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Etude génétique des populations de loutres captives dans différents zoos européens et relations avec les populations sauvages : implication pour de futurs projets de réintroduction

Auteur : Radermacher, Tom
Promoteur(s) : Michaux, Johan; 856
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March 2014

QIAamp® Fast DNA Stool Mini Handbook

For fast purification of genomic DNA from stool samples

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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Contents

Kit Contents

QIAamp® Fast DNA Stool Mini Kit	(50)
Catalog no.	**51604**
Number of preps	**50**
QIAamp Mini spin columns	50
Collection Tubes (2 ml)	200
InhibitEX® Buffer	140 ml
Buffer AL*	33 ml
Buffer AW1* (concentrate)	19 ml
Buffer AW2† (concentrate)	13 ml
Buffer ATE	12 ml
Proteinase K	1.4 ml

* Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. See page 5 for safety information.

† Contains sodium azide as a preservative.

Shipping and storage

The QIAamp Fast DNA Stool Mini Kit is shipped at room temperature and can be stored dry at room temperature (15–25°C) for up to 1 year without showing any reduction in performance. The QIAamp Fast DNA Stool Mini 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 temperature often exceeds 25°C, we suggest storing proteinase K at 2–8°C.

Product Use Limitations

The QIAamp Fast DNA Stool Mini Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Quality Control

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

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate 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.

The sample-preparation waste contains guanidine hydrochloride from Buffers AL and AW1, 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.

Technical Assistance

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

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Introduction

The QIAamp Fast DNA Stool Mini Kit provides fast and easy purification of total DNA from fresh or frozen stool samples. The purified DNA is of high quality and well suited for use in PCR and other downstream applications.

The simple QIAamp spin procedure yields pure DNA ready for direct use in as little as 25 minutes. The procedure can be automated on the QIAcube® for increased standardization and ease of use (see page 10). Purification requires no phenol–chloroform extraction or alcohol precipitation, and involves minimal handling. DNA is eluted in low-salt buffer and is free of protein, nucleases, and other impurities or inhibitors. The purified DNA is ready for use in PCR and other enzymatic reactions, or can be stored at –20°C for later use.

Stool samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. To ensure removal of these substances, the QIAamp Fast DNA Stool Mini Kit contains InhibitEX Buffer, which is specially formulated to separate inhibitory substances from DNA in stool samples.

Principle and procedure

The QIAamp Fast DNA Stool Mini Kit is designed for rapid purification of total DNA from up to 220 mg stool and is suitable for both fresh and frozen samples. A special protocol is provided for isolating DNA from larger amounts of stool. The fast and easy procedure comprises the following steps:

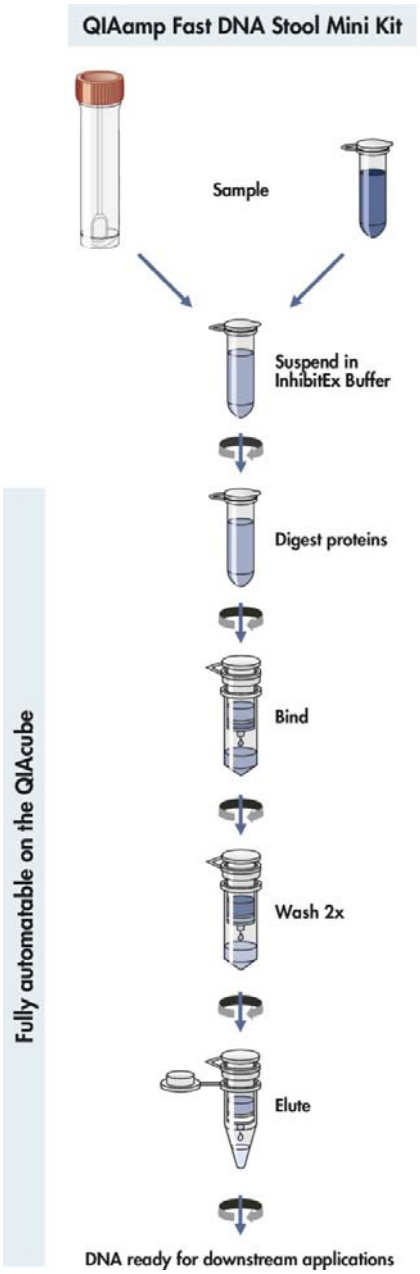
- Lysis of and separation of impurities from stool samples in InhibitEX Buffer
- Purification of DNA on QIAamp Mini spin columns (see flowchart)

Sample size

The QIAamp Fast DNA Stool Mini Kit is optimized for use with 180–220 mg fresh or frozen stool, but can also be used with larger amounts of stool. Starting with larger amounts of stool is recommended when the target DNA is not distributed homogeneously throughout the stool and/or is at a low concentration. A larger amount of starting material will increase the likelihood of purifying DNA from low-titer sources in stool samples. See "Protocol: Isolation of DNA from Larger Volumes of Stool", page 26.

The QIAamp Fast DNA Stool Mini protocols can also be used for samples of less than 180 mg (e.g., forensic samples). In such cases, follow one of the standard protocols, without reducing the amounts of buffers used.

For maximum flexibility, protocols are provided for use with samples collected in both 2 ml microcentrifuge tubes and 15 ml stool tubes.


QIAamp Fast DNA Stool Mini Kit
Sample
Suspend in InhibitEx Buffer
Digest proteins
Bind
Wash 2x
Elute
DNA ready for downstream applications
Fully automatable on the QIAcube

Lysis and removal of impurities in InhibitEX Buffer

In the first steps of the protocol, stool samples are lysed in InhibitEX Buffer. Human cells lyse efficiently at room temperature. Bacterial cells and those of other pathogens in the stool are effectively lysed by incubating the stool homogenate at 70°C (if necessary, this temperature can be increased to 95°C). This is recommended for detection of cells that are difficult to lyse (e.g., some bacteria and parasites).

During lysis, DNA-degrading substances and PCR inhibitors present in the stool sample are separated from the DNA by the InhibitEX buffer. The sample matrix is pelleted by centrifugation and the DNA in the supernatant is purified on QIAamp Mini spin columns.

Purification on QIAamp spin columns

The QIAamp DNA purification procedure involves digestion of proteins, binding DNA to the QIAamp silica membrane, washing away impurities, and eluting pure DNA from the spin column.

Proteins are digested and degraded under denaturing conditions during a 70°C incubation with proteinase K. Buffering conditions are then adjusted to allow optimal binding of DNA to the QIAamp membrane, and the sample is loaded onto the QIAamp spin column. DNA is adsorbed onto the QIAamp silica membrane during a brief centrifugation step. Optimized salt concentrations and pH conditions in the lysate ensure that remains of digested proteins and other impurities, which can inhibit PCR and other downstream enzymatic reactions, are not retained on the QIAamp membrane.

DNA bound to the QIAamp membrane is washed in two centrifugation steps. Optimized wash conditions using two wash buffers ensure complete removal of any residual impurities without affecting DNA binding.

Purified, concentrated DNA is eluted from the QIAamp Mini spin column in low-salt buffer equilibrated to room temperature. DNA yield is typically 10–50 µg but, depending on the individual stool sample and the way it was stored, may range from 5–100 µg. DNA concentration is typically 50–250 ng/µl. The eluted DNA is up to 20 kb long and is suitable for direct use in PCR and other enzymatic reactions. If the purified DNA is to be kept, storage at –20°C is recommended.

The convenient spin procedure is designed to ensure that no sample-to-sample cross-contamination occurs and to allow safe handling of potentially infectious samples.

QIAamp spin columns fit into most standard microcentrifuge tubes. Eluted DNA is collected in standard 1.5 ml microcentrifuge tubes (not provided).

Automated purification

Purification of DNA from stool samples using the QIAamp Fast DNA Stool Mini Kit can be automated on the QIAcube. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using the QIAamp Fast DNA Stool Mini Kit for purification of high-quality DNA. For more information about the automated procedure, see the relevant protocol sheet available at www.qiagen.com/MyQIAcube.

The QIAcube is 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 www.qiagen.com/MyQIAcube.

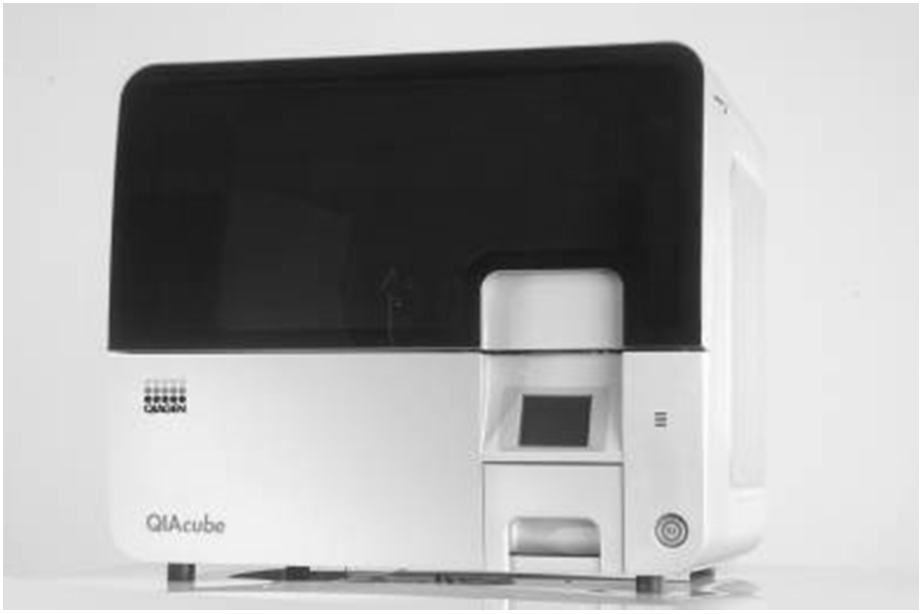


Figure 1. Automated DNA purification. DNA purification using the QIAamp Fast DNA Stool Mini Kit can be automated on the QIAcube.

Equipment and Reagents to Be Supplied by User

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

- Ethanol (96–100%)*
- 1.5 ml and 2 ml microcentrifuge tubes (e.g., Eppendorf Safe-Lock, cat. no. 0030120.094 or Sarstedt Safe-Seal, cat. no. 72.695)†
- Pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- Microcentrifuge (with rotor for 2 ml tubes)
- Thermomixer with 2ml inlays or a water bath for incubation at 70°C
- Spatula (e.g., Sarstedt cat. no. 81.970)†
- Vortexer
- Ice

Using stool tubes

- Stool tubes (for easier measurement of stool samples, stool tubes with integrated measuring spoons in their lids are available, e.g., Sarstedt cat. no. 80.734 or Böttger cat. no. 07.023.2007 and 07.033.2007)†

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

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

Important Notes

Preparation of reagents

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

Mix InhibitEX Buffer thoroughly by shaking. If a precipitate has formed, incubate at 37–70°C until it has fully dissolved.

InhibitEX Buffer will exhibit a color change (orange) during storage; this does not affect the functionality of the buffer.

Buffer AL* (store at room temperature)

Mix Buffer AL thoroughly by shaking before use. If a precipitate has formed, incubate at 70°C until it has fully dissolved.

Buffer AL is stable for 1 year when stored closed at room temperature.

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

Buffer AW1* (store at room temperature)

Buffer AW1 is supplied as a concentrate. Before using for the first time, add 25 ml ethanol (96–100%) as indicated on the bottle.

Buffer AW1 is stable for 1 year when stored closed at room temperature.

Buffer AW1 should be thoroughly mixed before use.

Buffer AW2† (store at room temperature)

Buffer AW2 is supplied as a concentrate. Before using for the first time, add 30 ml ethanol (96–100%) to Buffer AW2 concentrate as indicated on the bottle.

Buffer AW2 is stable for 1 year when stored closed at room temperature.

Buffer AW2 should be thoroughly mixed before use.

* Contains chaotropic salt. Take appropriate laboratory safety measure and wear gloves when handling. Not compatible with disinfecting agents that contain bleach. See page 5 for safety information.

† Contains sodium azide.

Handling of QIAamp Mini spin columns

Owing to the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling QIAamp Mini spin columns to avoid cross-contamination between samples.

- Carefully apply the sample or solution to the QIAamp Mini spin column. Pipet the sample into the QIAamp Mini spin column without moistening the rim of the column.
- Change pipet tips between all liquid transfers. The use of aerosol-barrier pipet tips is recommended.
- Avoid touching the QIAamp membrane with the pipet tip.
- To avoid cross-contamination, we recommend briefly centrifuging the microcentrifuge tubes after each vortexing step to remove drops from the inside of the lid.
- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.
- Close the QIAamp Mini spin column before placing it in the microcentrifuge. Centrifuge as described.
- Remove the QIAamp Mini spin column and collection tube from the microcentrifuge. Place the QIAamp Mini spin column in a new collection tube. Discard the filtrate and the collection tube. Please note that the filtrate may contain hazardous substances and should be disposed of appropriately.
- Open only one QIAamp Mini spin 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 spin columns can be transferred after centrifugation. Discard used collection tubes containing the filtrate and place the new collection tubes containing the QIAamp Mini spin columns directly into the microcentrifuge.

Centrifugation

QIAamp Mini spin columns fit into most standard 1.5 and 2 ml microcentrifuge tubes. Additional 2 ml collection tubes are available separately.

All centrifugation steps should be carried out at room temperature (15–25°C) at 20,000 x g (approximately 14,000 rpm). Increase the centrifugation time proportionately if your centrifuge cannot provide 20,000 x g (e.g., instead of centrifuging for 5 minutes at 20,000 x g, centrifuge for 10 minutes at 10,000 x g).

Protocol: Isolation of DNA from Stool for Human DNA Analysis

Lysis conditions in this protocol are optimized to increase the ratio of human DNA to nonhuman DNA. Nonhuman DNA is not excluded by this protocol.

Important points before starting

- All centrifugation steps should be carried out at room temperature (15–25°C) at 20,000 x g (approximately 14,000 rpm). Increase the centrifugation time proportionately if your centrifuge cannot provide 20,000 x g (e.g., instead of centrifuging for 5 min at 20,000 x g, centrifuge for 10 min at 10,000 x g).

Things to do before starting

- Prepare a thermomixer with 2 ml inlays or a water bath at 70°C for use in step 7
- Redissolve any precipitates in Buffer AL and InhibitEX Buffer by incubating at 37–70°C
- Add ethanol to Buffer AW1 and Buffer AW2 concentrates
- Mix all buffers before use

Procedure

1. **Weigh 180–220 mg stool in a 2 ml microcentrifuge tube (not provided) and place tube on ice.**

   This protocol is optimized for use with 180–220 mg stool but can also be used with smaller amounts. There is no need to reduce the amounts of buffers when using smaller amounts of stool. For samples >220 mg, see "Protocol: Isolation of DNA from Larger Volumes of Stool", page 26.

   If the sample is liquid, pipet 200 µl into the microcentrifuge tube. Cut the end of the pipet tip to make pipetting easier.

   If the sample is frozen, use a scalpel or spatula to scrape bits of stool into a 2 ml microcentrifuge tube on ice.

   Note: When using frozen stool samples, take care that the samples do not thaw until InhibitEX Buffer is added in step 2 to lyse the sample; otherwise the DNA in the sample may degrade. After addition of InhibitEX Buffer, all following steps can be performed at room temperature (15–25°C).

2. **Add 1 ml InhibitEX Buffer to each stool sample. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.**

   Note: It is important to vortex the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.

3. **Centrifuge sample at full speed for 1 min to pellet stool particles.**

   IMPORTANT: Do not transfer any solid material. If particles are still visible in the supernatant, centrifuge the sample again.

4. **Pipet 25 µl proteinase K into a new 2 ml microcentrifuge tube (not provided).**
5. **Pipet 600 µl supernatant from step 3 into the 2 ml microcentrifuge tube containing proteinase K.**
6. **Add 600 µl Buffer AL and vortex for 15 s.**

   Note: Do not add proteinase K directly to Buffer AL. It is essential that the sample and Buffer AL are thoroughly mixed to form a homogeneous solution.

7. **Incubate at 70°C for 10 min.**

   Centrifuge briefly to remove drops from the inside of the tube lid (optional).

8. **Add 600 µl of ethanol (96–100%) to the lysate, and mix by vortexing.**

   Centrifuge briefly to remove drops from the inside of the tube lid (optional).

9. **Carefully apply 600 µl lysate from step 8 to the QIAamp spin column. Close the cap and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate.**
10. **Repeat step 9 until all of the lysate has been loaded on the column.**

    Close each spin column in order to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.

11. **Carefully open the QIAamp spin column and add 500 µl Buffer AW1. Centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.**
12. **Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Centrifuge at full speed for 3 min. Discard the collection tube containing the filtrate.**

    Note: Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, causing the flow-through containing Buffer AW2 to come in contact with the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come in contact with the QIAamp spin column.

13. **Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 3 min.**

    This step helps to eliminate the chance of possible Buffer AW2 carryover.

14. **Transfer the QIAamp spin column into a new, labeled 1.5 ml microcentrifuge tube (not provided) and pipet 200 µl Buffer ATE directly onto the QIAamp membrane. Incubate for 1 min at room temperature, then centrifuge at full speed for 1 min to elute DNA.**

    If yield is to be quantified by UV absorbance, blank the measuring device using Buffer ATE to avoid false results. For more information about elution and how to determine DNA yield, purity, and length, see the Appendix on page 33. For long-term storage, we recommend keeping the eluate at –20°C.

Protocol: Using Stool Tubes for Isolation of DNA from Stool for Human DNA Analysis

For easier measurement of stool samples, stool tubes with integrated measuring spoons in their lids are available. The spoons in these tubes are designed to collect 200 mg stool. This protocol is optimized for usage with such stool tubes.

Lysis conditions in this protocol are optimized to increase the ratio of human DNA to nonhuman DNA. Nonhuman DNA is not excluded by this protocol.

Important points before starting

- All centrifugation steps should be carried out at room temperature (15–25°C) at 20,000 x g (approximately 14,000 rpm). Increase the centrifugation time proportionately if your centrifuge cannot provide 20,000 x g (e.g., instead of centrifuging for 5 min at 20,000 x g, centrifuge for 10 min at 10,000 x g).

Things to do before starting

- Prepare a thermomixer with 2 ml inlays or a water bath at 70°C for use in step 8
- Redissolve any precipitates in Buffer AL and InhibitEX Buffer by incubating at 37–70°C.
- Add ethanol to Buffer AW1 and Buffer AW2 concentrates
- Mix all buffers before use

Procedure

1. **Use the spoon integrated in the cap of a stool tube (not provided) to measure 180–220 mg of the stool sample. A level spoonful will correspond to approximately 200 mg stool. Close the tube and place it on ice.**

   A spatula should be used to remove excess stool from the spoon.

2. **Add 2 ml InhibitEX Buffer to each stool sample. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.**

   Note: It is important to vortex the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate. After InhibitEX Buffer has been added, all the following steps can be carried out at room temperature (15–25°C).

3. **Pipet 1.2 ml of the stool lysate into a labeled 2 ml microcentrifuge tube (not provided).**

   Cut the ends off the pipet tips to make pipetting viscous samples easier.

4. **Centrifuge sample at full speed for 1 min to pellet stool particles.**
   IMPORTANT: Do not transfer any solid material. If particles are still visible in the supernatant, centrifuge the sample again.
5. **Pipet 25 µl proteinase K into a new 2 ml microcentrifuge tube (not provided).**
6. **Pipet 600 µl supernatant from step 4 into the 2 ml microcentrifuge tube containing proteinase K.**
7. **Add 600 µl Buffer AL and vortex for 15 s.**
   Note: Do not add proteinase K directly to Buffer AL. It is essential that the sample and Buffer AL are thoroughly mixed to form a homogeneous solution.
8. **Incubate at 70°C for 10 min.**
   Centrifuge briefly to remove drops from the inside of the tube lid (optional).
9. **Add 600 µl of ethanol (96–100%) to the lysate, and mix by vortexing.**
   Centrifuge briefly to remove drops from the inside of the tube lid (optional).
10. **Carefully apply 600 µl lysate from step 9 to the QIAamp spin column. Close the cap and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate.**
11. **Repeat step 10 until all of the lysate has been loaded on the column.**
    Close each spin column in order to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.
12. **Carefully open the QIAamp spin column and add 500 µl Buffer AW1. Centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.**
13. **Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Centrifuge at full speed for 3 min. Discard the collection tube containing the filtrate.**
    Note: Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, causing the flow-through containing Buffer AW2 to come in contact with the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column.
14. **Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 3 min.**
    This step helps to eliminate the chance of possible Buffer AW2 carryover.

15. **Transfer the QIAamp spin column into a new, labeled 1.5 ml microcentrifuge tube (not provided) and pipet 200 µl Buffer ATE directly onto the QIAamp membrane. Incubate for 1 min at room temperature, then centrifuge at full speed for 1 min to elute DNA.**

    If yield is to be quantified by UV absorbance, blank the measuring device using Buffer ATE to avoid false results. For more information about elution and how to determine DNA yield, purity, and length, see the Appendix on page 33. For long-term storage, we recommend keeping the eluate at –20°C.

Protocol: Isolation of DNA from Stool for Pathogen Detection

Lysis conditions in this protocol are optimized to increase the ratio of nonhuman DNA to human DNA. Human DNA is not excluded by this protocol.

Important points before starting

- For detection of cells that are difficult to lyse, such as Gram-positive bacteria, the lysis temperature in step 3 can be increased to 95°C, if necessary.
- All centrifugation steps should be carried out at room temperature (15–25°C) at 20,000 x g (approximately 14,000 rpm). Increase the centrifugation time proportionately if your centrifuge cannot provide 20,000 x g (e.g., instead of centrifuging for 5 min at 20,000 x g, centrifuge for 10 min at 10,000 x g).

Things to do before starting

- Prepare a thermomixer with 2 ml inlays or a water bath at 70°C for use in steps 3 and 8
- Redissolve any precipitates in Buffer AL and InhibitEX Buffer by incubating at 37–70°C
- Add ethanol to Buffer AW1 and Buffer AW2 concentrates
- Mix all buffers before use

Procedure

1. **Weigh 180–220 mg stool in a 2 ml microcentrifuge tube (not provided) and place tube on ice.**

   This protocol is optimized for use with 180–220 mg stool but can also be used with smaller amounts. There is no need to reduce the amounts of buffers when using smaller amounts of stool. For samples >220 mg, see "Protocol: Isolation of DNA from Larger Volumes of Stool", page 26.

   If the sample is liquid, pipet 200 µl into the microcentrifuge tube. Cut the end of the pipet tip to make pipetting easier.

   If the sample is frozen, use a scalpel or spatula to scrape bits of stool into a 2 ml microcentrifuge tube on ice.

   Note: When using frozen stool samples, take care that the samples do not thaw until InhibitEX Buffer is added in step 2 to lyse the sample; otherwise the DNA in the sample may degrade. After addition of InhibitEX Buffer, all following steps can be performed at room temperature (15–25°C).

2. **Add 1 ml InhibitEX Buffer to each stool sample. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.**

   Note: It is important to vortex the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.

3. **Heat the suspension for 5 min at 70°C. Vortex for 15 s.**

   This heating step helps to lyse bacteria and other parasites. The lysis temperature can be increased to 95°C for cells that are difficult to lyse (such as Gram-positive bacteria).

4. **Centrifuge sample at full speed for 1 min to pellet stool particles.**

   IMPORTANT: Do not transfer any solid material. If particles are still visible in the supernatant, centrifuge the sample again.

5. **Pipet 15 µl proteinase K into a new 1.5 ml microcentrifuge tube (not provided).**
6. **Pipet 200 µl supernatant from step 4 into the 1.5 ml microcentrifuge tube containing proteinase K.**
7. **Add 200 µl Buffer AL and vortex for 15 s.**

   Note: Do not add proteinase K directly to Buffer AL. It is essential that the sample and Buffer AL are thoroughly mixed to form a homogeneous solution.

8. **Incubate at 70°C for 10 min.**

   Centrifuge briefly to remove drops from the inside of the tube lid (optional).

9. **Add 200 µl of ethanol (96–100%) to the lysate, and mix by vortexing.**

   Centrifuge briefly to remove drops from the inside of the tube lid (optional).

10. **Carefully apply 600 µl lysate from step 9 to the QIAamp spin column. Close the cap and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate.**

    Close each spin column in order to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.

11. **Carefully open the QIAamp spin column and add 500 µl Buffer AW1. Centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.**

12. **Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Centrifuge at full speed for 3 min. Discard the collection tube containing the filtrate.**

    Note: Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, causing the flow-through containing Buffer AW2 to come in contact with the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column.

13. **Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 3 min.**

    This step helps to eliminate the chance of possible Buffer AW2 carryover.

14. **Transfer the QIAamp spin column into a new, labeled 1.5 ml microcentrifuge tube (not provided) and pipet 200 µl Buffer ATE directly onto the QIAamp membrane. Incubate for 1 min at room temperature, then centrifuge at full speed for 1 min to elute DNA.**

    If yield is to be quantified by UV absorbance, blank the measuring device using Buffer ATE to avoid false results. For more information about elution and how to determine DNA yield, purity, and length, see the Appendix on page 33. For long-term storage, we recommend keeping the eluate at –20°C.

Protocol: Using Stool Tubes for Isolation of DNA from Stool for Pathogen Detection

For easier measurement of stool samples, stool tubes with integrated measuring spoons in their lids are available. The spoons in these tubes are designed to collect 200 mg stool. This protocol is optimized for usage with such stool tubes.

Lysis conditions in this protocol are optimized to increase the ratio of nonhuman DNA to human DNA. Human DNA is not excluded by this procedure.

Important points before starting

- For detection of cells that are difficult to lyse, such as Gram-positive bacteria, the lysis temperature in step 3 can be increased to 95°C, if necessary.
- All centrifugation steps should be carried out at room temperature (15–25°C) at 20,000 x g (approximately 14,000 rpm). Increase the centrifugation time proportionately if your centrifuge cannot provide 20,000 x g (e.g., instead of centrifuging for 5 min at 20,000 x g, centrifuge for 10 min at 10,000 x g).

Things to do before starting

- Prepare a thermomixer with 2 ml inlays or a water bath at 70°C for use in steps 4 and 9
- Redissolve any precipitates in Buffer AL and InhibitEX Buffer by incubating at 37–70°C
- Add ethanol to Buffer AW1 and Buffer AW2 concentrates
- Mix all buffers before use

Procedure

1. **Use the spoon integrated in the cap of a stool tube (not provided) to measure 180–220 mg of the stool sample. A level spoonful will correspond to approximately 200 mg stool. Close the tube and place it on ice.**

   A spatula should be used to remove excess stool from the spoon.

2. **Add 2 ml InhibitEX Buffer to each stool sample. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.**

   Note: It is important to vortex the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate. After InhibitEX Buffer has been added, all the following steps can be carried out at room temperature (15–25°C).

3. **Pipet 1.2 ml of the stool lysate into a labeled 2 ml microcentrifuge tube (not provided).**

   Cut the ends off the pipet tips to make pipetting viscous samples easier.

4. **Heat the suspension for 5 min at 70°C. Vortex for 15 s.**

   This heating step helps to lyse bacteria and other parasites. The lysis temperature can be increased to 95°C for cells that are difficult to lyse (such as Gram-positive bacteria).

5. **Centrifuge sample at full speed for 1 min to pellet stool particles.**

   IMPORTANT: Do not transfer any solid material. If particles are still visible in the supernatant, centrifuge the sample again.

6. **Pipet 15 µl proteinase K into a new 1.5 ml microcentrifuge tube (not provided).**

7. **Pipet 200 µl supernatant from step 5 into the 1.5 ml microcentrifuge tube containing proteinase K.**

8. **Add 200 µl Buffer AL and vortex for 15 s.**

   Note: Do not add proteinase K directly to Buffer AL. It is essential that the sample and Buffer AL are thoroughly mixed to form a homogeneous solution.

9. **Incubate at 70°C for 10 min.**

   Centrifuge briefly to remove drops from the inside of the tube lid (optional).

10. **Add 200 µl of ethanol (96–100%) to the lysate, and mix by vortexing.**

    Centrifuge briefly to remove drops from the inside of the tube lid (optional).

11. **Carefully apply 600 µl lysate from step 10 to the QIAamp spin column. Close the cap and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate.**

    Close each spin column in order to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.

12. **Carefully open the QIAamp spin column and add 500 µl Buffer AW1. Centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.**

13. **Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Centrifuge at full speed for 3 min. Discard the collection tube containing the filtrate.**

    Note: Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, causing the flow-through containing Buffer AW2 to come in contact with the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come in contact with the QIAamp spin column.

14. **Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 3 min.**

    This step helps to eliminate the chance of possible Buffer AW2 carryover.

15. **Transfer the QIAamp spin column into a new, labeled 1.5 ml microcentrifuge tube (not provided) and pipet 200 µl Buffer ATE directly onto the QIAamp membrane. Incubate for 1 min at room temperature, then centrifuge at full speed for 1 min to elute DNA.**

    If yield is to be quantified by UV absorbance, blank the measuring device using Buffer ATE to avoid false results. For more information about elution and how to determine DNA yield, purity, and length, see the Appendix on page 33. For long-term storage, we recommend keeping the eluate at –20°C.

Protocol: Isolation of DNA from Larger Volumes of Stool

This protocol is recommended when the target DNA is not distributed homogeneously in the stool. Using a relatively large amount of starting material enhances the chances of isolating DNA from low-titer sources in stool samples. Note that the yield and concentration of DNA isolated using this protocol are not greater than the yield and concentration of DNA isolated using the other protocols in this handbook.

Important points before starting

- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on the labels
- Mix all buffers before use
- If a precipitate has formed in InhibitEx Buffer or AL, dissolve by incubating at 37–70°C
- All centrifugation steps should be carried out at room temperature (15–25°C) at 20,000 x g (approximately 14,000 rpm). Increase the centrifugation time proportionately if your centrifuge cannot provide 20,000 x g (e.g., instead of centrifuging for 5 min at 20,000 x g, centrifuge for 10 min at 10,000 x g).

Procedure

1. **Weigh the stool sample and add 10 volumes of InhibitEx Buffer (e.g., add 10 ml InhibitEx buffer to 1 g stool).**

   If necessary, additional InhibitEx Buffer can be purchased separately (see Ordering Information on page 34).

2. **Vortex vigorously for 1 min or until the stool sample is thoroughly homogenized.**

   Note: It is important to vortex the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.

3. **Pipet 2 ml of lysate into a labeled 2 ml microcentrifuge tube (not provided).**

   Cut the ends off the pipet tips to make pipetting viscous samples easier.

4. **Depending on the downstream application, continue with step 4 of "Protocol using Stool Tubes for Isolation of DNA from Stool for Human DNA Analysis" (page 17) or step 4 of "Protocol using Stool Tubes for Isolation of DNA from Stool for Pathogen Detection" (page 23).**

Troubleshooting Guide

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

	Comments and suggestions
Little or no DNA in the eluate	
a) Sample stored incorrectly	Samples should be stored at 4°C or –20°C.
b) Insufficient homogenization of stool sample in InhibitEX Buffer	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and InhibitEX Buffer until the sample is thoroughly homogenized.
c) Insufficient mixing with Buffer AL	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer AL immediately and thoroughly by pulse-vortexing.
d) No alcohol added to the lysate before loading onto the QIAamp spin column	Repeat the purification procedure with a new sample.
e) Low-percentage alcohol used instead of 100%	Repeat the purification procedure with a new sample.
f) DNA not eluted efficiently	To increase elution efficiency, pipet Buffer ATE onto the QIAamp spin column and incubate the column for 5 min at room temperature before centrifugation.
g) Buffer AW1 or Buffer AW2 prepared incorrectly	Check that Buffer AW1 and Buffer AW2 concentrates were diluted with correct volumes of pure ethanol. Repeat the purification procedure with a new sample.

	Comments and suggestions
h) Buffer AW1 or Buffer AW2 prepared with 70% ethanol	Check that Buffer AW1 and Buffer AW2 concentrates were diluted with 96–100% ethanol. Repeat the purification procedure with a new sample.
i) Buffer AW1 and Buffer AW2 used in the wrong order	Ensure that Buffer AW1 and Buffer AW2 are used in the correct order in the protocol. Add 200 µl Buffer AL and 200 µl ethanol to the eluate, and continue with step 10 of "Protocol: Isolation of DNA from Stool for Pathogen Detection" (page 20).

A260/A280 ratio for purified nucleic acids is low

a) Insufficient mixing with Buffer AL	Repeat the procedure with a new sample. Be sure to mix the sample and Buffer AL immediately and thoroughly by pulse-vortexing.
b) Decreased proteinase activity	Repeat the DNA purification procedure with a new sample and proteinase K.
c) No alcohol added to the lysate before loading onto the QIAamp spin column	Repeat the purification procedure with a new sample.
d) Buffer AW1 or Buffer AW2 prepared with low-percentage ethanol.	Check that Buffer AW1 and Buffer AW2 concentrates were diluted with 96–100% ethanol. Repeat the purification procedure with a new sample.
e) Buffer AW1 or Buffer AW2 prepared incorrectly	Check that Buffer AW1 and Buffer AW2 concentrates were diluted with correct volumes of pure ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification procedure with a new sample.
f) Buffer AW1 and Buffer AW2 used in the wrong order	Ensure that Buffer AW1 and Buffer AW2 are used in the correct order in the protocol. Add 200 µl Buffer AL and 200 µl ethanol to the eluate, and continue with step 10 of "Protocol: Isolation of DNA from Stool for Pathogen Detection" (page 20).

A260/A280 ratio for purified nucleic acids is high

a) High level of residual RNA	Add 20 µl of RNase A (20 mg/ml) to the eluate and incubate for 10 minutes at room temperature (15–25°C).

DNA does not perform well in downstream applications

a) Too much DNA used in downstream reaction	The QIAamp Fast DNA Stool Mini Kit purifies total DNA, which could originate from many different organisms present in the original stool sample (e.g., human, animal, plant, bacterial). If the amount of total DNA is too high, PCR could be inhibited by excess total DNA. Reduce the amount of eluate used in the downstream reaction if possible.
b) Inefficient lysis of target cells	The amount of target DNA in the eluate may be low if the target cells are difficult to lyse, as is the case with some bacteria and parasites. In future preparations, increase lysis temperature to 95°C and/or incubation time as required.
c) Not enough DNA in eluate	Check "Little or no DNA in the eluate" for possible reasons.
d) Inhibitory substances in preparation	See "A260/A280 ratio for purified nucleic acids is low" for possible reasons. Bring the eluate volume to 200 µl if necessary, and repeat the purification procedure from step 5 of "Protocol: Isolation of DNA from Stool for Pathogen Detection" (page 14).
e) Residual Buffer AW2 in the eluate	Add 200 µl Buffer AL and 200 µl ethanol to the eluate, and continue with step 10 of "Protocol: Isolation of DNA from Stool for Pathogen Detection" (page 20).
f) Buffer AW1 and Buffer AW2 used in the wrong order	Ensure that Buffer AW1 and Buffer AW2 are used in the correct order in the protocol. Add 200 µl Buffer AL and 200 µl ethanol to the eluate, and continue with step 10 of "Protocol: Isolation of DNA from Stool for Pathogen Detection" (page 20).

	Comments and suggestions
g) Insufficient mixing with InhibitEX Buffer	Repeat the purification with new samples.
h) High level of residual RNA	Add 20 µl of RNase A (20 mg/ml) to the eluate and incubate for 10 min at room temperature (15–25°C). Add 200 µl Buffer AL and 200 µl ethanol to the eluate, and continue with step 10 of "Protocol: Isolation of DNA from Stool for Pathogen Detection" (page 20).
i) Reduced sensitivity of amplification reaction	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the reaction.

Little or no supernatant visible after initial centrifugation step

a) Insufficient centrifugal force	Increase the centrifugation time proportionately if your centrifuge cannot provide 20,000 x g (e.g., instead of centrifuging for 5 minutes at 20,000 x g, centrifuge for 10 minutes at 10,000 x g).

White precipitate in InhibitEX Buffer or Buffer AL

a) Storage at low temperature or prolonged storage	Any precipitate in InhibitEX Buffer or Buffer AL must be dissolved by incubating the buffer at 37–70°C.

Precipitate after addition of Buffer AL or ethanol

a) A precipitate may form upon addition of Buffer AL	In most cases, the precipitate will dissolve during incubation at 70°C. The precipitates do not interfere with the QIAamp procedure, or with any subsequent application.

	Comments and suggestions
b) A precipitate may form upon addition of ethanol	In most cases, the precipitate will dissolve after vortexing immediately following its appearance. The precipitates do not interfere with the QIAamp procedure, or with any subsequent application.

General handling

a) Lysate not completely passed through silica membrane	Centrifuge for 1 min at full speed or until all the lysate has passed through the membrane.
b) Cross-contamination between samples	To avoid cross-contamination when handling QIAamp spin columns, read "Handling of QIAamp Mini spin columns" on page 13. Repeat the purification procedure with new samples.

References

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

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

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

Determination of concentration, yield, and purity

DNA yields are determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an A260/A280 ratio of 1.7–1.9. Absorbance readings at 260 nm should lie between 0.1 and 1.0 to be accurate.

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

Determination of DNA length

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

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

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

Ordering Information

Product	Contents	Cat. no.
QIAamp Fast DNA Stool Mini Kit (50)	50 QIAamp Mini Spin Columns, Proteinase K, InhibitEX Buffer, wash and elution buffers, Collection Tubes (2ml)	51604
Related products		
QIAamp DNA Mini Kit — for genomic DNA purification from tissue, blood, and body fluids		
QIAamp DNA Mini Kit (50)*	50 QIAamp Mini Spin Columns, Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
QIAamp DNA Mini Kit (250)*	250 QIAamp Mini Spin Columns, Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51306
QIAamp DNA Blood Mini Kit — for genomic DNA purification from blood and body fluids		
QIAamp DNA Blood Mini Kit (50)*	50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104
QIAamp DNA Blood Mini Kit (250)*	250 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51106
QIAamp 96 DNA Blood Mini Kit — for high-throughput genomic DNA purification from blood and body fluids		
QIAamp 96 DNA Blood Kit (4)	4 QIAamp 96 Plates, QIAGEN Protease, Reagents, Buffers, Lysis Blocks, Tape Pads, Collection Vessels	51161
QIAamp 96 DNA Blood Kit (12)	12 QIAamp 96 Plates, QIAGEN Protease, Reagents, Buffers, Lysis Blocks, Tape Pads, Collection Vessels	51162
QIAamp RNA Blood Mini Kit — for total RNA purification from blood and body fluids		
QIAamp RNA Blood Mini Kit (20)*	20 QIAamp Mini Spin Columns, 20 QIAshredder Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-Free Reagents and Buffers	52303

Product	Contents	Cat. no.
QIAamp RNA Blood Mini Kit (50)*	50 QIAamp Mini Spin Columns, 50 QIAshredder Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-Free Reagents and Buffers	52304
QIAamp Viral RNA Mini Kit — for viral RNA purification from plasma, serum, and body fluids		
QIAamp Viral RNA Mini Kit (50)*	50 QIAamp Mini Spin Columns, Carrier RNA, Collection Tubes (2 ml), RNase-Free Buffers	52904
QIAamp Viral RNA Mini Kit (250)*	250 QIAamp Mini Spin Columns, Carrier RNA, Collection Tubes (2 ml), RNase-Free Buffers	52906
QIAcube — for fully automated sample preparation using QIAGEN spin-column kits		
QIAcube (110V)*	Robotic workstation for automated purification of nucleic acids or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor‡	9001292*
QIAcube (230V) †		9001293†

* US, Canada, and Japan.

† Rest of world.

‡ Agreements for comprehensive service coverage are available; please inquire.

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

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www.qiagen.com

Australia ■ techservice-au@qiagen.com

Austria ■ techservice-at@qiagen.com

Belgium ■ techservice-bnl@qiagen.com

Brazil ■ suportetecnico.brasil@qiagen.com

Canada ■ techservice-ca@qiagen.com

China ■ techservice-cn@qiagen.com

Denmark ■ techservice-nordic@qiagen.com

Finland ■ techservice-nordic@qiagen.com

France ■ techservice-fr@qiagen.com

Germany ■ techservice-de@qiagen.com

Hong Kong ■ techservice-hk@qiagen.com

India ■ techservice-india@qiagen.com

Ireland ■ techservice-uk@qiagen.com

Italy ■ techservice-it@qiagen.com

Japan ■ techservice-jp@qiagen.com

Korea (South) ■ techservice-kr@qiagen.com

Luxembourg ■ techservice-bnl@qiagen.com

Mexico ■ techservice-mx@qiagen.com

The Netherlands ■ techservice-bnl@qiagen.com

Norway ■ techservice-nordic@qiagen.com

Singapore ■ techservice-sg@qiagen.com

Sweden ■ techservice-nordic@qiagen.com

Switzerland ■ techservice-ch@qiagen.com

UK ■ techservice-uk@qiagen.com

USA ■ techservice-us@qiagen.com