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QIAamp® Fast DNA Tissue Kit Handbook

For rapid purification of DNA from tissue samples

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAamp Fast DNA Tissue Kit	
Catalog No.	**51404**
Number of preps	**50**
QIAamp Mini Spin Columns	50
Buffer AVE*	20 ml
Buffer VXL†	6 ml
Buffer MVL†‡ (concentrate)	14.7 ml
QIAGEN Proteinase K	1.25 ml
RNase A	280 µl
Buffer AW1†§ (concentrate)	19 ml
Buffer AW2§ (concentrate)	17 ml
Buffer ATE	20 ml
Reagent DX	1 ml
Collection Tubes (1.5 ml)	50
Collection Tubes (2 ml)	150
Tissue Disruption Tube	50
Quick-Start Protocol	1

***CAUTION**: Contains sodium azide as a preservative.

† **CAUTION**: Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 5 for safety information.

‡ Before using for the first time, add isopropanol as indicated on the bottle to obtain a working solution.

§ Before using for the first time, add ethanol (96–100%) as indicated on the bottle to obtain a working solution.

Storage

QIAamp Mini Spin Columns and buffers can be stored dry at room temperature (15–25°C) for up to 1 year without showing any reduction in performance.

QIAamp Fast DNA Tissue Kits contain ready-to-use proteinase K solution, which is dissolved in a specially formulated storage buffer. QIAGEN Proteinase K is stable for up to 1 year after delivery when stored at room temperature. To prolong the lifetime of QIAGEN Proteinase K, storage at 2–8°C is recommended.

Intended Use

The QIAamp Fast DNA Tissue Kit is intended for molecular biology applications. These products are 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.

Buffer MVL contains guanidine thiocyanate and Buffer VXL and Buffer AW1 contain guanidine hydrochloride, which can form highly reactive compounds if combined with bleach.

If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

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

Introduction

The QIAamp Fast DNA Tissue Kit allows rapid purification of DNA from solid tissue. Fibrous tissues such as heart or fatty tissues such as liver or brain are all easily lysed using this method, as are more difficult samples such as mouse tails, ear punches, trachea tissues and Allprotect®-stabilized tissue. The optimized combination of mechanical, chemical and enzymatic lysis developed for this kit provides high yields with minimal time investment.

Homogenization and lysis of tissue samples

The QIAamp Fast DNA Tissue Kit uses a combination of mechanical, chemical and enzymatic lysis to homogenize samples. The included Tissue Disruption Tubes contain a specially shaped bead that effectively disrupts tissue when agitated. As little as 5 minutes on a desktop vortexer or 30 seconds in a high-powered bead mill is sufficient to homogenize the tissue. The optimized chemistry allows homogenization of the tissue and simultaneous stabilization of DNA released from the disrupted tissue. The digestion buffer mix also contains proteinase K, which completely lyses the sample material in a short subsequent incubation and is not affected by mechanical homogenization. During disruption, there is no need to open the tubes to add buffers or other reagents, thus minimizing the risk of aerosol formation. For particularly tough samples, such as rodent tails or Allprotect-stabilized tissue, two rounds of homogenization and proteinase K digestion may be carried out without needing to open the tubes or perform any special procedures.

Purification on QIAamp Mini Spin Columns

After homogenization and lysis, lysate buffering conditions are adjusted to allow optimal binding of the DNA to the QIAamp membrane before the sample is loaded onto the QIAamp Mini Spin Column. DNA is adsorbed onto the QIAamp silica membrane during a brief centrifugation step. The salt and pH conditions in the lysate ensure that protein and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not retained on the QIAamp membrane. The QIAamp spin procedures can be fully

automated on the QIAcube® for increased standardization and ease of use (see below). The procedures are designed to ensure that there is no sample-to-sample cross-contamination and allow safe handling of potentially infectious samples.

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

Purified DNA is eluted from the QIAamp Mini Spin Column in a concentrated form in Buffer ATE. Elution buffer should be equilibrated to room temperature (15–25°C) before applying to the column. Yields are increased if the QIAamp Mini Spin Column is incubated with the elution buffer at room temperature for 5 minutes before centrifugation. Eluted DNA is ready for direct addition to downstream applications, for example, PCR or NGS. Alternatively, eluted DNA can be safely stored at –20°C for later use. The purified DNA is free of protein, nucleases and other contaminants or inhibitors. DNA purified using QIAamp Kits is up to 50 kb in size, with fragments of approximately 20–30 kb predominating.

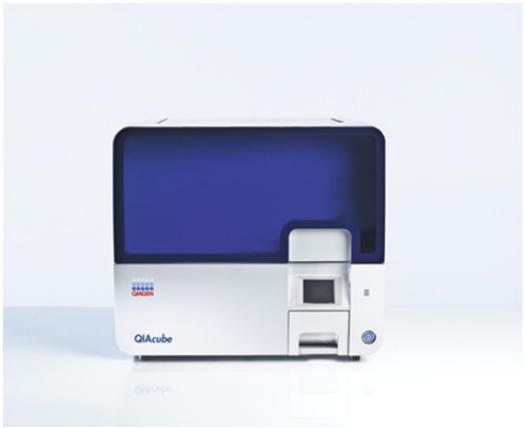
If the purified DNA is to be stored, elution in Buffer ATE and storage at –20°C is recommended. Buffer ATE has pH 8.3 and 0.1 mM EDTA. If this pH or EDTA concentration affects sensitive downstream applications, use water for elution. However, ensure that the pH of the water is at least 7.0 (deionized water from certain sources can be acidic). DNA stored in water is subject to degradation by acid hydrolysis.

Automated DNA purification on the QIAcube

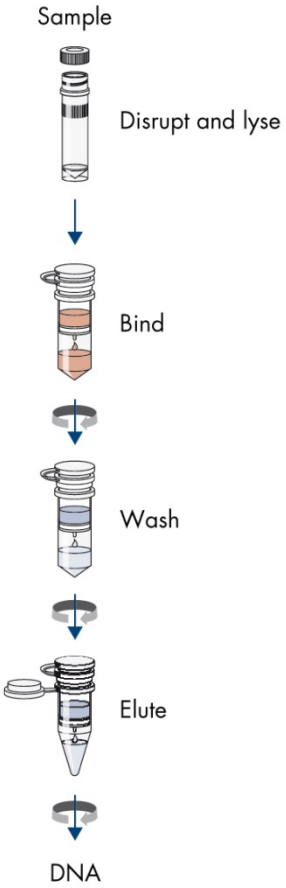
Purification of DNA from tissue using the QIAamp Fast DNA Tissue Kit can be fully automated on the QIAcube. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample preparation into your laboratory workflow. Sample 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 Tissue 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, proteins and 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**.



QIAamp Fast DNA Tissue Procedure



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.

- Pipets and disposable pipet tips with aerosol barriers (20–1000 µl)
- Isopropanol
- Ethanol (96–100%)*
- Vortexer with adapter or microtube holder e.g., the Horizontal (24) - Microtube Holder (cat. no. SI-H524) or Vertical (24) - Microtube Holder (cat. no. SI-V524) from Scientific Industries™ (www.scientificindustries.com)
- Thermomixer at 56°C, for example, the Thermomixer Comfort (cat. no. 5355 000.011) and a thermoblock for 24 x 2 ml tubes (cat. no. 5362 000.019) from Eppendorf® (www.eppendorf.com)
- Benchtop centrifuge (with rotor for 2 ml tubes)
- **Optional**: TissueLyser II (QIAGEN, cat. no. 85300) with a TissueLyser II Adapter Set 2 x 24 (QIAGEN, cat. no. 69982), TissueLyser LT (QIAGEN, cat. no. 85600) with the TissueLyser LT Adapter for 12 tubes (QIAGEN, cat. no. 69980) or other bead-mill homogenizers.

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

Important Notes

Starting material

The QIAamp Fast DNA Tissue Kit procedure is suitable for use with fresh, frozen or stabilized (e.g., Allprotect-stabilized tissues) samples. Different tissue types can vary widely with regard to texture and rigidity, cell types and content of host nucleic acids and inhibitory substances. Up to 25 mg of fresh, frozen or stabilized tissue can be used as a starting amount. For spleen or other tissues with very high DNA content, no more than 10 mg should be used.

Do not overload the QIAamp membrane, as this can lead to impaired nucleic acid extraction and/or performance in downstream assays. In some downstream applications such as PCR and RT-PCR, very high background concentrations of nucleic acids may impair the reaction. Use appropriate controls (e.g., an internal control) to verify successful PCR amplification.

Preparing reagents

Buffer AW1

Buffer AW1 is supplied as a concentrate. Before using for the first time, add ethanol (96–100%) as indicated on the bottle to obtain a working solution. Tick the check box on the bottle label to indicate that ethanol has been added. Mix well after adding ethanol.

Buffer AW2

Buffer AW2 is supplied as a concentrate. Before using for the first time, add ethanol (96–100%) as indicated on the bottle to obtain a working solution. Tick the check box on the bottle label to indicate that ethanol has been added. Mix well after adding ethanol.

Buffer MVL

Buffer MVL is supplied as a concentrate. Before using for the first time, add the appropriate amount of isopropanol (100%) to Buffer MVL concentrate as indicated on the bottle. Tick the check box on the bottle label to indicate that isopropanol has been added. Mix well after adding isopropanol. Buffer MVL is stable for 1 year when stored closed at room temperature.

QIAGEN Proteinase K

The QIAamp Fast DNA Tissue Kit contains ready-to-use QIAGEN Proteinase K supplied in a specially formulated storage buffer. The activity of the QIAGEN Proteinase K solution is 600 mAU/ml.

QIAGEN Proteinase K is stable for at least 1 year after delivery when stored at room temperature (15–25°C). To store for more than 1 year or if ambient temperature often exceeds 25°C, we recommend storing QIAGEN Proteinase K at 2–8°C.

Protocol: Purification of DNA from Tissue

This protocol is for the purification of DNA from tissue samples.

Notes before starting

- Add ethanol to Buffer AW1 and Buffer AW2 concentrates
- Add isopropanol to Buffer MVL concentrate
- Preheat a thermomixer to 56°C for use in steps 4 and 5
- Unless stated otherwise, all centrifugation steps should be performed at full speed (maximum 20,000 x *g*) for 30 s at room temperature in a conventional tabletop centrifuge

Procedure

1. Weigh and cut the tissue sample to a suitable size (5–25 mg), then place in the Tissue Disruption Tube (supplied).

   In general, it is not necessary to cut the tissue into small pieces prior to disruption. For particularly tough samples, such as rodent tails or Allprotect-stabilized tissues, it may increase the efficiency of disruption to cut the tissue into small pieces before proceeding with the disruption.

2. Add the following buffers and enzymes: 200 µl AVE, 40 µl VXL, 1 µl DX Reagent, 20 µl proteinase K, 4 µl RNase A (100 mg/ml). Tightly cap the lid and mix.

   Optional: When processing multiple samples simultaneously you may prepare a master digestion buffer mix. The master digestion buffer mix should not be stored; prepare a new master mix for each set of samples to be processed.

3. Homogenize using one of the following three options:

   a. Vortex-Genie®2 with appropriate 2 ml tube adapter: full speed for 5 min

   b. TissueLyser II with TissueLyser Adapter Set 2 x 24: 24 Hz for 30 s

c. TissueLyser LT: 45 Hz for 2 min

Note: Do not use frequencies higher than 24 Hz if using the TissueLyser II.

Note: Proceed with step 4 regardless of whether there is residual tissue visible or not.

Note: If residual tissue pieces are observed in the cap, briefly spin the microcentrifuge tube after homogenization.

Note: The lysis mixture may foam after mechanical homogenization. It is not necessary to take any action if this is observed; the foam will recede during the 56°C incubation.

4. Incubate in a thermomixer at 1000 rpm for 10 min at 56°C.
5. If the lysate is homogenous after step 4, proceed directly with step 6. If there is still residual tissue left after step 4, repeat steps 3 and 4 a single time with the same settings as for the first homogenization.

   If there is residual tissue left after repeating steps 3 and 4, increase the incubation time up to 1 h (1000 rpm at 56°C). Avoid longer incubation periods. Proceed with step 6 even if there is residual tissue remaining after the prolonged incubation.
6. Add 265 µl Buffer MVL and mix by pipetting or vortexing.
7. Apply the mixture from step 6 to the QIAamp Mini Spin Column and centrifuge for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.
8. Add 500 µl Buffer AW1 to the spin column and centrifuge. Place the spin column into a new 2 ml collection tube (supplied).
9. Add 500 µl Buffer AW2 to the spin column and centrifuge. Place the spin column into a new 2 ml collection tube (supplied).
10. Centrifuge for 2 min. Place the spin column into a clean 1.5 ml microcentrifuge tube (supplied).
11. Add 50–100 µl ATE directly onto the spin column membrane, incubate at room temperature for 1 min and then centrifuge for 1min.

    Optional: Repeat step 11 for increased yield.

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/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
Little or no DNA in the eluate		
a)	Buffer MVL not added to the lysate before loading onto the QIAamp Mini Spin Column	Repeat the purification procedure with a new sample.
b)	Buffer AW1 or AW2 prepared incorrectly	Check that Buffer AW1 and AW2 concentrates were diluted with the correct volumes of pure ethanol (see page 11). Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification procedure with a new sample.
c)	Buffer MVL prepared incorrectly	Check that Buffer MVL concentrate was diluted with the correct volumes of isopropanol (see page 12). Repeat the purification procedure with a new sample.
d)	QIAamp Mini Spin Column not incubated at room temperature (15-25°C) for 1 minute	After addition of Buffer ATE, the QIAamp Mini Spin Column should be incubated at room temperature for at least 1 minute.
e)	DNA not eluted efficiently	To increase elution efficiency, pipet Buffer ATE onto the center of the QIAamp Mini Spin Column and incubate the column for 5 min at room temperature before centrifugation.
f)	pH of water incorrect (acidic)	Low pH may reduce DNA yield. Ensure that the pH of the water is at least 7.0 or use Buffer ATE for elution.

		Comments and suggestions
A_{260}/A_{280} ratio for purified nucleic acids is low		
a)	Buffer AW1 or AW2 prepared incorrectly	Check that Buffer AW1 and AW2 concentrates were diluted with the correct volumes of pure ethanol (see page 11). Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification procedure with a new sample.
b)	Buffer AW1 or AW2 prepared incorrectly	Ensure that Buffers AW1 and AW2 are used in the correct order in the protocol. Repeat the purification procedure with a new sample.
DNA does not perform well in subsequent enzymatic reactions		
a)	Not enough DNA in sample	Check "Little or no DNA in the eluate" on page 15 for possible reasons. Increase the amount of eluate added to the reaction, if possible. If necessary, vacuum-concentrate the DNA or increase the amount of sample used, and repeat the purification procedure. If the amount of purified DNA is still expected to be low, reduce the elution volume to 50 µl. Lowering the elution volume will slightly reduce the overall yield, but will result in a higher concentration of nucleic acids in the eluate. DNA remaining on the QIAamp Mini Spin Column can be recovered in a subsequent elution step by applying the same eluate to the column.
b)	Inhibitory substances in preparation	Check "A_{260}/A_{280} ratio for purified nucleic acids is low" for possible reasons.
c)	Buffers AW1 and AW2 used in the wrong order	Ensure that Buffers AW1 and AW2 are used in the correct order in the protocol. Repeat the purification procedure with a new sample.
d)	Reduced sensitivity of amplification reaction	Adjust the volume of eluate added as template in the amplification reaction.
e)	Amplification reaction setup has been modified	Re-optimize the amplification system by adjusting the volume of eluate added.
General handling		
a)	Lysate not completely passed through the membrane	Centrifuge for 1 minute at full speed or until all the lysate has passed through the membrane.

Ordering Information

Product	Contents	Cat. no.
QIAamp Fast DNA Tissue Kit	For 50 preps: QIAamp spin Columns, QIAGEN Proteinase K, RNase A, Tissue Disruption Tubes, Buffers	51404
TissueLyser		
TissueLyser II	Bead mill, 100-120/220-240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96	85300
TissueLyser Adapter Set 2 x 24	2 sets of adapter plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II	69982
TissueLyser LT	Compact bead mill, 100-240 V AC, 50–60 Hz; requires the TissueLyser LT Adapter, 12-Tube	85600
TissueLyser LT Adapter, 12-Tube	Adapter for disruption of up to 12 samples in in 2 ml microcentrifuge tubes on the TissueLyser LT	69980
QIAcube and QIAcube accessories		
QIAcube	Robotic workstation for automated purification of nucleic acids or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor	9001292*

* QIAcube (110 V) cat. no. 9001292, or QIAcube (239V) cat. no. 9001293.

Product	Contents	Cat. no.
Starter Pack, QIAcube	Pack includes: reagent bottle racks (3); rack labeling strips (8); 200 µl filter-tips(1024); 1000 µl filter-tips (1024);1000 µl filter-tips, wide-bore (1024); 30 ml reagent bottles (18); rotor adapters (240); rotor adapter holder	990395
Filter-Tips, 200 µl (1024)	Sterile, Disposable Filter-Tips, racked	990332
Filter-Tips, 1000 µl (1024)	Sterile, Disposable Filter-Tips, racked	990352
Filter-Tips, 1000 µl, wide-bore (1024)	Sterile, Disposable Filter-Tips, wide-bore, racked; (8 x 128);not required for all protocols	990452
Rotor Adapters (10 x 24)	For 240 preps: 240 Disposable Rotor Adapters; for use with the QIAcube	990394

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.

Limited License Agreement for QIAamp Fast DNA Tissue Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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Technical Support

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