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QIAamp® DSP DNA Mini Kit Instructions for Use (Handbook)

Version 2

IVD

For In Vitro Diagnostic Use

REF 61304


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Sample to Insight

QIAGEN

Contents

Intended Use

The QIAamp DSP DNA Mini Kit is a system that uses silica-membrane technology (QIAamp technology) for isolation and purification of genomic DNA from biological specimens.

The product is intended to be used by professional users, such as technicians and physicians that are trained in molecular biological techniques.

The QIAamp DSP DNA Mini Kit is intended for in vitro diagnostic use.

Description and Principle

The QIAamp DSP DNA Mini Kit provides fast and easy methods for purification of total DNA for reliable PCR and Southern blotting. Total DNA (e.g., genomic, viral, mitochondrial) can be purified from whole blood, plasma, serum, buffy coat, bone marrow, other body fluids, lymphocytes, cultured cells, tissue, and forensic specimens. The simple QIAamp spin and vacuum procedures, which allow for simultaneous processing of multiple samples, yield purified DNA ready for direct amplification. Some of the QIAamp spin procedures can be automated on the QIAcube or QIAcube Connect MDx for increased standardization and ease of use. The QIAamp procedure is suitable for use with fresh or frozen whole blood and blood which has been treated with EDTA or citrate, but not with heparin. Prior separation of leukocytes is not necessary.

Purification requires no phenol/chloroform extraction or alcohol precipitation, and involves very little handling. DNA is eluted in Elution Buffer (AE), ready for direct addition to PCR or other enzymatic reactions. Alternatively, it can be safely stored at –20°C for later use.

Lysing with proteinase K

The QIAamp DSP DNA Mini Kit contains proteinase K, which is the enzyme of choice for SDS-containing lysis buffers used in the Tissue Protocol, but which performs equally well in the Blood and Body Fluid Protocol. The activity of the proteinase K solution is 600 mAU/ml solution (or 40 mAU/mg protein).

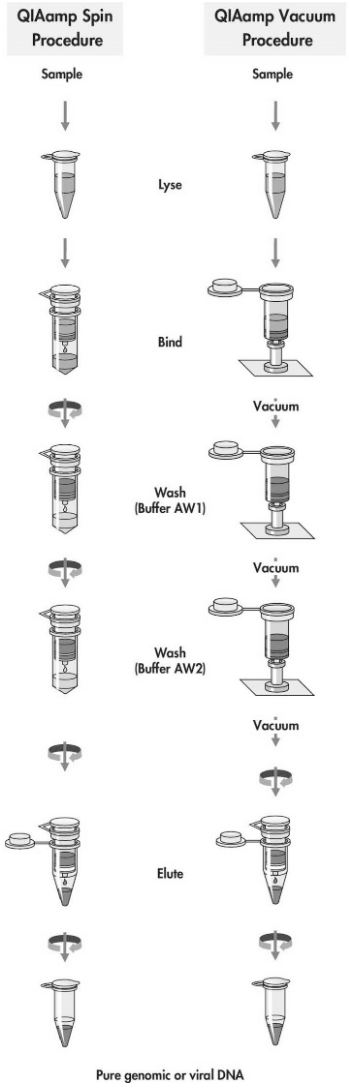

Figure 1. QIAamp DSP DNA Mini Spin and Vacuum Procedures

The QIAamp DSP Spin procedure can be automated on the QIAcube or the QIAcube Connect MDx.

Purification on QIAamp Mini spin columns

The QIAamp DNA purification procedure is carried out using QIAamp Mini spin columns in a standard microcentrifuge, on a vacuum manifold, or fully automated on the QIAcube or QIAcube Connect MDx (see page 7). The procedures are designed to minimize the potential for sample-to-sample cross-contamination and allow safe handling of potentially infectious samples.

QIAamp Mini spin columns fit into most standard microcentrifuge tubes. In the spin protocol, due to the volume of filtrate, 2 ml collection tubes (provided) are required to support the QIAamp Mini spin column during loading and wash steps. For the vacuum protocol, a vacuum manifold (e.g., QIAvac 24 Plus manifold; see "Materials Required But Not Provided", page 10) and a vacuum pump capable of producing a vacuum of –800 to –900 mbar are required. Eluted DNA can be collected in standard 1.5 ml microcentrifuge tubes (provided).

Adsorption to the QIAamp membrane

The lysate buffering conditions are adjusted to allow optimal binding of the DNA to the QIAamp membrane before the sample is loaded onto the QIAamp Mini spin column. DNA is adsorbed onto the QIAamp silica membrane during a brief centrifugation or vacuum step. Salt and pH conditions in the lysate ensure that protein and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not retained on the QIAamp membrane.

Removal of residual contaminants

DNA bound to the QIAamp membrane is washed in 2 centrifugation or vacuum steps. The use of 2 different wash buffers, Buffer AW1 and Buffer AW2, significantly improves the purity of the eluted DNA. Wash conditions ensure efficient removal of any residual contaminants without affecting DNA binding.

Elution of pure nucleic acids

Purified DNA is eluted from the QIAamp Mini spin column in a concentrated form in Elution Buffer (AE). The Elution buffer should be equilibrated to room temperature (15–25°C) before it is applied to the column.

Automated DNA purification on QIAcube/QIAcube Connect MDx

Some QIAamp DSP DNA Mini Kit protocols can be fully automated on the QIAcube and on the QIAcube Connect MDx instruments. The QIAcube and QIAcube Connect MDx perform automated isolation and purification of nucleic acids. It can process of up to 12 samples per single run.

Sample preparation using the QIAcube and the QIAcube Connect MDx follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using the QIAamp DSP DNA Mini Kit for purification of high-quality DNA.

If automating the QIAamp DSP DNA Mini Kit on QIAcube instruments, they instrument may process fewer than 50 samples due to dead volumes, evaporation, and additional reagent consumption by automated pipetting. QIAGEN only guarantees 50 sample preps with manual use of the QIAamp DSP DNA Mini Kit.

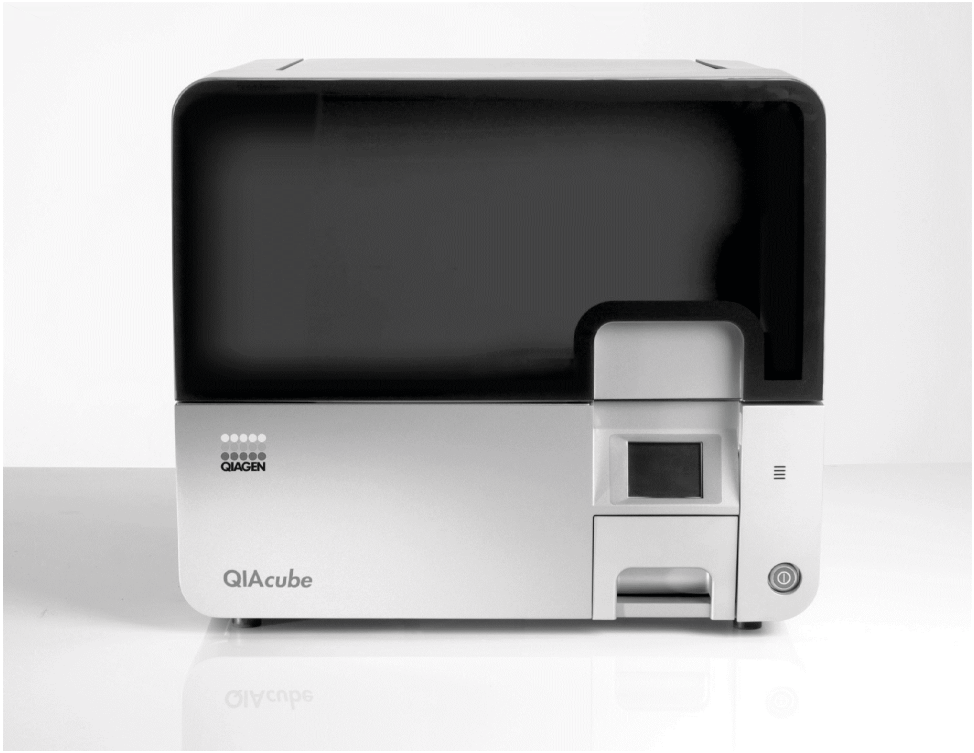

Figure 2. The QIAcube.

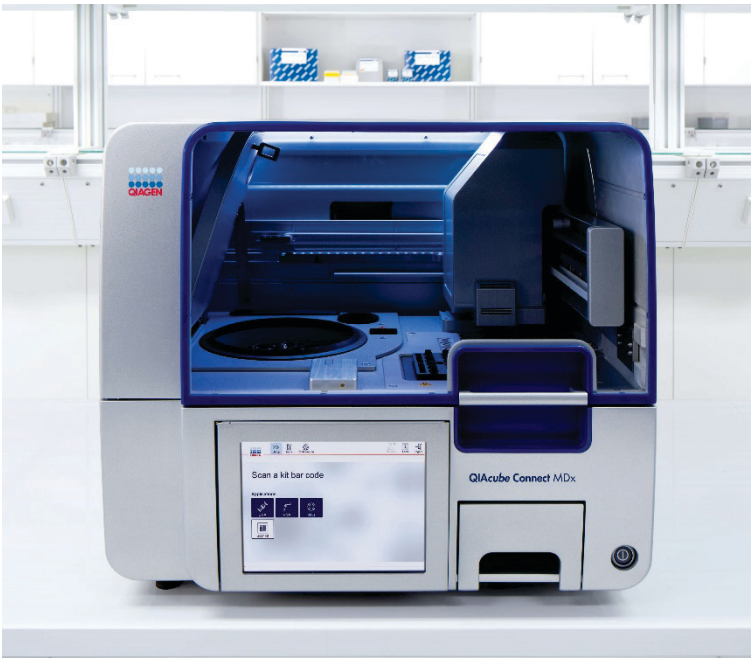

Figure 3. Automated DNA purification. DNA purification using the QIAamp DSP DNA Mini Kit can be automated on the QIAcube Connect MDx.

Summary and explanation

QIAamp DSP DNA Mini Kits provide fast and easy methods for purification of total DNA. Total DNA (e.g., genomic, viral, mitochondrial) can be purified from whole blood, plasma, serum, buffy coat, bone marrow, other body fluids, lymphocytes, cultured cells, and tissue.

The simple QIAamp spin and vacuum procedures (shown on Figure 1) are suitable for simultaneous processing of multiple samples. Some of the QIAamp spin procedures can be fully automated on the QIAcube or QIAcube Connect MDx for increased standardization and ease of use (see page 9).

Materials Provided

Kit contents

QIAamp DSP DNA Mini Kit			
Catalog no.			**61304**
Number of preparations			**50***
QIAamp Mini Spin	QIAamp Mini Spin Columns with Wash Tubes	COL	50
WT	Wash Tubes (2 ml)	WASH TUBE	3 x 50
LT	Lysis Tubes (2 ml)	LYS TUBE	50
ET	Elution Tubes (1.5 ml)	ELU TUBE	50
AL	Lysis Buffer†	LYS BUF	12 ml
ATL	Tissue Lysis Buffer	TIS LYS BUF	10 ml
AW1	Wash Buffer 1† (concentrate)	WASH BUF 1 CONC	19 ml
AW2	Wash Buffer 2‡ (concentrate)	WASH BUF 2 CONC	13 ml
AE	Elution Buffer	ELU BUF	22 ml
	Proteinase K	PROTK	2 ml
	Instructions for Use (Handbook)		

* If automating the QIAamp DSP DNA Mini Kit on the QIAcube or QIAcube Connect MDx instrument, the instrument may process fewer than 50 samples due to dead volumes, evaporation, and additional reagent consumption by automated pipetting. QIAGEN only guarantees 50 sample preps with manual use of the QIAamp DSP DNA Mini Kit.

† Contains chaotropic salt. Not compatible with disinfectants containing bleach. See page 11 for warnings and precautions.

‡ Contains sodium azide as a preservative.

Materials Required but Not Provided

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.

- Ethanol (96–100%)*
- Pipettes and pipette tips with aerosol barrier
- Microcentrifuge (with rotor for 2 ml tubes)
- Vortexer
- Water bath or heating block at 56°C
- Phosphate-buffered saline (PBS) may be required for some samples

For vacuum protocols

- QIAvac 24 Plus vacuum manifold (cat. no. 19413) or equivalent
- VacConnectors (cat. no. 19407)
- Vacuum Regulator (cat. no. 19530) for easy monitoring of vacuum pressures and easy releasing of vacuum
- Vacuum Pump (cat. no. 84010) or equivalent pump capable of producing a vacuum of –800 to –900 mbar
- For buccal swabs or large volumes: Extension Tubes (cat. no. 19587)
- **Optional**: VacValves (cat. no. 19408)
- **Optional**: QIAvac Connecting System (cat. no. 19419)
- **Optional**: RNase A (100 mg/ml; cat. no. 19101)

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

For the automated procedure only

- Rotor Adapters, cat. no. 990394
- Rotor Adapter Holder, cat. no. 990392
- Sample Tubes CB, cat. no. 990382 (sample input tube)
- Sample Tubes RB, cat. no. 990381 (sample input tube for bacterial DNA, tissue)
- Shaker Rack Plugs, cat. no. 9017854 (only if screw cap tubes are used for samples)
- Reagent Bottles, 30 ml, cat. no. 990393
- Filter Tips, 1000 µl, cat. no. 990352
- Filter Tips, 200 µl, cat. no. 990332
- SafeSeal Tube, 1.5 mL, Sarstedt® (cat. no. 72.706)

For tissues

- Additional water bath or heating block at 70°C
- Optional: Equipment for mechanical disruption, such as the TissueRuptor II® (cat. no. 9002755) or mortar, pestle, and liquid nitrogen

For buccal swabs

- 2 ml microcentrifuge tubes
- For cotton or DACRON® swabs: Scissors or appropriate cutting device

Warnings and Precautions

Please be aware that you may be required to report serious incidents that have occurred in relation to the device to the manufacturer and the regulatory authority in which the user and/or the patient is established.

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.

Lysis Buffer (AL) and Wash Buffer 1 (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.

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid personal injury or injury to others.

QIAGEN has not tested the liquid waste generated by the QIAamp DSP DNA Mini procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

Precautions

The following hazard and precautionary statements apply to components of the QIAamp DSP DNA Mini Kit.

Buffer AL

Contains: guanidine hydrochloride; maleic acid. Warning! May be harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. May cause an allergic skin reaction. If eye irritation persists: Get medical advice/attention. Take off contaminated clothing and wash it before reuse. Wear protective gloves/protective clothing/eye protection/face protection.

Buffer ATL

Warning! Causes mild skin irritation. If skin irritation occurs: Get medical advice/attention.

Buffer AW1

Contains: guanidine hydrochloride. Warning! Harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. Call a POISON CENTER or doctor/physician if you feel unwell. Dispose of contents/container to an approved waste disposal plant. Take off contaminated clothing and wash it before reuse. Wear protective gloves/protective clothing/eye protection/face protection.

Proteinase K

Contains: Proteinase K. Danger! Causes mild skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Dispose of contents/container to an approved waste disposal plant. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/ physician. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. Wear respiratory protection.

Reagent Storage and Handling

QIAamp Mini spin columns should be stored at 2–8°C upon arrival. When stored properly, the QIAamp Mini spin columns are stable until the expiration date on the kit box. All buffers and Proteinase K can be stored at room temperature (15–25°C) and are stable until the expiration date on the kit box.

Reconstituted Wash Buffer 1 (AW1) and reconstituted Wash Buffer 2 (AW2) are stable for up to 1 year when stored at room temperature (15–25°C), but only until the expiration date on the kit box.

Specimen Storage and Handling

The QIAamp procedure is suitable for use with fresh or frozen whole blood and blood that has been treated with citrate or EDTA. Prior separation of leukocytes is not necessary. Purification requires no phenol/chloroform extraction or alcohol precipitation, and involves very little handling.

Procedure

Important points before starting

- After receiving the kit, check the kit components for damage. If the blister packs or the buffer bottles are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to "Warnings and Precautions", page 15. Do not use damaged kit components, since their use may lead to poor kit performance.
- Always use RNase-free equipment.
- Always change pipette tips between liquid transfers. To minimize cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- All centrifugation steps are carried out at room temperature (15–25°C).
- Always use disposable gloves and regularly check that they are not contaminated with sample material. Discard gloves if they become contaminated.
- To minimize cross-contamination, open only one tube at a time.
- Do not use kit components from other kits with the kits you are currently using, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To ensure safety from potentially infectious material, we recommend working under laminar airflow conditions until the samples are lysed.
- This kit should only be used by personnel trained in in vitro diagnostic laboratory practice.

Preparing reagents and buffers

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

Buffer AW1 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle. Reconstituted Buffer AW1 is stable for 1 year when stored closed at room temperature (15–25°C), but only until the kit expiration date.

Note: Always mix the reconstituted Wash Buffer 1 (AW1) by inverting the bottle several times before starting the procedure.

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

Buffer AW2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) to Buffer AW2 concentrate as indicated on the bottle. Reconstituted Buffer AW2 is stable for 1 year when stored closed at room temperature (15–25°C), but only until the kit expiration date.

Note: Always mix the reconstituted Wash Buffer 2 (AW2) by inverting the bottle several times before starting the procedure.

* Contains chaotropic salt. Take appropriate laboratory safety measures, and wear gloves when handling. Not compatible with disinfecting agents that contain bleach. See page 14 for Warnings and Precautions.

† Contains sodium azide as a preservative.

Amounts of starting material

Use the amounts of starting material indicated in Table 1.

Table 1. Amounts of starting material for QIAamp Mini procedures

Sample	Amount
Blood, plasma, serum	200 µl
Buffy coat	200 µl
Tissue	25 mg*
Cells (diploid)	5 x 10^6 cells

* When isolating DNA from spleen, 10 mg samples should be used.

Preparation of buffy coat

Buffy coat is a leukocyte-enriched fraction of whole blood. Preparing a buffy-coat fraction from whole blood is simple and yields approximately 5–10 times more DNA than an equivalent volume of whole blood.

Prepare buffy coat by centrifuging whole blood at approximately 2500 x *g* for 10 minutes ± 1 minute at room temperature. After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes.

Handling of QIAamp Mini columns

Due to the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling QIAamp Mini columns to avoid cross contamination between sample preparations:

- Carefully apply the sample or solution to the QIAamp Mini column. Pipet the sample into the QIAamp Mini column without wetting the rim of the column.
- Always change pipette tips between liquid transfers. We recommend the use of aerosol-barrier pipette tips.
- Avoid touching the QIAamp Mini column membrane with the pipette tip.
- After all pulse-vortexing steps, briefly centrifuge the microcentrifuge tubes to remove drops from the inside of the lids.
- Open only one QIAamp Mini column at a time, and take care to avoid generating aerosols.
- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

Centrifugation

All centrifugation steps should be carried out at room temperature (15–25°C).

Centrifugation of QIAamp Mini columns is performed at approximately 6000 x *g* to reduce centrifuge noise. Centrifugation at full speed will not affect DNA yield. Centrifugation at lower speeds is also acceptable, provided that nearly all of each solution is transferred through the QIAamp membrane.

When preparing DNA from buffy coat or lymphocytes, full-speed centrifugation is recommended to avoid clogging.

Processing QIAamp Mini columns using a microcentrifuge (spin protocols)

Close the QIAamp Mini column before placing it in the microcentrifuge. Centrifuge as described above.

- Remove the QIAamp Mini column and collection tube from the microcentrifuge. Place the QIAamp Mini column in a new collection tube. Discard the filtrate and the collection tube. Note that the filtrate may contain hazardous waste and should be disposed of appropriately.
- Open only one QIAamp Mini column at a time, and take care to avoid generating aerosols.
- For efficient parallel processing of multiple samples, fill a rack with collection tubes to which the QIAamp Mini columns can be transferred after centrifugation. Used collection tubes containing the filtrate can be discarded, and the new collection tubes containing the QIAamp Mini columns can be placed directly in the microcentrifuge.

The QIAvac 24 Plus

The QIAvac 24 Plus is designed for fast and efficient vacuum processing of up to 24 QIAGEN spin columns in parallel. Samples and wash solutions are drawn through the column membranes by vacuum instead of centrifugation, providing greater speed and reduced hands-on time in purification procedures.

In combination with the QIAvac Connecting System (optional), the QIAvac 24 Plus can be used as a flow-through system. The sample flow-through is collected in a separate waste bottle.

For maintenance of the QIAvac 24 Plus, please refer to the handling guidelines in the *QIAvac 24 Plus Handbook*.

Processing QIAamp Mini columns on the QIAvac 24 Plus (vacuum protocols)

QIAamp Mini spin columns are processed on the QIAvac 24 Plus using disposable VacConnectors and reusable VacValves. VacValves (optional) are inserted directly into the luer slots of the QIAvac 24 Plus manifold and ensure a steady flow rate, facilitating parallel processing of samples of different natures (e.g., blood and body fluids), volumes, or viscosities. They should be used if sample flow rates differ significantly in order to ensure consistent vacuum. VacConnectors are disposable connectors that fit between QIAamp Mini columns and VacValves or between the QIAamp Mini columns and the luer slots of the QIAvac 24 Plus. They prevent direct contact between the spin column and VacValve during purification and reduce the risk of cross-contamination between samples. VacConnectors are discarded after a single use.

Handling Guidelines for the QIAvac 24 Plus

- Always place the QIAvac 24 Plus on a secure bench top or work area. If dropped, the QIAvac 24 Plus manifold may crack.
- Always store the QIAvac 24 Plus clean and dry. For cleaning procedures see the *QIAvac 24 Plus Handbook.*
- The components of the QIAvac 24 Plus are not resistant to certain solvents (Table 2). If these solvents are spilled on the unit, rinse it thoroughly with water.
- To ensure consistent performance, do not apply silicone or vacuum grease to any part of the QIAvac 24 Plus manifold.
- Always use caution and wear safety glasses when working near a vacuum manifold under pressure.
- Contact QIAGEN Technical Services or your local distributor for information concerning spare or replacement parts.

- The vacuum pressure is the pressure differential between the inside of the vacuum manifold and the atmosphere (standard atmospheric pressure 1013 millibar or 760 mm Hg) and can be measured using the QIAvac Connecting System or a vacuum regulator (see Figure 1). The vacuum protocol requires a vacuum pump capable of producing a vacuum of –800 to –900 mbar (e.g., QIAGEN, Vacuum Pump). Higher vacuum pressures must be avoided. Use of vacuum pressures lower than recommended may reduce DNA yield and purity and increase the frequency of clogged membranes.

Table 2. Chemical resistance properties of the QIAvac 24 Plus

Resistant to:		Not resistant to:
Acetic acid	Chaotropic salts	Benzene
Chromic acid	Concentrated alcohols	Phenol
SDS	Sodium chloride	Chloroform
Tween® 20	Urea	Toluene
Chlorine bleach	Hydrochloric acid	Ethers
Sodium hydroxide		

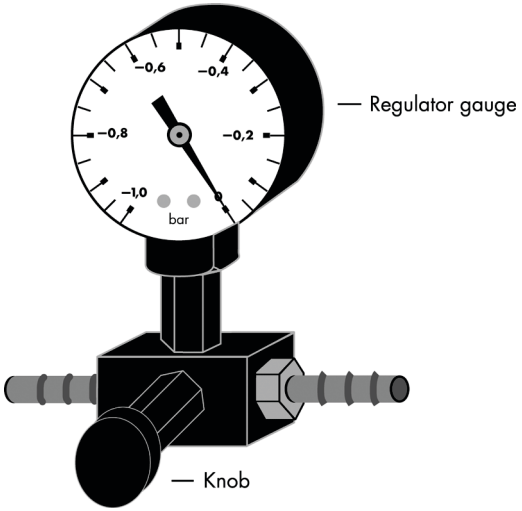

Figure 4. Schematic diagram of the Vacuum Regulator.

Setup of the QIAvac 24 Plus vacuum manifold

1. Connect the QIAvac 24 Plus to a vacuum source. If using the QIAvac Connecting System, connect the system to the manifold and vacuum source as described in "Appendix A" of the *QIAvac 24 Plus Handbook*.
2. **Recommended**: Insert a VacValve into each luer slot of the QIAvac 24 Plus that is to be used (see Figure 4).

   VacValves should be used if flow rates of samples differ significantly to ensure consistent vacuum.
3. Insert a VacConnector into each VacValve (see Figure 4).

   Perform this step directly before starting the purification to avoid exposure of VacConnectors to potential contaminants in the air.
4. Place the QIAamp Mini columns into the VacConnectors on the manifold (see Figure 4).
5. If necessary, insert an Extension Tube into each QIAamp Mini column (see Figure 5).

   Extension Tubes are required for processing buccal swabs or large volumes.
6. For nucleic acid purification, follow the instructions in the vacuum protocols. Discard the VacConnectors appropriately after use.

   Leave the lid of the QIAamp Mini column open while applying vacuum. Switch off the vacuum between steps to ensure that a consistent, even vacuum is applied during processing. For faster vacuum release, a vacuum regulator should be used (see Figure 3).

   Note: Each VacValve can be closed individually when the sample is completely drawn through the spin column, allowing parallel processing of samples of different volumes or viscosities.
7. After processing samples, clean the QIAvac 24 Plus (see "Cleaning and Decontaminating the QIAvac 24 Plus" in the *QIAvac 24 Plus Handbook*).

   Note: Buffers AL and AW1 used in QIAamp DSP DNA Mini procedures are not compatible with disinfecting agents containing bleach. See page 15 for Warnings and Precautions.

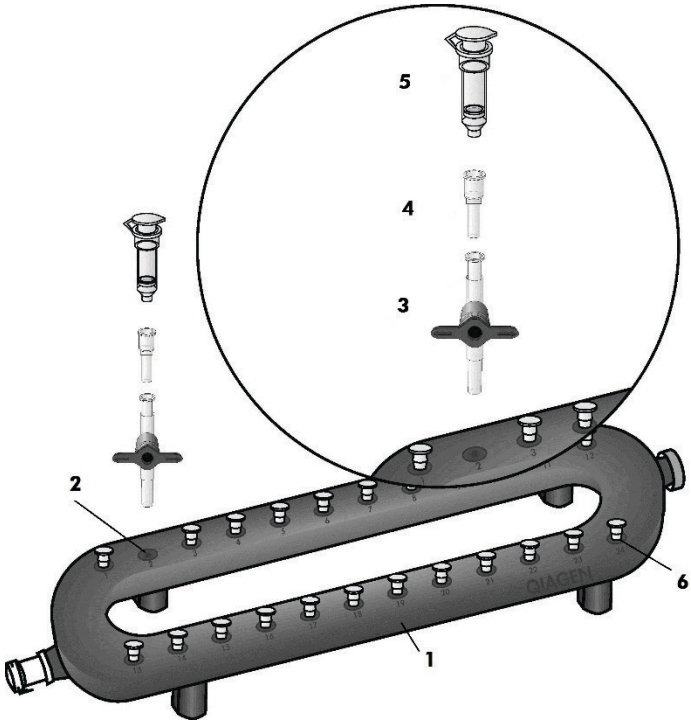

Figure 5. Setting up the QIAvac 24 Plus with QIAamp Mini columns using VacValves and VacConnectors.

1. QIAvac 24 Plus vacuum manifold
2. Luer slot of the QIAvac 24 Plus
3. VacValve*
4. VacConnector*
5. QIAamp column
6. Luer slot closed with luer plug

* Must be purchased separately.

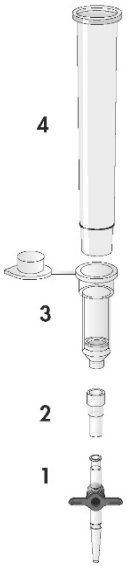

Figure 6. Assembly of QIAamp Mini columns with extension tubes (for buccal swabs or large volumes)

1. VacValve*
2. VacConnector*
3. QIAamp Mini column
4. Extension tube*

* Must be purchased separately.

Processing QIAamp Mini columns on the QIAcube

Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute). For more information about the automated procedure, see the relevant protocol sheet available at **www.qiagen.com/qiacubeprotocols**.

Copurification of RNA

QIAamp Mini spin columns copurify DNA and RNA when both are present in the sample. If RNA-free genomic DNA is required, 4 µl of an RNase A stock solution (100 mg/ml) should be added to the sample prior to the addition of Buffer AL. RNase A is not supplied with the kits and should be purchased separately (see Materials Required but Not Provided, page 13). Ensure that the RNase A used is free of DNase activity.

Protocol: DNA purification from blood or body fluids using a microcentrifuge or QIAcube/QIAcube Connect MDx instruments

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from whole blood, plasma, serum, buffy coat, lymphocytes, and body fluids using a microcentrifuge or by automation on QIAcube or QIAcube Connect MDx instruments. For total DNA purification using a vacuum manifold, see "Protocol: DNA purification from blood or body fluids (vacuum protocol)", page 31.

Important points before starting

- All centrifugation steps are carried out at room temperature.
- Automated processing of 2 – 10 or 12 samples can be performed on QIAcube instruments.
- For automation, follow the instructions from the Protocol Sheets (QIAcube) or on the software screen (QIAcube Connect MDx) and the *QIAcube or QIAcube connect MDx User Manual*.

Things to do before starting

- Equilibrate samples to room temperature.
- Heat a water bath or heating block to 56°C for use in step 4.
- Equilibrate Elution Buffer (AE) to room temperature for elution in step 11.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on page 19.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C ± 3°C.

Procedure

- For the manual procedure with a microcentrifuge follow steps 1–11.
- This procedure can be automated in two different versions:
  - Standard: full automation starting from step 1
  - Manual lysis: partly automated with off-board manual lysis (starting after step 5)

1. Pipet 20 µl proteinase K into the bottom of the lysis tube.
2. Add 200 µl sample to the lysis tube. Use up to 200 µl whole blood, plasma, serum, buffy coat, or body fluids, or up to 5 x 10^6 lymphocytes in 200 µl PBS.

   QIAamp Mini spin columns copurify RNA and DNA when both are present in the sample. If RNA-free genomic DNA is required, 4 µl of an RNase A stock solution (100 mg/ml) should be added to the sample before addition of Buffer AL.

   Note: It is possible to add proteinase K to samples that have already been dispensed into microcentrifuge tubes. In this case, it is important to ensure proper mixing after adding the enzyme.
3. Add 200 µl Buffer AL to the sample. Mix by pulse-vortexing for ≥15 s.

   In order to ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

   Note: Do not add proteinase K directly to Buffer AL.
4. Incubate at 56°C ± 3°C for 10 min ± 1 min.
5. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

   Note: If manual lysis (steps 1–5) was done off-board, the following steps (steps 6–11) can be automated on the QIAcube or QIAcube Connect MDx following the instructions on the instrument screen for the Protocol: Blood or Body Fluid Manual Lysis.
6. Add 200 µl of ethanol (96–100%) to the sample, and mix by pulse-vortexing for ≥15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.
7. Carefully apply the mixture of the solution from step 6 to the QIAamp Mini column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at approximately 6000 x *g* for ≥1 min. Place the QIAamp Mini column into a clean 2 ml wash tube (provided), and discard the tube containing the filtrate.

   Close each spin column in order to avoid aerosol formation during centrifugation.

Centrifugation is performed at approximately 6000 x *g* in order to limit microcentrifuge noise. Centrifugation at full speed will not affect the yield or purity of the DNA. If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the QIAamp Mini spin column is empty.

Note: When preparing DNA from buffy coat or lymphocytes, centrifugation at full speed is recommended to avoid clogging.

8. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at approximately 6000 x *g* for ≥1 min. Place the QIAamp Mini spin column in a clean 2 ml wash tube (provided), and discard the collection tube containing the filtrate.*
9. Carefully open the QIAamp Mini column, and add 500 µl of Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (approximately 20,000 x g) for 3 min ± 30 s.
10. Place the QIAamp Mini spin column in a new 2 ml wash tube and discard the old collection tube with the filtrate. Centrifuge at full speed for ≥1 min.

    This step helps to minimize the chance of possible Buffer AW2 carryover.
11. Place the QIAamp Mini spin column in an elution tube (provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200 µl Elution Buffer (AE). Incubate at room temperature for 1 min, and then centrifuge at approximately 6000 x *g* for ≥1 min.

    Important note: In automated procedures, remove the eluates from the instrument directly after the finished run and store them properly.

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 14 for Warnings and Precautions.

Protocol: DNA purification from blood or body fluids (vacuum protocol)

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from whole blood, plasma, serum, lymphocytes, and body fluids using the QIAvac 24 Plus or equivalent vacuum manifold. For total DNA purification using a microcentrifuge, see "Protocol: DNA purification from blood or body fluids using a microcentrifuge or QIAcube/QIAcube Connect MDx instruments", page 28.

Important points before starting

- All centrifugation steps are carried out at room temperature.
- For setup of the QIAvac 24 Plus, see page 25.
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during protocol steps.

Things to do before starting

- Equilibrate samples to room temperature.
- Heat a water bath or heating block to 56°C for use in step 4.
- Equilibrate Elution Buffer (AE) to room temperature for elution in step 11.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on page19.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C ± 3°C.

Procedure

1. Pipet 20 µl proteinase K into the bottom of the lysis tube.
2. Add 200 µl sample to the lysis tube. Use up to 200 µl whole blood, plasma, serum, or body fluids, or up to 5 x 106 lymphocytes in 200 µl PBS.

   If the sample volume is less than 200 µl, add the appropriate volume of PBS.

QIAamp Mini columns copurify RNA and DNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 µl of an RNase A stock solution (100 mg/ml) should be added to the sample before addition of Buffer AL.

Note: It is possible to add proteinase K to samples that have already been dispensed into microcentrifuge tubes. In this case, it is important to ensure proper mixing after adding the enzyme.

3. Add 200 µl Buffer AL to the sample. Mix by pulse-vortexing for ≥15 s.

   In order to ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

   Note: Do not add proteinase K directly to Buffer AL.

4. Incubate at 56°C ± 3°C for 10 min ± 1 min.
5. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
6. Add 200 µl of ethanol (96–100%) to the sample, and mix again by pulse-vortexing for ≥15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.
7. Insert the QIAamp Mini column into the VacConnector on the QIAvac vacuum manifold. Carefully apply the mixture from step 6 to the QIAamp Mini column without wetting the rim. Switch on the vacuum pump. Be sure to leave the lid of the QIAamp Mini column open while applying vacuum. After all lysates have been drawn through the spin column, switch off the vacuum pump.

   The collection tube from the blister pack can be saved for the centrifugation in step 10.

   If at this stage all of the solution has not passed through the membrane, place the QIAamp Mini column into a clean 2 ml collection tube (provided), close the cap, and centrifuge at approximately 6000 x *g* for ≥3 min or until all the liquid has completely passed through. Place the QIAamp Mini column into another clean 2 ml collection tube, and discard the tube containing the filtrate.* Continue with step 8 of the.microcentrifuge protocol, page 30.

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach.

8. Carefully apply 750 µl Buffer AW1 to the QIAamp Mini column without wetting the rim. Leave the lid of the QIAamp Mini column open and switch on the vacuum pump.

   After all of Buffer AW1 has been drawn through the QIAamp Mini column, switch off the vacuum pump.

9. Add 750 µl Buffer AW2 without wetting the rim of the QIAamp Mini column. Leave the lid of the QIAamp Mini column open and switch on the vacuum pump. After all of Buffer AW2 has been drawn through the QIAamp Mini column, switch off the vacuum pump.

10. Close the lid of the QIAamp Mini column, remove it from the vacuum manifold, and discard the VacConnector. Place the QIAamp Mini column into a clean 2 ml wash tube and centrifuge at approximately 20,000 x *g* for ≥1 min to dry the membrane completely.

11. Place the QIAamp Mini spin column in an elution tube (provided). Discard the collection tube containing the filtrate. Carefully open the QIAamp Mini column. Add 200 µl Elution Buffer (AE) equilibrated to room temperature. Incubate at room temperature for 1 min, and then centrifuge at approximately 6000 x *g* for ≥1 min.

Protocol: DNA purification from tissues using a microcentrifuge or QIAcube/QIAcube Connect MDx instruments

For purification of total (genomic, mitochondrial, and viral) DNA from tissues using a microcentrifuge or automation on the QIAcube/QIAcube Connect MDx

Important points before starting

- All centrifugation steps are carried out at room temperature.
- Avoid repeated freezing and thawing of stored samples, since this leads to reduced DNA size.
- Transcriptionally active tissues, such as liver and kidney, contain high levels of RNA, which will copurify with genomic DNA. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR. If RNA-free genomic DNA is required, include the RNase A digest, as described in step 5a of the protocol.
- Automated processing of 2 – 10 or 12 samples can be performed on QIAcube or QIAcube Connect MDx instruments.
- For automation, follow the instructions from the Protocol Sheets (QIAcube) or on the software screen (QIAcube Connect MDx) and the *QIAcube or QIAcube connect MDx User Manual*.

Things to do before starting

- Equilibrate sample to room temperature.
- Heat 2 water baths or heating blocks: one to 56°C for use in step 3 and one to 70°C for use in step 5.
- Equilibrate Elution Buffer (AE) to room temperature for elution in step 11.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on page 19.
- If a precipitate has formed in Buffer ATL or Buffer AL, dissolve by incubating at 56°C ± 3°C.

Procedure

- For the manual procedure with a microcentrifuge follow steps 1–12.
- This procedure can be partly automated starting from step 5b.

1. Excise the tissue sample or remove it from storage. Determine the amount of tissue. Do not use more than 25 mg (10 mg spleen).

   Weighing tissue is the most accurate way to determine the amount.

   If DNA is prepared from spleen tissue, no more than 10 mg should be used.

   The yield of DNA will depend on both the amount and the type of tissue processed.

2. Cut up (step 2a), grind (step 2b), or mechanically disrupt (step 2c) the tissue sample.

   The QIAamp procedure requires no mechanical disruption of the tissue sample, but lysis time will be reduced if the sample is ground in liquid nitrogen (step 2b) or mechanically homogenized (step 2c) in advance.

   2a. Cut up to 25 mg of tissue (up to 10 mg spleen) into small pieces. Place in the lysis tube, and add 180 µl of Buffer ATL. Proceed with step 3.
   It is important to cut the tissue into small pieces to decrease lysis time.

   2b. Place up to 25 mg of tissue (10 mg spleen) in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into the lysis tube. Allow the liquid nitrogen to evaporate (do not allow the tissue to thaw) and add 180 µl of Buffer ATL. Proceed with step 3.

   2c. Add up to 25 mg of tissue (10 mg spleen) to the lysis tube containing no more than 80 µl PBS. Homogenize the sample using the TissueRuptor or equivalent rotor–stator homogenizer. Add 100 µl Buffer ATL, and proceed with step 3.
   Some tissues require undiluted Buffer ATL for complete lysis. In this case, grinding in liquid nitrogen is recommended. Samples cannot be homogenized directly in Buffer ATL, which contains detergent.

3. Add 20 µl proteinase K, mix by vortexing, and incubate at 56°C ± 3°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a 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. Overnight lysis is possible and does not influence the preparation. In order to ensure efficient lysis, a shaking water bath or a rocking platform should be used. If not available, vortexing 2–3 times per hour during incubation is recommended.

4. Briefly centrifuge the lysis tube to remove drops from the inside of the lid.

5. If RNA-free genomic DNA is required, follow step 5a. Otherwise, follow step 5b.

   Transcriptionally active tissues, such as liver and kidney, contain high levels of RNA which will copurify with genomic DNA. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR.

   5a. Add 4 µl RNase A (100 mg/ml), mix by pulse-vortexing for ≥15 s, and incubate for 2 min ± 30 s at room temperature. Briefly centrifuge the lysis tube to remove drops from inside the lid before adding 200 µl Buffer AL to the sample. Mix again by pulse-vortexing for ≥15 s, and incubate at 70°C ± 3°C for 10 min ± 1 min. Briefly centrifuge the lysis tube to remove drops from inside the lid.
   It is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

   A white precipitate may form on addition of Buffer AL. In most cases, it will dissolve during incubation at 70°C ± 3°C. The precipitate does not interfere with the QIAamp procedure or with any subsequent application.

   Note: The following steps (steps 5b–12) can be automated on the QIAcube or QIAcube Connect MDx. For QIAcube Connect MDx, follow the instructions on the instrument screen for the Protocol: Tissue_Standard

5b. Add 200 µl Buffer AL to the sample, mix by pulse-vortexing for ≥15 s, and incubate at 70°C ± 3°C for 10 min ± 1 min. Briefly centrifuge the lysis tube to remove drops from inside the lid.

It is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

A white precipitate may form on addition of Buffer AL, which in most cases will dissolve during incubation at 70°C ± 3°C. The precipitate does not interfere with the QIAamp procedure or with any subsequent application.

Note: The following steps (steps 6–12) can be automated on the QIAcube or QIAcube Connect MDx.

6. Add 200 µl ethanol (96–100%) to the sample, and mix by pulse-vortexing for ≥15 s. After mixing, briefly centrifuge the lysis tube to remove drops from inside the lid.

   It is essential that the sample, Buffer AL, 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 QIAamp Mini spin column. This precipitate does not interfere with the QIAamp procedure or with any subsequent application.

   Do not use alcohols other than ethanol since this may result in reduced yields.

7. Carefully apply the mixture from step 6 (including the precipitate) to the QIAamp Mini spin column (in a 2 ml wash tube) without wetting the rim. Close the cap, and centrifuge at approximately 6000 x *g* for ≥1 min. Place the QIAamp Mini spin column in a clean 2 ml wash tube (provided), and discard the tube containing the filtrate.*

   Close each spin column to avoid aerosol formation during centrifugation.

   It is essential to apply all of the precipitate to the QIAamp Mini spin column.

   Centrifugation is performed at approximately 6000 x *g* in order to reduce noise. Centrifugation at full speed will not affect the yield or purity of the DNA. If the solution has not completely passed through the membrane, centrifuge again at a higher speed until all the solution has passed through.

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 14 for Warnings and Precautions.

8. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap, and centrifuge at approximately 6000 x *g* for ≥1 min. Place the QIAamp Mini spin column in a clean 2 ml wash tube (provided), and discard the old tube containing the filtrate.
9. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (approximately 20,000 x *g*) for 3 min ± 30 s.
10. **Recommended**: Place the QIAamp Mini spin column in a new 2 ml collection tube (provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for ≥1 min.

    This step helps to minimize the chance of possible Buffer AW2 carryover.
11. Place the QIAamp Mini spin column in a clean 1.5 ml elution tube, and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200 µl Elution Buffer (AE). Incubate at room temperature for ≥1 min, and then centrifuge at approximately 6000 x *g* for ≥1 min.
12. Repeat step 11.

    Important note: For automated procedures, remove the eluates from the instrument directly after the finished run and store them properly.

Protocol: DNA purification from buccal swabs (spin protocol)

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from buccal swabs using a microcentrifuge. For total DNA purification using a vacuum manifold, see "Protocol: DNA purification from buccal swabs (vacuum protocol)" on page 42.

Important points before starting

- Due to the increased volume of Buffer AL that is required for the buccal swab protocol, fewer preparations can be performed.
- This protocol is recommended for the following swab types: C.E.P. (Omni Swabs from Whatman® Bioscience), cotton, and DACRON (Daigger, Puritan® applicators with plastic stick and cotton or DACRON tip from Hardwood Products Company or from Hain Diagnostika).
- To collect a sample, scrape the swab firmly against the inside of each cheek 6 times. Air-dry the swab for at least 2 h after collection. Ensure that the person providing the sample has not consumed any food or drink in the 30 min prior to sample collection.
- All centrifugation steps are carried out at room temperature.

Things to do before starting

- Prepare 56°C ± 3°C water bath for use in step 3.
- Equilibrate Elution Buffer (AE) to room temperature for elution in step 9.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on page 19.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C ± 3°C.

Procedure

1. Place buccal swab in the 2 ml lysis tube. Add 400 µl (cotton and DACRON swab) or 600 µl (Omni Swab) PBS to the sample.

   The Omni Swab is ejected into the tube by pressing the stem end towards the swab. Cotton or DACRON swabs are separated from the stick by hand or with scissors. QIAamp Mini spin columns copurify RNA and DNA in parallel when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 µl of an RNase A stock solution (100 mg/ml) should be added to the sample prior to the addition of Buffer AL.

2. Add 20 µl proteinase K and 400 µl (cotton or DACRON swab) or 600 µl (Omni Swab) Buffer AL to the sample. Mix immediately by vortexing for ≥15 s.

   In order to ensure efficient lysis, it is essential that the sample and Buffer AL are mixed immediately and thoroughly.

   Note: Do not add proteinase K directly to Buffer AL.

3. Incubate at 56°C ± 3°C for 10 min ±1 min. Briefly centrifuge to remove drops from inside the lid.

4. Add 400 µl (cotton or DACRON swab) or 600 µl (Omni Swab) ethanol (96–100%) to the sample, and mix again by vortexing. Briefly centrifuge to remove drops from inside the lid.

5. Carefully apply 700 µl of the mixture from step 4 to the QIAamp Mini spin column (in a 2 ml wash tube) without wetting the rim. Close the cap, and centrifuge at approximately 6000 x *g* for ≥1 min. Place the QIAamp Mini spin column in a clean 2 ml wash tube (provided), and discard the tube containing the filtrate.*

   Close each spin column in order to avoid aerosol formation during centrifugation.

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 14 for Warnings and Precautions.

6. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at approximately 6000 x *g* for ≥1 min. Place the QIAamp Mini spin column in a clean 2 ml wash tube (provided), and discard the tube containing the filtrate.*
7. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (approximately 20,000 x *g*) for 3 min at 30 s.
8. **Recommended**: Place the QIAamp Mini spin column in a new 2 ml wash tube and discard the old collection tube with the filtrate. Centrifuge at full speed for ≥1 min.

   This step helps to minimize the chance of possibly Buffer AW2 carryover.
9. Place the QIAamp Mini spin column in a clean Elution Tube (ET) (provided). Discard the wash tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 150 µl Elution Buffer (AE). Incubate at room temperature for ≥1 min, and then centrifuge at approximately 6000 x *g* for ≥1 min.

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 14 for Warnings and Precautions.

Protocol: DNA purification from buccal swabs (vacuum protocol)

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from buccal swabs using a microcentrifuge. For total DNA purification using a vacuum manifold, see "Protocol: DNA purification from buccal swabs (spin protocol)", page 39.

Important points before starting

- Due to the increased volume of Buffer AL that is required for the buccal swab protocol, fewer preparations can be performed.
- This protocol is recommended for the following swab types: C.E.P. (Omni Swabs from Whatman Bioscience), cotton, and DACRON (Daigger, Puritan applicators with plastic stick and cotton or DACRON tip from Hardwood Products Company or from Hain Diagnostika).
- To collect a sample, scrape the swab firmly against the inside of each cheek 6 times. Air-dry the swab for at least 2 h after collection. Ensure that the person providing the sample has not consumed any food or drink in the 30 min prior to sample collection.
- All centrifugation steps are carried out at room temperature.
- For setup of the QIAvac 24 Plus, see page 25.
- Switch off the vacuum between steps to ensure that a consistent, even vacuum is applied during protocol steps.

Things to do before starting

- Prepare 56°C ± 3°C water bath for use in step 3.
- Equilibrate Elution Buffer (AE) or distilled water to room temperature for elution in step 9.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on page 19.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C ± 3°C.

Procedure

1. Place buccal swab in the 2 ml lysis tube. Add 400 µl (cotton and DACRON swab) or 600 µl (Omni Swab) PBS to the sample.

   The Omni Swab is ejected into the tube by pressing the stem end towards the swab. Cotton or DACRON swabs are separated from the stick by hand or with scissors. QIAamp Mini spin columns copurify RNA and DNA in parallel when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 µl of an RNase A stock solution (100 mg/ml) should be added to the sample prior to the addition of Buffer AL.

2. Add 20 µl proteinase K stock solution and 400 µl (cotton or DACRON swab) or 600 µl (Omni Swab) of Buffer AL to the sample. Mix immediately by vortexing for ≥15 s.

   In order to ensure efficient lysis, it is essential that the sample and Buffer AL are mixed immediately and thoroughly.

   Note: Do not add proteinase K directly to Buffer AL.

3. Incubate at 56°C ± 3°C for 10 min ±1 min. Briefly centrifuge to remove drops from inside the lid.

4. Add 400 µl (cotton or DACRON swab) or 600 µl (Omni Swab) ethanol (96–100%) to the sample, and mix again by vortexing.

5. Insert the QIAamp Mini column into a VacConnector on the QIAvac vacuum manifold. Place an extension tube (see Ordering Information, page 56) on the column. Seal unused luer Adapters with luer plugs.

6. Apply the mixture from step 4 to the QIAamp Mini column. Switch on the vacuum pump to draw the lysate through the QIAamp Mini column. After the lysate has passed through the QIAamp Mini column, switch off the vacuum pump.

7. Add 750 µl Buffer AW1 into the extension tube. Switch on the vacuum pump to draw Buffer AW1 through the QIAamp Mini column. Switch off the vacuum pump.

   Carefully remove the extension tube from the QIAamp Mini column and discard.

8. Add 750 µl Buffer AW2 without wetting the rim of the QIAamp Mini column. Leave the lid of the QIAamp Mini column open and switch on the vacuum pump. After all of Buffer AW2 has been drawn through the spin column, switch off the vacuum pump.*
9. Close the lid of the QIAamp Mini column, remove it from the vacuum manifold, and discard the VacConnector. Place the QIAamp Mini column into a clean 2 ml wash tube and centrifuge at approximately 20,000 x *g* for ≥1 min to dry the membrane completely.
10. Place the QIAamp Mini column in a clean Elution Tube (ET) (provided). Discard the wash tube and the filtrate. Carefully open the QIAamp Mini column. Elute the DNA with 150 µl Elution Buffer (AE). Incubate at room temperature for ≥1 min, and then centrifuge at approximately 6000 x *g* for ≥1 min.

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 14 for Warnings and Precautions.

Protocol: Purification of total DNA from cultured cells

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from cultured cells using a microcentrifuge.

Additional equipment and reagents required

- Phosphate buffered saline (PBS)*
- Equipment for harvesting cells. Depending on the method chosen, one or more of the following are required:
  - Microcentrifuge
  - Trypsin and culture media*
  - Cell scraper

Important points before starting

- Do not use more than 5 x 10^6 cells (with a normal set of chromosomes).
- All centrifugation steps are carried out at room temperature.

Things to do before starting

- Heat a water bath or heating block to 56°C.
- Equilibrate Elution Buffer (AE) to room temperature for elution.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on page 19.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C ± 3°C.

* 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.

Procedure

1. Harvest cells according to step 2 (for cells grown in suspension) or step 3 (for cells grown in a monolayer).
2. Cells grown in suspension (do not use more than 5 x 10^6 cells with a normal set of chromosomes): Determine the number of cells. Centrifuge the appropriate number of cells for 5 min ± 30 s at approximately 300 x *g* in a 1.5 ml microcentrifuge tube. Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Continue with step 4.
3. Cells grown in a monolayer (do not use more than 5 x 10^6 cells with a normal set of chromosomes): Cells grown in a monolayer can be detached from the culture flask by either trypsinization or using a cell scraper.

   To trypsinize cells:

   Determine the number of cells. Aspirate the medium and wash cells with PBS. Aspirate the PBS, and add 0.10–0.25% trypsin. After cells have detached from the dish or flask, collect them in medium, and transfer the appropriate number of cells (do not use more than 5 x 10^6 cells with a normal set of chromosomes) to a 1.5 ml microcentrifuge tube (not provided). Centrifuge for 5 min ±30 s at approximately 300 x *g*. Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Continue with step 4.

   Using a cell scraper:

   Detach cells from the dish or flask. Transfer the appropriate number of cells (do not use more than 5 x 10^6 cells with a normal set of chromosomes) to a 1.5 ml microcentrifuge tube (not provided) and centrifuge for 5 min ± 30 s at approximately 300 x *g*. Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Continue with step 4.
4. Resuspend cell pellet in PBS to a final volume of 200 µl.
5. Add 20 µl proteinase K.
6. Continue with step 3 of "Protocol: DNA purification from blood or body fluids using a microcentrifuge or QIAcube/QIAcube Connect MDx instruments", page 28.

Protocol: Isolation of bacterial DNA from biological fluids

For purification of bacterial DNA using a microcentrifuge or the QIAcube/QIAcube Connect MDx.

Some bacteria, particularly Gram-positive bacteria, require pre-incubation with specific enzymes such as lysozyme* or lysostaphin (e.g., staphylococci) to lyse the rigid multilayered cell wall. In these cases, "Protocol: Isolation of genomic DNA from Gram-positive bacteria", page 50, should be used.

Important points before starting

- All centrifugation steps are carried out at room temperature.
- Avoid repeated freezing and thawing of stored samples, since this leads to reduced DNA size.
- Automated processing of 2 – 10 or 12 samples can be performed on QIAcube or QIAcube Connect MDx instruments.
- For automation, follow the instructions from the Protocol Sheets (QIAcube) or on the software screen (QIAcube Connect MDx) and the *QIAcube or QIAcube connect MDx User Manual*.

Things to do before starting

- Equilibrate sample to room temperature.
- Heat 2 water baths or heating blocks: one to 56°C and one to 70°C.
- Equilibrate Elution Buffer (AE) to room temperature for elution.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on page 19.
- If a precipitate has formed in Buffer ATL or Buffer AL, dissolve by incubating at 56°C ± 3°C.

* 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.

Procedure

- For the manual procedure with a microcentrifuge follow steps 1–3.
- This procedure can be partly automated starting from step 2 (Protocol: Bacterial Pellet_Bacterial DNA).

1. Pellet bacteria by centrifugation for 10 min at 5,000 x *g* (7500 rpm).
2. Resuspend bacteria pellet in 180 µl Buffer ATL.
3. Follow “Protocol: DNA purification from tissues using a microcentrifuge or QIAcube/QIAcube Connect MDx instruments”, page 34, beginning at step 3.

Protocol: Isolation of bacterial DNA from eye, nasal, pharyngeal, or other swabs

Some bacteria (particularly Gram-positive bacteria), require pre-incubation with specific enzymes such as lysozyme* or lysostaphin* (e.g., staphylococci) to lyse the rigid multilayered cell wall. In these cases, "Protocol: Isolation of genomic DNA from Gram-positive bacteria", page 50, should be used.

Important points before starting

- All centrifugation steps are carried out at room temperature.
- Avoid repeated freezing and thawing of stored samples, since this leads to reduced DNA size.

Things to do before starting

- Equilibrate sample to room temperature.
- Heat 2 water baths or heating blocks: one to 56°C and one to 70°C.
- Equilibrate Elution Buffer (AE) to room temperature for elution.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on page21.
- If a precipitate has formed in Buffer ATL or Buffer AL, dissolve by incubating at 56°C ± 3°C.

Procedure

1. Collect samples and place in the 2 ml lysis tube.
2. Add 1 ml PBS and incubate for 2 h ± 10 min at room temperature.
3. Pellet bacteria by centrifugation for 10 min at 5000 x *g* (7500 rpm).
4. Resuspend bacterial pellet in 180 µl Buffer ATL.
5. Follow "Protocol: DNA purification from tissues using a microcentrifuge or QIAcube/QIAcube Connect MDx instruments", page 35, beginning at 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.

Protocol: Isolation of genomic DNA from Gram-positive bacteria

For use with a microcentrifuge or automated on QIAcube/QIAcube Connect MDx instruments

Important points before starting

- All centrifugation steps are carried out at room temperature.
- Avoid repeated freezing and thawing of stored samples, since this leads to reduced DNA size.
- Automated processing of 2 – 10 or 12 samples can be performed on QIAcube or QIAcube Connect MDx instruments.
- For automation follow the instructions from the Protocol Sheets (QIAcube) or on the software screen (QIAcube Connect MDx) and the *QIAcube or QIAcube connect MDx User Manual*.

Things to do before starting

- Equilibrate sample to room temperature.
- Heat 2 water baths or heating blocks: one to 56°C and one to 95°C.
- Equilibrate Elution Buffer (AE) to room temperature for elution.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on page 19.
- If a precipitate has formed in Buffer ATL or Buffer AL, dissolve by incubating at 56°C ± 3°C.
- Prepare appropriate enzyme solution (not provided): 20 mg/ml lysozyme or 200 µg/ml lysostaphin; 20 mM Tris·HCl, pH 8.0; 2 mM EDTS; 1.2% Triton®.

Procedure

- For the manual procedure with a microcentrifuge, follow steps 1–8.
- This procedure can be partly automated starting after step 7 (Protocol: Bacteria (Gram+) or Yeast_Enzymatic Lysis)

1. Collect samples and place in the 2 ml lysis tube.
2. Pellet bacteria by centrifugation for 10 min at 5000 x *g* (75,000 rpm).
3. Suspend bacterial pellet in 180 µl of the appropriate enzyme solution (20 mg/ml lysozyme or 200 µg/ml lysostaphin; 20 mM Tris·HCl, pH 8.0; 2 mM EDTA; 1.2% Triton).
4. Incubate for at least 30 min at 37°C.
5. Add 20 µl proteinase K and 200 µl Buffer AL. Mix by vortexing.
6. Incubate at 56°C for 30 min and then incubate at 95°C for an additional 15 min.
7. Centrifuge for a few seconds.
8. Follow "Protocol: DNA purification from tissues using a microcentrifuge or QIAcube/QIAcube Connect MDx instruments", page 34, beginning at step 6.

Quality Control

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

Limitations

The system performance has been established using whole blood, bacterial cultures, and tissue for isolation of genomic DNA.

It is the user's responsibility to validate system performance for any procedures used in their laboratory which are not covered by the QIAGEN performance studies.

To minimize the risk of a negative impact on the diagnostic results, adequate controls for downstream applications should be used. For further validation, the guidelines of the International Conference on Harmonization of Technical Requirements (ICH) in ICH Q2(R1) Validation Of Analytical Procedures: Text And Methodology are recommended.

Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

Symbols

The following symbols may appear in the instructions for use or on the packaging and labeling:

Symbol	Symbol definition
 <N>	Contains reagents sufficient for <N> reactions
	Use by
IVD	In vitro diagnostic medical device
REF	Catalog number
LOT	Lot number
MAT	Material number (i.e., component labeling)
COMP	Components
CONT	Contains
NUM	Number
GTIN	Global Trade Item Number
VOL	Volume
Rn	R is for revision of the Instructions for Use and n is the revision number
	Temperature limitation
	Leads to

Symbol	Symbol definition
	Upon arrival
	Open on delivery; store QIAamp Mini Spin Columns at 2–8°C.
 ___	Write down the current date after adding ethanol to the bottle
	Ethanol
	Adding
	Guanidine hydrochloride
	Maleic acid
	Manufacturer
	Consult instructions for use
	Keep away from sunlight
	Warning/caution

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support** (for contact information, visit **www.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
QIAamp DSP DNA Mini Kit (50)	For 50 DNA preps: QIAamp Mini spin columns, proteinase K, Reagents, Buffers, and collection tubes	61304
Related products		
QIAcube Connect MDx*	Instrument and 1-year warranty on parts and labor	9003070
Accessories		
Extension Tubes (3 ml)†	For use with QIAGEN spin columns on vacuum manifolds: 100 per pack	19587
QIAvac 24 Plus vacuum manifold‡	Vacuum manifold for processing 1–24 spin columns: QIAvac 24 Plus Vacuum manifold, Luer Plugs, Quick Couplings	19413
Vacuum Pump‡	Universal vacuum pump	84010
VacConnectors‡	500 disposable connectors for use with QIAamp spin columns on luer connectors	19407
Rotor Adapters	For 240 preps: 240 Disposable Rotor Adapters and 240 Elution Tubes (1.5 ml); for use with the QIAcube	990394
Rotor Adapter Holder	Holder for 12 disposable rotor adapters; for use with the QIAcube	990392
Sample Tubes CB	1000 conical screw-cap tubes without skirted base (2 ml) for use with the QIAcube and QIAcube Connect MDx	990382

Product	Contents	Cat. no.
Sample Tubes RB	1000 safe-lock microcentrifuge tubes (2 ml) for use with the QIAcube and QIAcube Connect	990381
Shaker Rack Plugs	For loading the QIAcube shaker rack	9017854
Reagent Bottles, 30 ml	Reagent Bottles (30 ml) with lids; pack of 6; for use with the QIAcube	990393
Filter-Tips, 1000 µl	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube	990352
Filter-Tips, 1000 µl, wide-bore	Disposable Filter-Tips, wide-bore, racked; (8 x 128); not required for all protocols. For use with the QIAcube	990452
Filter-Tips, 200 µl	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube and the QIAsymphony SP/AS instruments	990332

* The QIAcube connect MDx is not available in all countries. For further details, please contact QIAGEN technical services.

† For use with buccal swabs or protocols with large volumes.

‡ For use with vacuum protocols.

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Document Revision History

Revision	Description
R4, 01/2021	Updates to the following sections: Automated DNA purification on QIAcube/QIAcube Connect MDx, Materials Required but Not Provided, Warnings and Precautions, Protocol: DNA purification from blood or body fluids using a microcentrifuge or QIAcube/QIAcube Connect MDx instruments, Protocol: DNA purification from tissues using a microcentrifuge or QIAcube/QIAcube Connect MDx instruments,**Error! Reference source not found.** Protocol: Isolation of bacterial DNA from biological fluids, Protocol: Isolation of genomic DNA from Gram-positive bacteria, Symbols, and Ordering Information. Removal of protocol for dried blood spots. Added references to the QIAcube Connect MDx and its accessories. Editorial and layout changes.

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