Second Edition June 2012

QIAamp® DNA Stool Handbook

For DNA purification from stool samples

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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

QIAGEN sets standards in:

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

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

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Kit Contents

QIAamp DNA Stool Mini Kit	
Catalog no.	**51504**
Number of preps	**50**
QIAamp Mini Spin Columns	50
Collection Tubes (2 ml)	200
InhibitEX® Tablets	50
Buffer ASL	140 ml
Buffer AL*	33 ml
Buffer AW1* (concentrate)	19 ml
Buffer AW2† (concentrate)	13 ml
Buffer AE	15 ml
Selection guide	1

* 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.

Storage

The QIAamp DNA Stool Mini Kit can be stored dry at room temperature (15–25°C) for up to 1 year without showing any reduction in performance. The proteinase K solution can be stored at room temperature or at 2–8°C.

Intended Use

The QIAamp 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.

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.

24-hour emergency information

Emergency medical information can be obtained 24 hours a day from:
Poison Information Center Mainz, Germany
Tel.: +49-6131-19240

Quality Control

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

Introduction

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

The simple QIAamp spin procedure yields pure DNA ready for direct use in less than 1 hour. The procedure can be automated on the QIAcube® for increased standardization and ease of use (see page 9). 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 DNA Stool Mini Kit contains InhibitEX Tablets, a unique reagent provided in a convenient tablet form. InhibitEX Tablets efficiently adsorb these substances early in the purification process so that they can easily be removed by a quick centrifugation step. In addition, the kit contains Buffer ASL, which is specially developed to remove inhibitory substances from stool samples.

DNA of up to 20 kb is purified by the QIAamp DNA Stool Mini Kit. DNA of this length denatures completely during thermal cycling and can be amplified with the highest efficiency.

Robustness and specificity of downstream PCR

To increase robustness of PCR assays using DNA isolated from stool samples, we strongly recommend adding BSA to PCR mixtures to a final concentration of 0.1 µg/µl (e.g., Serva cat. no. 11920 or New England Biolabs® BSA, cat. no. BSA-007).

In DNA eluates from stool samples, the ratio of target DNA to background DNA is often very low. To increase the specificity of downstream PCR assays, we recommend the use of HotStarTaq® *Plus* DNA Polymerase (see ordering information on page 38). The combination of QIAamp DNA Stool sample preparation and use of BSA and HotStarTaq *Plus* DNA Polymerase in downstream amplification maximizes PCR robustness and specificity when amplifying DNA prepared from stool samples.

Principle and procedure

The QIAamp 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 stool samples in Buffer ASL
- Adsorption of impurities to InhibitEX matrix
- Purification of DNA on QIAamp Mini spin columns (see flowchart)

Sample size

The QIAamp 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 30.

The QIAamp 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 or InhibitEX matrix used.

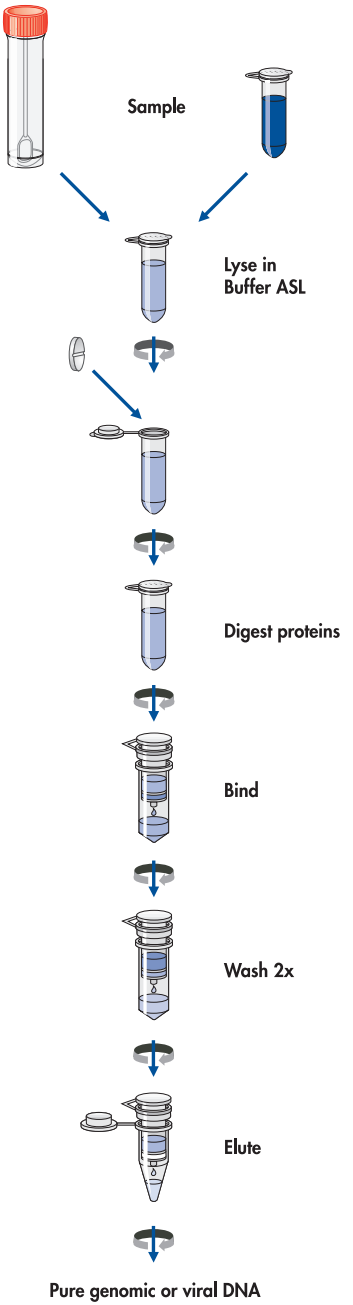
For maximum flexibility, protocols are provided for use with samples collected in both 2 ml microcentrifuge tubes and 15 ml stool tubes.

Lysis and adsorption of impurities to InhibitEX matrix

In the first steps of the protocol, stool samples are lysed in Buffer ASL. Human cells lyse efficiently at temperature (15–25°C). 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). However, note that total DNA concentration in the lysate will be increased 3- to 5-fold by lysis at 70°C and the ratio of nonhuman to human DNA will increase.

After lysis, DNA-damaging substances and PCR inhibitors present in the stool sample are adsorbed to InhibitEX matrix. InhibitEX matrix is provided in a convenient tablet form. After inhibitors and DNA-degrading substances have been adsorbed to InhibitEX matrix, the InhibitEX matrix is pelleted by centrifugation and the DNA in the supernatant is purified on QIAamp Mini spin columns.

QIAamp DNA Stool Mini Procedure



Fully automatable on the QIAcube

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 elution of 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 a low-salt buffer equilibrated to temperature (15–25°C). DNA yield is typically 15–60 µg, but, depending on the individual stool sample and the way it was stored, may range from 5–100 µg. DNA concentration is typically 75–300 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 stored for longer periods, storage at –20°C is recommended. It is highly recommended that 0.1 µg/µl BSA is added to downstream PCR assays to maximize robustness.

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 DNA purification on the QIAcube

Purification of DNA from stool samples using the QIAamp 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 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 DNA Stool Mini Kit can be automated on the QIAcube.

Application of purified DNA in downstream PCR

The QIAamp DNA Stool Mini Kit can purify large amounts of DNA. PCR can be inhibited by excess total DNA (in general >1 µg DNA). Depending on the individual stool sample, yield may be up to 100 µg total DNA (up to 500 ng/µl). In general, for optimum PCR results, use the minimum amount of DNA eluate possible in PCR. The volume of eluate used as template should not exceed 10% of the final volume of the PCR mixture. For maximum PCR robustness, we highly recommend adding BSA to a final concentration of 0.1 µg/µl to the PCR mixture. To maximize PCR specificity, we recommend using HotStarTaq *Plus* DNA Polymerase.

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%)*
- BSA, for increased robustness in downstream PCR (e.g., Serva cat. no. 11920 or New England Biolabs BSA, cat. no. B9001S)†
- 1.5 ml and 2 ml microcentrifuge tubes (the 2 ml tubes should be wide enough to accommodate an InhibitEX tablet, for example, 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)
- 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

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

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

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

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.

Handling of InhibitEX Tablets

InhibitEX Tablets are blister-packed for easy aseptic handling. InhibitEX Tablets should be popped out of their packet directly into a suitable 2 ml tube without being touched, to prevent cross-contamination.

The 2 ml tubes used should be wide enough to accommodate an InhibitEX Tablet.

* 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.
- After all vortexing steps, to avoid cross-contamination, we recommend briefly centrifuging the microcentrifuge tubes 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 in 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 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 those of some bacteria and parasites, 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*).
- The 2 ml tubes used in step 5 should be wide enough to accommodate an InhibitEX Tablet.

Things to do before starting

- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on the labels.
- Mix all buffers before use.
- If a precipitate has formed in Buffer ASL or Buffer AL, dissolve by incubating at 70°C.
- Prepare a 70°C water bath for use in steps 3 and 12.

Procedure

1. **Weigh 180–220 mg stool in a 2 ml microcentrifuge tube (not provided) and place the 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 or InhibitEX matrix when using smaller amounts of stool. For samples >220 mg, see "Protocol: Isolation of DNA from Larger Volumes of Stool", page 30.

   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 Buffer ASL is added in step 2 to lyse the sample; otherwise the DNA in the sample may degrade. After addition of Buffer ASL, all following steps can be performed at room temperature (15–25°C).

2. **Add 1.4 ml Buffer ASL 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.**

   This heating step increases total DNA yield 3- to 5-fold and 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. **Vortex for 15 s and centrifuge sample at full speed for 1 min to pellet stool particles.**

5. **Pipet 1.2 ml of the supernatant into a new 2 ml microcentrifuge tube (not provided) and discard the pellet.**

   Note: The 2 ml tubes used should be wide enough to accommodate an InhibitEX Tablet.

   Transfer of small quantities of pelleted material will not affect the procedure.

6. **Add 1 InhibitEX Tablet to each sample and vortex immediately and continuously for 1 min or until the tablet is completely suspended. Incubate suspension for 1 min at room temperature to allow inhibitors to adsorb to the InhibitEX matrix.**

7. **Centrifuge sample at full speed for 3 min to pellet inhibitors bound to InhibitEX matrix.**

8. **Pipet all the supernatant into a new 1.5 ml microcentrifuge tube (not provided) and discard the pellet. Centrifuge the sample at full speed for 3 min.**

   Transfer of small quantities of pelleted material from step 7 will not affect the procedure.

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

10. **Pipet 200 µl supernatant from step 8 into the 1.5 ml microcentrifuge tube containing proteinase K.**

11. **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.

12. **Incubate at 70°C for 10 min.**

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

13. **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).

14. **Label the lid of a new QIAamp spin column placed in a 2 ml collection tube. Carefully apply the complete lysate from step 13 to the QIAamp spin column without moistening the rim. 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 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.

15. **Carefully open the QIAamp spin column and add 500 µl Buffer AW1. 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 collection tube containing the filtrate.**

16. **Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Close the cap and 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, resulting in the flow-through, which contains Buffer AW2, contacting 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.

17. **Recommended: 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 1 min.**

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

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

Note: When using eluates in PCR, for maximum PCR robustness we highly recommend adding BSA to a final concentration of 0.1 µg/µl to the PCR mixture. For maximum PCR specificity we recommend using HotStarTaq *Plus* DNA Polymerase (see ordering information on page 38). For best results in downstream PCR, use the minimum amount of eluate possible in PCR; the volume of eluate used as template should not exceed 10% of the final volume of the PCR mixture. Also, note that high amounts of template DNA may inhibit the PCR.

DNA yield is typically 15–60 µg but, depending on the individual stool sample and the way it was stored, may range from 5 to 100 µg. DNA concentration is typically 75–300 ng/µl.

For more information about elution and how to determine DNA yield, purity, and length, see the Appendix, page 36.

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 those of some bacteria and parasites, the lysis temperature in step 4 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*).
- The 2 ml tubes used in step 6 should be wide enough to accommodate an InhibitEX Tablet.

Things to do before starting

- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on the labels.
- Mix all buffers before use.
- If a precipitate has formed in Buffer ASL or Buffer AL, dissolve by incubating at 70°C.
- Prepare a 70°C water bath for use in steps 4 and 13.

Procedure

1. **Use the spoon integrated into 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 Buffer ASL to each stool tube. Use the pipet to wash the stool sample from the spoon while transferring the buffer. 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 Buffer ASL has been added, all the following steps can be carried out at room temperature (15–25°C).

3. **Pipet 1.6 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.**

   This heating step increases total DNA yield 3- to 5-fold and helps to lyse bacteria and parasites. The lysis temperature can be increased to 95°C for cells that are difficult to lyse (such as Gram-positive bacteria).

5. **Vortex for 15 s and centrifuge sample at full speed for 1 min to pellet stool particles.**

6. **Pipet 1.2 ml of the supernatant into a new 2 ml microcentrifuge tube (not provided) and discard the pellet.**

   Note: The 2 ml tubes used should be wide enough to accommodate an InhibitEX Tablet.

   Transfer of small quantities of pelleted material will not affect the procedure.

7. **Add 1 InhibitEX Tablet to each sample and vortex immediately and continuously for 1 min or until the tablet is completely suspended. Incubate suspension for 1 min at room temperature to allow inhibitors to adsorb to the InhibitEX matrix.**

8. **Centrifuge sample at full speed for 3 min to pellet stool particles and inhibitors bound to InhibitEX matrix.**

9. **Pipet all the supernatant into a new 1.5 ml microcentrifuge tube (not provided) and discard the pellet. Centrifuge the sample at full speed for 3 min.**

   Transfer of small quantities of pelleted material from step 8 will not affect the procedure.

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

11. **Pipet 200 µl supernatant from step 9 into the 1.5 ml microcentrifuge tube containing proteinase K.**
12. **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.
13. **Incubate at 70°C for 10 min.**

    Centrifuge briefly to remove drops from the inside of the tube lid (optional).
14. **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).
15. **Label the lid of a new QIAamp spin column placed in a 2 ml collection tube. Carefully apply the complete lysate from step 14 to the QIAamp spin column without moistening the rim. 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 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.
16. **Carefully open the QIAamp spin column and add 500 µl Buffer AW1. 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 collection tube containing the filtrate.**
17. **Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Close the cap and 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, resulting in the flow-through, which contains Buffer AW2, contacting 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.
18. **Recommended: 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 1 min.**

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

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

Note: When using eluates in PCR, for maximum PCR robustness we highly recommend adding BSA to a final concentration of 0.1 µg/µl to the PCR mixture. For maximum PCR specificity we recommend using HotStarTaq *Plus* DNA Polymerase (see ordering information on page 38). For best results in downstream PCR, use the minimum amount of eluate possible in PCR; the volume of eluate used as template should not exceed 10% of the final volume of the PCR mixture. Also, note that high amounts of template DNA may inhibit the PCR.

DNA yield is typically 15–60 µg but, depending on the individual stool sample and the way it was stored, may range from 5 to 100 µg. DNA concentration is typically 75–300 ng/µl.

For more information about elution and how to determine DNA yield, purity, and length, see the Appendix, page 36.

For long-term storage, we recommend keeping the eluate at –20°C.

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 procedure.

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*).
- The 2 ml tubes used in step 4 should be wide enough to accommodate an InhibitEX Tablet.

Things to do before starting

- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on the labels.
- Mix all buffers before use.
- If a precipitate has formed in Buffer ASL or Buffer AL, dissolve by incubating at 70°C.
- Prepare a 70°C water bath for use in step 11.

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 or InhibitEX matrix when using smaller amounts of stool. For samples >220 mg, see "Protocol: Isolation of DNA from Larger Volumes of Stool", page 30.

   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 Buffer ASL is added in step 2 to lyse the sample; otherwise the DNA in the sample may degrade. After addition of Buffer ASL, all following steps can be performed at room temperature (15–25°C).

2. **Add 1.6 ml Buffer ASL 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.**

4. **Pipet 1.4 ml of the supernatant into a new 2 ml microcentrifuge tube (not provided) and discard the pellet.**

   Note: The 2 ml tubes used should be wide enough to accommodate an InhibitEX Tablet.

   Transferring small quantities of pelleted material will not affect the procedure.

5. **Add 1 InhibitEX Tablet to each sample and vortex immediately and continuously for 1 min or until the tablet is completely suspended. Incubate suspension for 1 min at room temperature (15–25°C) to allow inhibitors to adsorb to the InhibitEX matrix.**

6. **Centrifuge sample at full speed for 3 min to pellet stool particles and inhibitors bound to InhibitEX matrix.**

   Note: For most samples, 3 min centrifugation is sufficient. With some samples, however, centrifugation for 3 min may result in a pellet that is not sufficiently compact. Therefore it may be difficult to remove enough supernatant to transfer 600 µl supernatant after the next centrifugation step (step 9). In these cases, we recommend to centrifuge for 6 min.

   Note: When processing more than 12 samples, for this step and step 7 we recommend processing batches of no more than 12 samples each. This is because the pellets formed after centrifugation will break up quickly if the supernatant is not removed immediately.

7. **Immediately after the centrifuge stops, pipet all of the supernatant into a new 1.5 ml microcentrifuge tube (not provided) and discard the pellet. Centrifuge the sample at full speed for 3 min.**

   Transferring small quantities of pelleted material from step 6 will not affect the procedure.

8. **Pipet 25 µl proteinase K into a new 2 ml microcentrifuge tube (not provided).**

9. **Pipet 600 µl supernatant from step 7 to the 2 ml microcentrifuge tube containing proteinase K.**

10. **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.

11. **Incubate at 70°C for 10 min.**

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

12. **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).

13. **Label the lid of a new QIAamp spin column provided in a 2 ml collection tube. Carefully apply 600 µl lysate from step 12 to the QIAamp spin column without moistening the rim. 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 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.

14. **Carefully open the QIAamp spin column, apply a second aliquot of 600 µl lysate 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 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.

15. **Repeat step 14 to load the third aliquot of the lysate onto the spin column.**

16. **Carefully open the QIAamp spin column and add 500 µl Buffer AW1. 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 collection tube containing the filtrate.**

17. **Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Close the cap and 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, resulting in the flow-through, which contains Buffer AW2, contacting 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.

18. **Recommended: 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 1 min.**

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

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

Note: When using eluates in PCR, for maximum PCR robustness we highly recommend adding BSA to a final concentration of 0.1 µg/µl to the PCR mixture. For maximum PCR specificity we recommend using HotStarTaq *Plus* DNA Polymerase (see ordering information on page 38). For best results in downstream PCR, use the minimum amount of eluate possible in PCR; the volume of eluate used as template should not exceed 10% of the final volume of the PCR mixture. Also, note that high amounts of template DNA may inhibit the PCR.

DNA yield is typically 15–60 µg but, depending on the individual stool sample and the way it was stored, may range from 5 to 100 µg. DNA concentration is typically 75–300 ng/µl.

For more information about elution and how to determine DNA yield, purity, and length, see the Appendix, page 36.

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 use 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 procedure.

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*).
- The 2 ml tubes used in step 6 should be wide enough to accommodate an InhibitEX Tablet.

Things to do 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 Buffer ASL or AL, dissolve by incubating at 70°C.
- Prepare a 70°C water bath for use in step 12.

Procedure

1. **Use the spoon integrated into 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 2.6 ml Buffer ASL to each stool tube. Use the pipet to wash the stool sample from the spoon while transferring the buffer. Vortex vigorously and 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 Buffer ASL has been added, all the following steps can be carried out at room temperature (15–25°C).

3. **Pipet 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.**

5. **Pipet 1.4 ml of the supernatant into a new 2 ml microcentrifuge tube (not provided) and discard the pellet.**

   Note: The 2 ml tubes used should be wide enough to accommodate an InhibitEX Tablet.

   Transferring small quantities of pelleted material will not affect the procedure.

6. **Add 1 InhibitEX Tablet to each sample and vortex immediately and continuously for 1 min or until the tablet is completely suspended. Incubate suspension for 1 min at room temperature to allow inhibitors to adsorb to the InhibitEX matrix.**

7. **Centrifuge sample at full speed for 3 min to pellet stool particles and inhibitors bound to InhibitEX matrix.**

   Note: For most samples, 3 min centrifugation is sufficient. With some samples, however, centrifugation for 3 min may result in a pellet that is not sufficiently compact. Therefore it may be difficult to remove enough supernatant to transfer 600 µl supernatant after the next centrifugation step (step 10). In these cases, we recommend to centrifuge for 6 min.

   Note: When processing more than 12 samples, for this step and step 8 it is recommended to process batches of no more than 12 samples each. This is because the pellets formed after centrifugation will break up quickly if the supernatant is not removed immediately.

8. **Immediately after the centrifuge stops, pipet all of the supernatant completely into a new 1.5 ml microcentrifuge tube (not provided) and discard the pellet. Centrifuge the sample at full speed for 3 min.**

   Transferring small quantities of pelleted material from step 7 will not affect the procedure.

9. **Pipet 25 µl proteinase K into a new 2 ml microcentrifuge tube (not provided).**

10. **Pipet 600 µl supernatant from step 8 into the 2 ml microcentrifuge tube containing proteinase K.**

11. **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.

12. **Incubate at 70°C for 10 min.**

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

13. **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).

14. **Label the lid of a new QIAamp spin column provided in a 2 ml collection tube. Carefully apply 600 µl lysate from step 13 to the QIAamp spin column without moistening the rim. 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 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.

15. **Carefully open the QIAamp spin column, apply a second aliquot of 600 µl lysate 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 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.

16. **Repeat step 15 to load the third aliquot of lysate onto the spin column.**

17. **Carefully open the QIAamp spin column and add 500 µl Buffer AW1. 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 collection tube containing the filtrate.**

18. **Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Close the cap and 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, resulting in the flow-through, which contains Buffer AW2, contacting 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. In these cases, the optional step below should be performed.

19. **Recommended: 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 1 min.**

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

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

Note: When using eluates in PCR, for maximum PCR robustness we highly recommend adding BSA to a final concentration of 0.1 µg/µl to the PCR mixture. For maximum PCR specificity we recommend using HotStarTaq *Plus* DNA Polymerase (see ordering information on page 38). For best results in downstream PCR, use the minimum amount of eluate possible in PCR; the volume of eluate used as template should not exceed 10% of the final volume of the PCR mixture. Also, note that high amounts of template DNA may inhibit the PCR.

DNA yield is typically 15–60 µg but, depending on the individual stool sample and the way it was stored, may range from 5 to 100 µg. DNA concentration is typically 75–300 ng/µl.

For more information about elution and how to determine DNA yield, purity, and length, see the Appendix, page 36.

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

- 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*).
- The 2 ml tubes used should be wide enough to accommodate an InhibitEX Tablet.

Things to do before starting

- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on the labels.
- Mix all buffers before use.
- If a precipitate has formed in Buffer ASL or Buffer AL, dissolve by incubating at 70°C.

Procedure

1. **Weigh the stool sample and add 10 volumes of Buffer ASL (e.g. add 10 ml Buffer ASL to 1 g stool). Vortex vigorously for 1 min or until the stool sample is thoroughly homogenized.**

   If necessary, additional Buffer ASL can be purchased separately (see ordering information on page 39).

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

2. **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.

3. **Depending on the downstream application, continue with step 4 of "Protocol: Using Stool Tubes for Isolation of DNA from Stool for Pathogen Detection", page 19, or step 4 of "Protocol: Using Stool Tubes for Isolation of DNA from Stool for Human DNA Analysis", page 27.**

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 Buffer ASL	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer ASL 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 AE onto the QIAamp spin column and incubate the column for 5 minutes at room temperature (15–25°C) 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.
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.

		Comments and suggestions
i)	Buffer AW1 and Buffer AW2 used in the wrong order	Ensure that Buffer AW1 and Buffer AW2 are user in the correct order in the protocol. Add 200 µl Buffer AL and 200 µl ethanol to the eluate, and continue with step 14 of "Protocol: Isolation of DNA from Stool for Pathogen Detection" (page 18).
A_{260}/A_{280} ratio for purified nucleic acids is low		
a)	Inefficient elimination of inhibitory substances due to insufficient mixing with InhibitEX matrix	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and InhibitEX matrix until the sample is thoroughly homogenized.
b)	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.
c)	Decreased proteinase activity	Repeat the DNA purification procedure with a new sample and with proteinase K.
d)	No alcohol added to the lysate before loading onto the QIAamp spin column	Repeat the purification procedure with a new sample.
e)	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.
f)	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.
g)	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 14 of "Protocol: Isolation of DNA from Stool for Pathogen Detection" (page 18).

A_{260}/A_{280} ratio for purified nucleic acids is high

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)	BSA not added to PCR mixture	When using eluates in PCR, for maximum PCR robustness add BSA to a final concentration of 0.1 µg/µl to the PCR mixture.
b)	Too much DNA used in downstream reaction	The QIAamp 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.
c)	Nonspecific bands in downstream PCR	It is likely that only a low quantity of target DNA is present in stool-sample eluates, together with high amounts of background DNA. To maximize PCR specificity, we recommend the use of HotStarTaq *Plus* DNA Polymerase (see ordering information on page 38).
d)	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.
e)	Not enough DNA in eluate	Check "Little or no DNA in the eluate" for possible reasons.
f)	Inhibitory substances in preparation	See "A_{260}/A_{280} 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 9 of "Protocol: Isolation of DNA from Stool for Pathogen Detection" (page 16).

		Comments and suggestions
g)	Residual Buffer AW2 in the eluate	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 14 of "Protocol: Isolation of DNA from Stool for Pathogen Detection" (page 16).
h)	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 14 of "Protocol: Isolation of DNA from Stool for Pathogen Detection" (page 16).
i)	Insufficient mixing with Buffer ASL	Repeat the purification with new samples.
j)	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 14 of "Protocol: Isolation of DNA from Stool for Pathogen Detection" (page 16).
k)	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.
l)	Amplification reaction setup has been modified	Re-optimize your amplification system by adjusting the volume of eluate added.
Little or no supernatant visible after initial centrifugation step		
	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*).

Little or no supernatant visible after precipitation of InhibitEX matrix

Human DNA protocols: Insufficient centrifugal force	With some samples, centrifugation to precipitate the InhibitEX matrix (step 6 on page 23; step 7 on page 27) may result in a pellet that is not sufficiently compact. In these cases, we recommend to increase the centrifugation time for precipitation of InhibitEX matrix to 6 minutes.

White precipitate in Buffer ASL or Buffer AL

Storage at low temperature or prolonged storage	Any precipitate in Buffer ASL or Buffer AL must be dissolved by incubation of the buffer at 70°C.

Precipitate after addition of Buffer AL or ethanol

a)	A precipitate may form on 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.
b)	A precipitate may form on 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 minute 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.
c)	InhibitEX Tablet does not fit in 2 ml tube	The 2 ml tubes used should be wide enough to accommodate an InhibitEX Tablet (e.g., Eppendorf Safe-Lock, cat. no. 0030120.094 or Sarstedt Safe-Seal, cat. no. 72.695).*

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

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 A_{260}/A_{280} ratio of 1.7–1.9.

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

Determination of DNA length

The length of genomic DNA can be determined by pulsed-field gel electrophoresis (PFGE) through an agarose gel. The DNA should be concentrated by alcohol* precipitation and reconstituted by gentle agitation in approximately 30 µl TE buffer, pH 8.0,* for at least 30 minutes at 60°C. Avoid drying the DNA pellet for more than 10 minutes at room temperature (15–25°C) 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 safety data sheets (SDSs), available from the product supplier.

Ordering Information

Product	Contents	Cat. no.
QIAamp DNA Stool Mini Kit (50)*	50 QIAamp Mini Spin Columns, 50 InhibitEX Tablets, Proteinase K, Buffers, Collection Tubes (2 ml)	51504
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 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 96 DNA Blood 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 RNA Blood Mini Kit — for total RNA purification from blood and body fluids		
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

* Automatable on the QIAcube. See www.qiagen.com/MyQIAcube for protocols.

† Other kit sizes are available; see www.qiagen.com.

‡ Fully automatable on the QIAcube. See www.qiagen.com/MyQIAcube for protocols.

Ordering Information

Product	Contents	Cat. no.
QIAamp 96 DNA Blood BioRobot® Kit — for automated, high-throughput purification of genomic, mitochondrial, and viral DNA from blood and other body fluids		
QIAamp 96 DNA Blood BioRobot Kit (12)	For 12 x 96 DNA preps: 12 QIAamp 96 Plates, Buffers, QIAGEN Protease, AirPore Tape Sheets, Tape Pad, S-Blocks, Racks with Collection Microtubes (1.2 ml), Caps	965142
QIAamp 96 Virus BioRobot Kit — for automated, high-throughput purification of viral RNA and DNA from cell-free body fluids		
QIAamp 96 Virus BioRobot Kit (12)	For 12 x 96 nucleic acid preps: 12 QIAamp 96 Plates, RNase-free Buffers, QIAGEN Protease, AirPore Tape Sheets, Tape Pad, S-Blocks, Racks with Collection Microtubes (1.2 ml), Carrier RNA, Caps	965642
HotStarTaq *Plus* DNA Polymerase — for highly specific hot-start PCR without optimization		
HotStarTaq *Plus* DNA Polymerase (250)*	250 units HotStarTaq *Plus* DNA Polymerase, 10x PCR Buffer, 10x CoralLoad® PCR Buffer, 5x Q-Solution®, 25 mM $MgCl_2$	203603
HotStarTaq *Plus* Master Mix Kit (250)*	For 250 x 20 µl reactions: 3 x 0.85 ml HotStarTaq *Plus* Master Mix, containing 250 units of HotStarTaq *Plus* DNA Polymerase total, 1 x 0.55 ml CoralLoad Concentrate, 2 x 1.9 ml RNase-Free Water	203643

* Other kit sizes are available; see www.qiagen.com.

Ordering Information

Product	Contents	Cat. no.
QIAcube — for fully automated sample preparation using QIAGEN spin-column kits		
QIAcube (110 V)* QIAcube (230 V)†	Robotic workstation for automated purification of nucleic acids or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor‡	9001292* 9001293†
Accessories		
Buffer ASL	560 ml Stool Lysis Buffer	19082
Buffer AL	216 ml for 1000 preparations	19075
Buffer AW1 (concentrate)	242 ml Wash Buffer 1 Concentrate for 1000 preparations	19081
Buffer AW2 (concentrate)	324 ml Wash Buffer 2 Concentrate for 1000 preparations	19072
Buffer AE	240 ml Elution Buffer for 1000 preparations	19077
Collection Tubes (2 ml)	1000 Collection Tubes (2 ml)	19201
InhibitEX Tablets (100)	PCR inhibitor absorption matrix to be used with Buffer ASL during DNA purification	19590
QIAGEN Proteinase K (2)	2 ml (>600 mAU/ml, solution)	19131
QIAGEN Proteinase K (10)	10 ml (>600 mAU/ml, solution)	19133
QIAamp DNA Accessory Set A	Additional Buffers and Reagents; for use with at least 8 x QIAamp DNA Stool Mini Kits (50) on the QIAcube	1048145

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.

* US, Canada, and Japan.

† Rest of world.

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

Notes

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Trademarks:

QIAGEN®, QIAamp®, QIAcube®, BioRobot®, CoralLoad®, HotStarTaq®, InhibitEX®, Q-Solution® (QIAGEN Group); Eppendorf® (Eppendorf AG); New England Biolabs® (New England Biolabs);.

Limited License Agreement for QIAamp DNA Stool Kit

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