volume of Buffer AE/C from the 15 mL bottle into a glass bottle larger than 120 mL, either by using a pipette or by pouring. Add 110 mL ultrapure water such as nuclease- free water (1000 mL, cat. no. 129115; 5 L, cat. no. 129117) to obtain a final volume of 120 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- Preassemble DNeasy Mini Spin Columns with Waste Tubes (provided).
- Preheat a thermomixer, shaking water bath, or rocking platform to 56°C for use in step 2.

Procedure

1. For blood with nonnucleated erythrocytes, follow step 1a; for blood with nucleated erythrocytes, follow step 1b; for cultured cells, follow step 1c. Blood from mammals contains nonnucleated erythrocytes. Blood from animals, such as birds, fish, or frogs, contains nucleated erythrocytes.

   a. Nonnucleated: Pipette 20 µL Proteinase K into a 1.5 or 2 mL microcentrifuge tube (not provided). Add 50–100 µL anticoagulated blood. Adjust the volume to 220 µL with PBS. Continue with step 2.

      Optional: If RNA-free genomic DNA is required, add 4 µL RNase A (100 mg/mL) and incubate for 2 min at room temperature before continuing with step 2.

   b. Nucleated: Pipette 20 µL Proteinase K into a 1.5 or 2 mL microcentrifuge tube (not provided). Add 5–10 µL anticoagulated blood. Adjust the volume to 220 µL with PBS. Continue with step 2.

      Optional: If RNA-free genomic DNA is required, add 4 µL RNase A (100 mg/mL) and incubate for 2 min at room temperature before continuing with step 2.

   c. Cultured cells: Centrifuge the appropriate number of cells (maximum 5 x 10^6) for 5 min at 300 x *g*. Resuspend the pellet in 200 µL PBS. Add 20 µL Proteinase K. Continue with step 2.

      When using a frozen cell pellet, allow cells to thaw before adding PBS until the pellet can be dislodged by gently flicking the tube.

      Ensure that an appropriate number of cells is used in the procedure. For cell lines with a high degree of ploidy (e.g., HeLa cells), it is recommended to use less than the maximum number of cells listed in Table 1 (page 28).

Optional: If RNA-free genomic DNA is required, add 4 µL RNase A (100 mg/mL), mix by vortexing, and incubate for 2 min at room temperature before continuing with step 2.

2. Add 200 µL Buffer AL (without added ethanol). Mix thoroughly by vortexing, and incubate at 56°C for 10 min.

   Note: Ensure that ethanol has not been added to Buffer AL (see "Buffer AL" on page 23). Buffer AL can be purchased separately (see "Ordering Information" starting on page 49).

   Important: The sample and Buffer AL are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution.

3. Add 200 µL ethanol (96–100%) to the sample, and mix thoroughly by vortexing.

   Important: The sample and the ethanol are mixed thoroughly to yield a homogeneous solution.

4. Pipette the mixture from step 3 into the DNeasy Mini Spin Column placed in a 2 mL Waste Tube (provided). Centrifuge at ≥6000 x *g* (8000 rpm) for 1 min. Discard the flow-through and reuse the Waste Tube*.

5. Place the DNeasy Mini Spin Column in the Waste Tube from step 4, add 500 µL Buffer AW1, and centrifuge for 1 min at ≥6000 x *g* (8000 rpm). Discard the flow-through and reuse the Waste Tube*.

6. Place the DNeasy Mini Spin Column in the Waste Tube from step 5, add 500 µL Buffer AW2, and centrifuge for 3 min at 20,000 x *g* (14,000 rpm) to dry the DNeasy membrane. Discard the flow-through and Waste Tube.

*Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 5 for Safety Information.

Important: Dry the membrane of the DNeasy Mini Spin Column because residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.

Following the centrifugation step, remove the DNeasy Mini Spin Column carefully so that the column does not come into contact with the flow-through, because this will result in carryover of ethanol. If carryover of ethanol occurs, empty the Waste Tube, then reuse it in another centrifugation for 1 min at 20,000 x *g* (14,000 rpm).

7. Place the DNeasy Mini Spin Column in a clean 1.5 or 2 mL microcentrifuge tube (not provided), and pipette 200 µL Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥6000 x *g* (8000 rpm) to elute.

   Elution with 100 µL (instead of 200 µL) not only increases the final DNA concentration in the eluate but also decreases the overall DNA yield (see Table 2 on page 21).

8. **Recommended**: For maximum DNA yield, repeat elution once as described in step 7. This step leads to increased overall DNA yield.

   A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluates, the microcentrifuge tube from step 7 can be reused for the second elution step.

   Note: Do not elute more than 200 µL into a 1.5 mL microcentrifuge tube because the DNeasy Mini Spin Column will come into contact with the eluate.

Protocol: Purification of Total DNA from Animal Tissues

This protocol is designed for purification of total DNA from animal tissues, including rodent tails.

Important points before starting

- If using the QIAwave DNA Blood & Tissue Kit for the first time, read "Important Notes" on page 15.
- All centrifugation steps are carried out at room temperature in a microcentrifuge.
- Vortexing should be performed by pulse-vortexing for 5–10 s.
- **Optional**: RNase A may be used to digest RNA during the procedure. RNase A is not provided in the QIAwave DNA Blood & Tissue (see "Copurification of RNA" on page 24).

Things to do before starting

Table 6. Preparation of Buffer concentrates

Kit (cat. no.)	Final buffer	Buffer*	Ultrapure water (mL)	Ethanol (96–100%) (mL)	Final volume (mL)
69554	AW1	AW1/C	–	20	35
	AW2	AW2/C	15	40	56.5
	AE	AE/C	22	–	24
69556	AW1	AW1/C	–	130	228
	AW2	AW2/C	60	160	226
	AE	AE/C	110	–	120

* Use entire volume.

- Buffer ATL and Buffer AL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.
- Preparation of Buffer AW1 (kit cat. no. 69554): Transfer the entire volume of Buffer AW1/C from the 15 mL bottle into a glass bottle larger than 35 mL, either by using a pipette or by pouring. Add 20 mL ethanol (96–100%) to Buffer AW1 /C to obtain a final volume of 35 mL. Cap the glass bottle tightly and mix thoroughly by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- Preparation of Buffer AW1 (kit cat. no. 69556): Transfer the entire volume of Buffer AW1/C from the 125 mL bottle into a glass bottle larger than 230 mL, either by using a pipette or by pouring. Add 130 mL ethanol (96–100%) to Buffer AW1/C to obtain a final volume of 228 mL. Cap the glass bottle tightly and mix thoroughly by inverting the bottle several times. To label the glass bottle,use the enclosed label and transfer it onto the glass bottle.
- Preparation of Buffer AW2 (kit cat. no. 69554): Transfer the entire volume of Buffer AW2/C from the 2 mL tube into a glass bottle larger than 57 mL, either by using a pipette or by pouring. Add 15 mL ultrapure water such as Nuclease-Free Water (1000 mL, cat. no. 129115; or 5 L, cat. no. 129117) and 40 mL ethanol (96–100%) to obtain a final volume of 56.5 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- Preparation of Buffer AW2 (kit cat. no. 69556) : Transfer the entire volume of Buffer AW2/C from the 15 mL bottle into a glass bottle larger than 230 mL, either by using a pipette or by pouring. Add 60 mL ultrapure water such as Nuclease-Free water (1000 mL, cat. no. 129115; 5 L, cat. no. 129117) and 160 mL ethanol (96–100%) to obtain a final volume of 226 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.

- Preparation of Buffer AE (kit cat. no. 69554): Transfer the entire volume of Buffer AE/C from the 2 mL tube into a glass bottle larger than 24 mL, either by using a pipette or by pouring. Add 22 mL ultrapure water such as Nuclease- free Water (1000 mL, cat. no.129115; or 5 L, cat. no. 129117) to obtain a final volume of 24 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- Preparation of Buffer AE (kit cat. no. 69556): Transfer the entire volume of Buffer AE/C from the 15 mL bottle into a glass bottle larger than 120 mL, either by using a pipette or by pouring. Add 110 mL ultrapure water such as nuclease-free water (1000 mL, cat. no. 129115; 5 L, cat. no. 129117) to obtain a final volume of 120 mL. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, use the enclosed label and transfer it onto the glass bottle.
- Preheat a thermomixer, shaking water bath, or rocking platform to 56°C for use in step 2. If using frozen tissue, equilibrate the sample to room temperature.
- Avoid repeated thawing and freezing of samples because this will lead to reduced DNA size.
- Preassemble DNeasy Mini Spin Columns with Waste Tubes.
- Preheat a thermomixer, shaking water bath, or rocking platform to 56°C for use in step 2.

Procedure

1. Cut up to 25 mg tissue (up to 10 mg spleen) into small pieces, and place in a 1.5 mL microcentrifuge tube. For rodent tails, place one (rat) or two (mouse) 0.4–0.6 cm lengths of tail into a 1.5 mL microcentrifuge tube. Add 180 µL Buffer ATL. Earmark the animal appropriately.

Ensure that the correct amount of starting material is used (see "Starting amounts of samples" on page 18). For tissues with a very high number of cells for a given mass of tissue, such as spleen, no more than 10 mg starting material should be used.

We strongly recommend cutting the tissue into small pieces to enable more efficient lysis. If desired, lysis time can be reduced by grinding the sample in liquid nitrogen* before addition of Buffer ATL and Proteinase K. Alternatively, tissue samples can be effectively disrupted before Proteinase K digestion using a rotor–stator homogenizer, such as the TissueRuptor II, or a bead mill, such as the TissueLyser III or TissueLyser II (see "Proteinase K" starting on page 23). A supplementary protocol for simultaneous disruption of up to 48 tissue samples using the TissueLyser can be obtained by contacting QIAGEN Technical Services (see the back cover). For rodent tails, a maximum of 1.2 cm (mouse) or 0.6 cm (rat) tail should be used. When purifying DNA from the tail of an adult mouse or rat, it is recommended to use only 0.4–0.6 cm.

2. Add 20 µL Proteinase K. Mix thoroughly by vortexing, and incubate at 56°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample or place in a thermomixer, shaking water bath or on a rocking platform.

   Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h or, for rodent tails, 6–8 h. If it is more convenient, samples can be lysed overnight; this will not affect them adversely.

   After incubation, the lysate may appear viscous but should not be gelatinous as it may clog the DNeasy Mini Spin Column. If the lysate appears very gelatinous, see the "Troubleshooting Guide" for recommendations.

   Optional: If RNA-free genomic DNA is required, add 4 µL RNase A (100 mg/mL), mix by vortexing, and incubate for 2 min at room temperature before continuing with step 3.

*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.

Transcriptionally active tissues, such as liver and kidney, contain high levels of RNA, which will copurify with genomic DNA. For tissues that contain low levels of RNA, such as rodent tails, or, if residual RNA is not a concern, RNase A digestion is not necessary.

3. Vortex for 15 s. Add 200 µL Buffer AL to the sample, and mix thoroughly by vortexing. Then add 200 µL ethanol (96–100%), and mix again thoroughly by vortexing.

   Important: The sample, Buffer AL, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples.

   A white precipitate may form on addition of Buffer AL and ethanol. This precipitate does not interfere with the DNeasy procedure. Some tissue types (e.g., spleen and lung) may form a gelatinous lysate after addition of Buffer AL and ethanol. In this case, vigorously shaking or vortexing the preparation is recommended.

4. Pipette the mixture from step (including any precipitate) into the DNeasy Mini Spin Column placed in a 2 mL Waste Tube (provided). Centrifuge at ≥6000 x *g* (8000 rpm) for 1 min. Discard the flow-through and reuse the Waste Tube*.

5. Place the DNeasy Mini Spin Column in the Waste Tube from step 4, add 500 µL Buffer AW1, and centrifuge for 1 min at ≥6000 x *g* (8000 rpm). Discard the flow-through and reuse the Waste Tube*

6. Place the DNeasy Mini Spin Column the Waste Tube from step 5, add 500 µL Buffer AW2, and centrifuge for 3 min at 20,000 x *g* (14,000 rpm) to dry the DNeasy membrane. Discard the flow-through and Waste Tube.

*Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 5 for Safety Information.

Important: Dry the membrane of the DNeasy Mini Spin Column because residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.

Following the centrifugation step, remove the DNeasy Mini Spin Column carefully so that the column does not come into contact with the flow-through because this will result in carryover of ethanol. If carryover of ethanol occurs, empty the Waste Tube, then reuse it in another centrifugation for 1 min at 20,000 x *g* (14,000 rpm).

Place the DNeasy Mini Spin Column in a clean 1.5 mL or 2 mL microcentrifuge tube (not provided), and pipette 200 µL Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥6000 x *g* (8000 rpm) to elute.

Elution with 100 µL (instead of 200 µL) not only increases the final DNA concentration in the eluate but also decreases the overall DNA yield (see Table 2 on page 21).

7. **Recommended**: For maximum DNA yield, repeat elution once as described in step 7. This step leads to increased overall DNA yield.

   A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluates, the microcentrifuge tube from step 7 can be reused for the second elution step.

   Note: Do not elute more than 200 µL into a 1.5 mL microcentrifuge tube because the DNeasy Mini Spin Column will come into contact with the eluate.

Protocol: Pretreatment for Gram-Negative Bacteria

This protocol is designed for purification of total DNA from Gram-negative bacteria, such as *E. coli*. The protocol describes the preliminary harvesting of bacteria before DNA purification.

Important point before starting

- See "Quantification of starting material" on page 20 for details of how to collect and store samples, and how to determine the number of cells in a bacterial culture

Procedure

1. Harvest cells (maximum 2 x 10^9 cells) in a microcentrifuge tube by centrifuging for 10 min at 5000 x *g* (7500 rpm). Discard supernatant.
2. Resuspend pellet in 180 µL Buffer ATL.
3. Continue with step of "Purification of Total DNA from Animal Tissues" on page 34.

Protocol: Pretreatment for Gram-Positive Bacteria

This protocol is designed for purification of total DNA from Gram-positive bacteria, such as *Corynebacterium* spp. and *Bacillus subtilis*. The protocol describes the preliminary harvesting of bacteria and incubation with lysozyme to lyse their cell walls before DNA purification.

Important points before starting

- See "Quantification of starting material" on page 20 for details of how to collect and store samples, and how to determine the number of cells in a bacterial culture.
- Ensure that ethanol has not been added to Buffer AL (see "Buffer AL" on page 23). Buffer AL can be purchased separately (see "Ordering Information" starting on page 49).

Things to do before starting

- Prepare enzymatic lysis buffer as described in "Equipment and Reagents to Be Supplied by User" on page 13.
- Preheat a heating block or water bath to 37°C for use in step 3.

Procedure

1. Harvest cells (maximum 2 x 10^9 cells) in a microcentrifuge tube by centrifuging for 10 min at 5000 x *g* (7500 rpm). Discard supernatant.
2. Resuspend bacterial pellet in 180 µL enzymatic lysis buffer.
3. Incubate for at least 30 min at 37°C.

   After incubation, heat the heating block or water bath to 56°C if it is to be used for the incubation in step 5.

4. Add 25 µL Proteinase K and 200 µL Buffer AL (without ethanol). Mix by vortexing.

   Note: Do not add Proteinase K directly to Buffer AL. Ensure that ethanol has not been added to Buffer AL (see "Buffer AL" on page 23). Buffer AL can be purchased separately (see "Ordering Information" starting on page 49).

5. Incubate at 56°C for 30 min.

   Optional: If required, incubate at 95°C for 15 min to inactivate pathogens. Note that incubation at 95°C can lead to some DNA degradation.

   Add 200 µL ethanol (96–100%) to the sample, and mix thoroughly by vortexing.

   It is important that the sample and the ethanol are mixed thoroughly to yield a homogeneous solution.

   A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the DNeasy Mini Spin Column. This precipitate does not interfere with the DNeasy procedure.

6. Continue with step 4 of "Protocol: Purification of Total DNA from Animal Tissues" on page 34.

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** (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Low yield	
a) Storage of starting material	DNA yield is dependent on the type, size, age, and storage of starting material. Lower yields will be obtained from material that has been inappropriately stored (see "Sample collection and storage" on page 17).
b) Too much starting material	In future preparations, reduce the amount of starting material used (see "Quantification of starting material" on page 20).
c) Insufficient mixing of sample with Buffer AL and ethanol before binding	DNeasy Mini Spin Column protocols: In future preparations, mix sample first with Buffer AL and then with ethanol by pulse vortexing for 15 seconds each time before applying the sample to the DNeasy Mini Spin Column.
d) DNA inefficiently eluted	Increase elution volume to 200 µL and perform another elution step. See also "Elution of pure nucleic acids" on page 24). Check that ethanol was added before applying the sample to the DNeasy Mini Spin Column. Check that any precipitate in Buffer ATL and/or Buffer AL was dissolved before use.
e) Buffer AW1 or Buffer AW2 or Buffer AE prepared incorrectly	Make sure that ultrapure water and/or ethanol has been added to Buffer AW1/C, Buffer AW2/C, and Buffer AE/C before use (see "Things to do before starting", pages 29 and 34.
f) Water used instead of Buffer AE for elution	The low pH of deionized water from some water purifiers may reduce DNA yield. When eluting with water, ensure that the pH of the water is at least 7.0.

	Comments and suggestions
g) Animal tissue: Insufficient lysis	In future preparations, reduce the amount of starting material used (see "Quantification of starting material" on page 20). Cut tissue into smaller pieces to facilitate lysis. After lysis, vortex sample vigorously; this will not damage or reduce the size of the DNA. If a substantial gelatinous pellet remains after incubation and vortexing, extend incubation time at 56°C for Proteinase K digest and/or increase amount of Proteinase K to 40 µL. Ensure that the sample is fully submerged in the buffer containing Proteinase K. If necessary, double the amount of Buffer ATL and Proteinase K, and use a 2 mL microcentrifuge tube for lysis. Remember to adjust the amount of Buffer AL and ethanol proportionately in subsequent steps. (For example, a lysis step with 360 µL Buffer ATL plus 40 µL Proteinase K will require 400 µL Buffer AL plus 400 µL ethanol to bind DNA to the DNeasy membrane). Pipette the sample into the DNeasy Mini Spin Column in two sequential loading steps. Discard flow-through between these loading steps.
h) Bacteria: Insufficient lysis	In future preparations, extend incubation with cell-wall–lysing enzyme and/or increase amount of lysing enzyme. Harvest bacteria during early log phase of growth (see "Sample collection and storage" on page 17).
i) DNA not bound to DNeasy Mini Spin Column	Check that ethanol was added before applying the sample to the DNeasy Mini Spin Column.
DNeasy Mini SPin Column clogged	
Too much starting material and/or insufficient lysis	Increase *g*-force and/or duration of centrifugation step. In future pre- parations, reduce thse amount of starting material used (see "Quantification of starting material" on page 20). For rodent tails or bacteria, see also "Insufficient lysis" in the "Low yield" section above.
Low concentration of DNA in the eluate	
Second elution step diluted in the DNA	Use a new collection tube for the second eluate to prevent dilution of the first eluate. Reduce elution volume to 50–100 µL. See "Elution of pure nucleic acids" on page 24.
$A_{260}/A280$ ratio of purified DNA is low	
a) Water used instead of buffer to measure A_{260}/A_{280}	Use 10 mM Tris·Cl, pH 7.5 instead of water to dilute the sample before measuring purity. See "Appendix: Determination of Yield, Purity, and Length of DNA" on page 47.

	Comments and suggestions
b) Inefficient cell lysis	See “Low yield” above.
A260/A280 ratio of purified DNA is high	
High level of residual RNA	Perform the optional RNase treatment in the protocol.
DNA does not perform well in downstream applications	
a) Salt carryover	Ensure that Buffer AW2 has been used at room temperature. Ensure that Buffer AW1 and Buffer AW2 were added in the correct order.
b) Ethanol carryover	Ensure that, when washing with Buffer AW2, the column is centrifuged for 3 min at 20,000 x *g* (14,000 rpm) to dry the DNeasy membrane. Following the centrifugation step, remove the DNeasy Mini Spin Column carefully so that the column does not come into contact with the flow-through. If ethanol is visible in the DNeasy Mini Spin Column (as either drops or a film), discard the flow-through, keep the Waste tube, and centrifuge for an additional 1 min at 20,000 x *g*.
C) Too much DNA used	For PCR applications, a single-copy gene can typically be detected after 35 PCR cycles with 100 ng template DNA.
Sheared DNA	
a) Sample repeatedly frozen and thawed	Avoid repeated freezing and thawing of starting material.
b) Sample too old	Old samples often yield only degraded DNA.
White precipitate in Buffer ATL or Buffer AL	
White precipitate may form at low temperature after prolonged storage	Any precipitate formed when Buffer ATL or Buffer AL are added must be dissolved by incubating the buffer at 56°C until it disappears.
Discolored membrane after wash with Buffer AW2 or colored eluate	
a) Rodent tails: Hair not removed from rodent tails during preparation	In future preparations, centrifuge lysate for 5 min at 20,000 x *g* after digestion with Proteinase K. Transfer supernatant into a new tube before proceeding with step 3.
b) Animal blood: Contamination with hemoglobin	Reduce amount of blood used and/or double the amount of Proteinase K used per preparation. Try using buffy coat instead of whole blood.

References

1. Wilfinger, W.W., Mackey, M., and Chomcynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *BioTechniques 22*, 474.

2. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., & Struhl, K. (2003). Current protocols in molecular biology, vol. 1 John Wiley & Sons. Inc, Brooklyn, New York, 3(1), 1994-2005.

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

Determination of yield and purity

DNA yield is determined by measuring the concentration of DNA in the eluate by its absorbance at 260 nm. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly. Measure the absorbance at 260 nm or scan absorbance from 220 to 330 nm (a scan will show if there are other factors affecting absorbance at 260 nm; for instance, absorbance at 325 nm would indicate contamination by particulate matter or a dirty cuvette). An A_{260} value of 1 (with a 1 cm detection path) corresponds to 50 µg DNA per milliliter water. Water should be used as diluent when measuring DNA concentration since the relationship between absorbance and concentration is based on extinction coefficients calculated for nucleic acids in water (Wilfinger et al., 1997). Both DNA and RNA are measured with a spectrophotometer at 260 nm; to measure only DNA in a mixture of DNA and RNA, a fluorimeter must be used.

An example of the calculations involved in DNA quantification is shown below.

Volume of DNA sample = 100 µL

Dilution = 20 µL of DNA sample + 180 µL distilled water (1/10 dilution)

Measure absorbance of diluted sample in a 0.2 mL cuvette

A_{260} = 0.2

Concentration of DNA sample = 50 µg/mL x A_{260} x dilution factor

Concentration of DNA sample = 50 µg/mL x 0.2 x 10

Concentration of DNA sample = 100 µg/mL

Total amount = concentration x volume of sample in milliliters

Total amount = 100 µg/mL x 0.1 mL

Total amount = 10 µg DNA

The ratio of the readings at 260 and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of DNA with respect to contaminants that absorb UV, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination. For accurate values, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5, in which pure DNA has an A_{260}/A_{280} ratio of 1.8–2.0. Always be sure to calibrate the spectrophotometer with the same solution.

Determination of length

The precise length of genomic DNA should be determined by pulse-field gel electrophoresis (PFGE) through an agarose gel. To prepare the sample for PFGE, the DNA should be concentrated by alcohol precipitation and the DNA pellet dried briefly at room temperature for 5–10 minutes. Avoid drying the DNA pellet for more than 10 minutes because overdried genomic DNA is very difficult to redissolve. Redissolve in approximately 30 µL Buffer TE, pH 8.0, for at least 30 minutes at 60°C. Load 3–5 µg of DNA per well. Standard PFGE conditions are as follows:

- 1% agarose gel in 0.5x TBE electrophoresis buffer*
- Switch intervals = 5–40 seconds
- Run time = 17 hours
- Voltage = 170 V

*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.

Ordering Information

Product	Contents	Cat. no.
QIAwave DNA Blood & Tissue Kit (50)	50 DNeasy Mini Spin Columns, Waste Tubes (2 mL), Proteinase K, Buffers	69554
QIAwave DNA Blood & Tissue Kit (250)	250 DNeasy Mini Spin Columns, Waste Tubes (2 mL), Proteinase K, Buffers	69556
QIAwave RNA Mini Kit (50)	50 RNeasy Mini Spin Columns, Waste Tubes (2 mL), RNase-free Reagents and Buffers	74534
QIAwave RNA Mini Kit (250)	250 RNeasy Mini Spin Columns, Waste Tubes (2 mL), RNase-free Reagents and Buffers	74536
QIAwave Plasmid Miniprep Kit (50)	50 QIAprep® 2.0 Spin Columns, Waste Tubes (2 mL), Reagents	27204
QIAwave Plasmid Miniprep Kit (250)	250 QIAprep® 2.0 Spin Columns, Waste Tubes (2 mL), Reagents	27206
Blood & Cell Culture DNA Kit	10 Genomic-tip 500/G, Protease, Buffers	13362
Genomic-tips	25 columns	10223
MagAttract HMW DNA Kit	For 48 DNA preps: MagAttract Suspension G, Buffer ATL, Buffer AL, Buffer MB, Buffer MW1, Buffer PE, Proteinase K, RNase A, Buffer AE, Nuclease-Free Water	67563
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect*	Instrument, connectivity package, 1 year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Reagent bottle racks (3); 200 µL filter-tips (1024); 1000 µL filter-tips (1024); 30 mL reagent bottles (12); rotor adapters (240); rotor adapter holder	990395
Accessories		
Waste Tubes (2 mL)	1000 Waste Tubes (2 mL)	19211

Product	Contents	Cat. no.
Nuclease-Free Water (1000 mL)	1000 mL nuclease-free water prepared without the use of diethylpyrocarbonate (DEPC); provided in a plastic bottle	129115
Nuclease-Free Water (5 liters)	5 liters nuclease-free water prepared without the use of diethylpyrocarbonate (DEPC); Provided in five 1 L bottles, delivered in a cardboard box	129117
TissueRuptor II	Handheld rotor–stator homogenizer	Inquire
TissueRuptor Disposable Probes	25 nonsterile plastic disposable probes for use with the TissueRuptor II	990890
TissueLyser III†	Bead mill; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96 (available separately)	9003240
TissueLyser Adapter Set 2 x 24†	2 sets of Adapter Plates and 2 racks or use with 2.0 mL microcentrifuge tubes on the TissueLyser II	69982
TissueLyser Adapter Set 2 x 96†	2 sets of Adapter Plates for use with Collection Microtubes (racked) on the TissueLyser II	69984
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with the TissueLyser II system	69989
QIAGEN Proteinase K (2 mL)	2 mL (>600 mAU/mL, solution)	19131
QIAGEN Proteinase K (10 mL)	10 mL (>600 mAU/mL, solution)	19133
RNase A (17,500 U)	2.5 mL (100 mg/mL; 7000 units/mL, solution)	19101
Buffer AL (216 mL)	216 mL Lysis Buffer	19075
Starter Pack, QIAcube	Reagent bottle racks (3); 200 µL filter-tips (1024); 1000 µL filter-tips adapter holder	990395

*All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

† Visit **www.qiagen.com/products/accessories** for details on the TissueLyser and accessories.

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Document Revision History

Date	Description
01/2022	Initial release
06/2023	Updated component list and instructions. Updated Ordering Information.
03/2024	Updated "Automated purification of DNA on QIAcube instruments" section with "The QIAwave kits can be run on the QIAcube Connect using either the DNeasy Blood&Tissue Kit protocols or the dedicated QIAwave DNA Blood&Tissue Kit protocols…".
05/2025	Updated the “Glassware” section under “Important Notes”.

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