April 2010

QIAsymphony® DNA Handbook

QIAsymphony DNA Mini Kit
QIAsymphony DNA Midi Kit

For purification of genomic DNA from
human whole blood
buffy coat
tissues
cultured cells
bacterial cultures
and purification of viral DNA from
human whole blood
using the QIAsymphony SP

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.

QIAGEN is a member of the Forest Stewardship Council (FSC). For the production of printed materials, including handbooks, QIAGEN has a policy to select suppliers that comply with FSC standards for printing processes and well-managed forests.

Contents

Kit Contents

QIAsymphony DNA Kits	Mini (192)	Midi (96)
Catalog no.	**931236**	**931255**
Number of preps	**192**	**96***
Reagent Cartridge†‡	2	2
Enzyme Rack	2	2
Piercing Lid	2	2
Buffer ATE‡	20 ml	20 ml
Reuse Seal Set§	2	2
Handbook	1	1

* For 96 x 1000 µl preps or 144 x 400 µl preps.

† Contains guanidine salts. Not compatible with disinfectants containing bleach. See page 7 for safety information.

‡ Contains sodium azide as a preservative.

§ A Reuse Seal Set contains 8 Reuse Seal Strips.

Storage

QIAsymphony DNA Kits should be stored at room temperature (15–25°C). Do not store reagent cartridges at temperatures below 15°C.

QIAsymphony DNA Kits contain ready-to-use proteinase K solution that can be stored at room temperature.

Partially used reagent cartridges can be stored for a maximum of 2 weeks, enabling cost-efficient reuse of reagents and more flexible sample processing. If a reagent cartridge is partially used, replace the cover of the trough containing the magnetic particles, seal the buffer troughs with the provided Reuse Seal Strips, and close the enzyme tubes with screw caps immediately after the end of the protocol run to avoid evaporation.

To avoid reagent evaporation, the reagent cartridge should be open for a maximum of 15 hours (including run times) at a maximum environmental temperature of 30°C.

Running batches with low sample numbers (<24) will increase both the time that the reagent cartridge is open and the required buffer volumes, potentially reducing the total number of sample preparations possible per cartridge.

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).

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 sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding QIAsymphony DNA Mini or Midi Kits 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 see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAsymphony DNA Mini and Midi 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 material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/Support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Buffers in the reagent cartridge contain guanidine salts, 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.

The following risk and safety phrases apply to components of QIAsymphony DNA Kits:

QSL1

Contains guanidine hydrochloride: harmful and irritant. Risk and safety phrases:* R22-36/38, S13-26-36-46

QSB1

Contains isopropanol and guanidine thiocyanate: highly flammable, harmful, irritant. Risk and safety phrases:* R11-20/21/22-32-36-67, S13-26-36/37/39-46

QSW1

Contains guanidine hydrochloride and ethanol: highly flammable, harmful, irritant. Risk and safety phrases:* R11-22-36/38, S13-26-36/37/39-46

QSW2

Contains ethanol: highly flammable. Risk and safety phrases:* R11, S7-16

* R11: Highly flammable; R20/21/22: Harmful by inhalation, in contact with skin, and if swallowed; R22: Harmful if swallowed; R32: Contact with acids liberates very toxic gas; R36: Irritating to eyes; R36/38: Irritating to eyes and skin; R67: Vapors may cause drowsiness and dizziness; S7: Keep container tightly closed; S13: Keep away from food, drink, and animal feedingstuffs; S16: Keep away from sources of ignition – No smoking; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S36/37/39: Wear suitable protective clothing, gloves, and eye/face protection; S46: If swallowed, seek medical advice immediately and show container or label.

Proteinase K

Contains proteinase K: sensitizer, irritant. Risk and safety phrases:* R36/37/38-42/43, S23-24-26-36/37

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Product Use Limitations

QIAsymphony DNA Kits are 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.

* R36/37/38: Irritating to eyes, respiratory system, and skin; R42/43: May cause sensitization by inhalation and skin contact; S23: Do not breathe vapor; S24: Avoid contact with the skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36/37: Wear suitable protective clothing and gloves.

Introduction

QIAsymphony DNA Kits are designed for automated purification of total DNA from human whole blood, buffy coat, human and animal tissues, cultured cells, and bacterial cultures as well as viral DNA from human whole blood. Proven, performance-leading magnetic-particle technology provides high-quality DNA, that is suitable for direct use in downstream applications, such as amplification or other enzymatic reactions or storage for later use. Purified DNA is free of proteins, nucleases, and other impurities. The QIAsymphony SP performs all steps of the purification procedure. Up to 96 samples are processed in a single run. For tissues, cultured cells, and bacteria protocols, manual sample pretreatment is required.

Principle and procedure

QIAsymphony technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles (Figure 1). The purification procedure is designed to ensure safe and reproducible handling of potentially infectious samples, and comprises 4 steps: lyse, bind, wash, and elute (see flowchart on next page). The user can choose between different elution volumes depending on the protocol. DNA yields depend on sample type and storage.

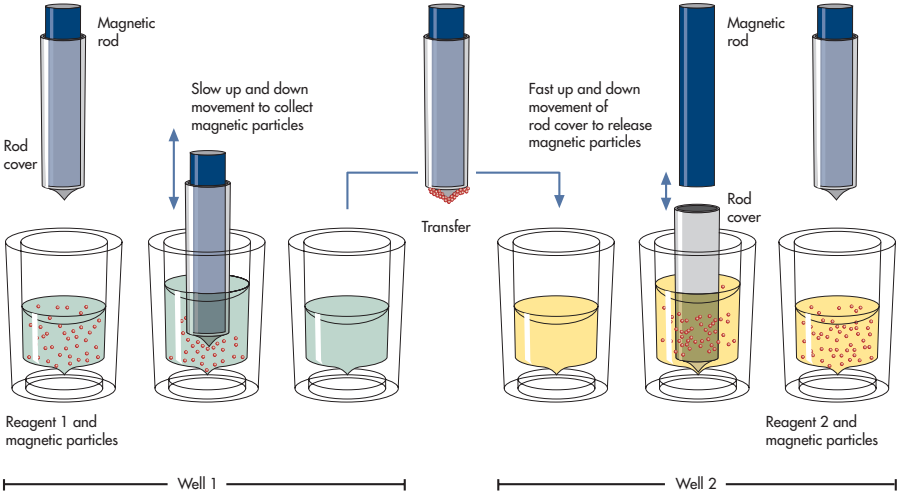
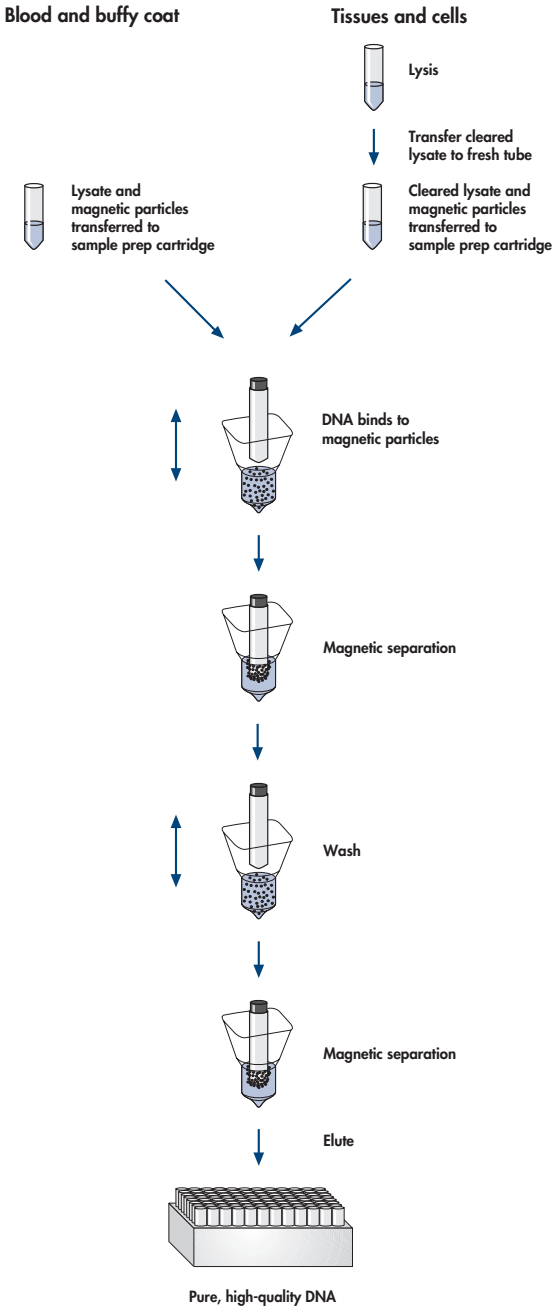


Figure 1. Schematic of the QIAsymphony SP principle. The QIAsymphony SP processes a sample containing magnetic particles as follows: A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another well and the magnetic particles are released. The QIAsymphony SP uses a magnetic head containing an array of 24 magnetic rods, and can therefore process up to 24 samples simultaneously. Steps 1 and 2 are repeated several times during sample processing.

QIAsymphony DNA Procedures



Equipment and Reagents to Be Supplied by User

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

All protocols

- Sample Prep Cartridges, 8-well cartridges (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Filter-Tips, 200 µl and 1500 µl (cat. nos. 990332 and 997024)
- Sample tubes or plates (e.g., 2 ml sample tubes with screw caps, Sarstedt cat. no. 72.693, or without caps, Sarstedt cat. no. 72.608, or S-Blocks, QIAGEN cat. no. 19585). Compatible primary and secondary tube and plate formats are listed at www.qiagen.com/QIAsymphony/Resources
- Elution tubes or plates. Compatible elution tube and plate formats are listed at www.qiagen.com/QIAsymphony/Resources
- Phosphate-buffered saline (PBS, may be required for diluting samples)
- Vortexer
- Optional: DNase-free RNase A (if RNA-free DNA is required)

Tissues

- Buffer ATL (cat. no. 19076)
- Thermomixer or shaker–incubator

Cultured cells

- Buffer P1 (cat. no. 19051)
- Thermomixer or shaker–incubator

Bacterial cultures

- For Gram-negative bacteria: Buffer ATL (cat. no. 19076)
- For Gram-positive bacteria:
  - Buffer P1 (cat. no. 19051)
  - Lysozyme
- Thermomixer or shaker–incubator

Human whole blood (viral DNA)

- Sample tubes, 14 ml (17 x 100 mm polystyrene, round-bottom tubes from Becton Dickinson, cat. no. 352051, www.bd.com) or 2 ml (Sarstedt, cat. no. 72.693 or 72.608, www.sarstedt.com)

Important Notes

Automated purification on the QIAsymphony SP

The QIAsymphony SP makes automated sample preparation easy and convenient. Samples, reagents and consumables, and eluates are separated in different drawers. Simply load samples, reagents provided in special cartridges, and preracked consumables in the appropriate drawer before a run. Start the protocol and remove purified DNA from the "Eluate" drawer after processing. Refer to the *QIAsymphony SP User Manual* for operating instructions.

Loading reagent cartridges into the "Reagents and Consumables" drawer

Reagents for purification of DNA are contained in an innovative reagent cartridge (see Figure 2). Each trough of the reagent cartridge contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer. Partially used reagent cartridges can be reclosed with Reuse Seal Strips for later reuse, which avoids generation of waste due to leftover reagents at the end of the purification procedure.

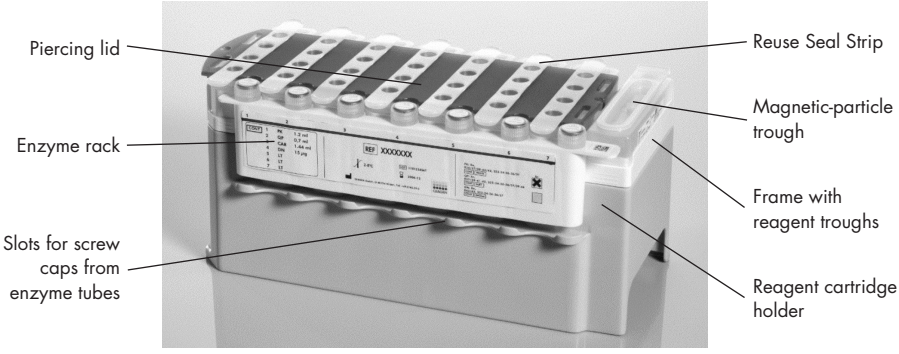


Figure 2. QIAsymphony reagent cartridge. The reagent cartridge contains all reagents required for the protocol run.

Before starting the procedure, ensure that the magnetic particles are fully resuspended. Remove the magnetic-particle trough from the reagent cartridge frame, vortex it vigorously for at least 3 minutes, and replace it in the reagent cartridge frame before the first use. Place the reagent cartridge into the reagent cartridge holder. Place the enzyme rack into the reagent cartridge holder. Before using a reagent cartridge for the first time, place the piercing lid on top of the reagent cartridge (Figure 3).

Important: The piercing lid is sharp. Take care when placing it onto the reagent cartridge. Make sure to place the piercing lid onto the reagent cartridge in the correct orientation.

After the magnetic-particle trough cover is removed and the enzyme rack tubes are opened (screw caps can be stored in dedicated slots, see Figure 2), the reagent cartridge is subsequently loaded into the "Reagents and Consumables" drawer.

Figure 3. Easy worktable setup with reagent cartridges.

Partially used reagent cartridges can be stored until needed again, see "Storage", page 5.

Loading plasticware into the "Reagents and Consumables" drawer

Sample prep cartridges, 8-Rod Covers (both preracked in unit boxes), and disposable filter-tips (200 µl tips provided in blue racks, 1500 µl tips provided in gray racks) are loaded into the "Reagents and Consumables" drawer.

See Tables 1 and 2 (pages 14 and 15) for the consumables required for DNA protocols. For plasticware ordering information, see page 42.

Note: Both types of tips have filters to help prevent cross-contamination.

Tip rack slots on the QIAsymphony worktable can be filled with either type of tip rack. The QIAsymphony SP will identify the type of tips loaded during the inventory scan.

Note: Do not refill tip racks before starting another protocol run. The QIAsymphony SP can use partially used tip racks.

Loading the "Waste" drawer

Sample prep cartridges and 8-Rod Covers used during a run are re-racked in empty unit boxes in the "Waste" drawer. Make sure that the "Waste" drawer contains sufficient empty unit boxes for plastic waste generated during the protocol run.

Note: Ensure that the covers of the unit boxes are removed before loading the unit boxes into the "Waste" drawer. If you are using 8-Rod Cover boxes for collecting used sample prep cartridges and 8-Rod Covers, ensure that the box spacer has been removed.

A bag for used filter-tips must be attached to the front side of the "Waste" drawer.

Note: The presence of a tip disposal bag is not checked by the system. Make sure that the tip disposal bag is properly attached before starting a protocol run. For more information, see the *QIAsymphony SP User Manual*.

A waste container collects liquid waste generated during the purification procedure. The "Waste" drawer can only be closed if the waste container is in place. Furthermore, a liquid-level sensor detects the level of liquid in the waste container. The system notifies the user if there is not enough capacity left in the container for liquid waste from the queued batch.

Loading the "Eluate" drawer

Load the required elution rack into the "Eluate" drawer. Do not load a 96-well plate onto "Elution slot 4". If eluates should be cooled, use "Elution slot 1" with the corresponding cooling adapter. As long-term storage of eluates in the "Eluate" drawer may lead to evaporation of eluates, we strongly recommend using the cooling position.

Inventory scan

Before starting a run, the instrument checks that sufficient consumables for the queued batch(es) have been loaded into the corresponding drawers (Tables 1 and 2).

Table 1. Consumables required for QIAsymphony DNA blood protocols

Protocol	Virus Blood 200		Blood 200		BC 200 FIX		Blood 400 BC 400 FIX		Blood 1000 Blood 1000 FIX	
Number of samples	**24**	**96**	**24**	**96**	**24**	**96**	**24**	**96**	**24**	**96**
Reagent cartridges	1	1	1	1	1	1	1	2	1	2
Sample prep cartridges*	21	84	18	72	18	72	18	72	18	72
8-Rod Covers†	3	12	3	12	3	12	3	12	3	12
1500 µl tips‡§	96	360	84	312	108	408	112	432	116	448
200 µl tips‡§	30	114	30	114	6	18	4	8	4	8

* 28 sample prep cartridges/unit box.

† Twelve 8-Rod Covers/unit box.

‡ 32 tips/tip rack.

§ Number of required tips includes tips for 1 inventory scan per reagent cartridge.

Table 2. Consumables required for QIAsymphony DNA tissue protocols

Protocol	Tissue LC 200		Tissue HC 200	
Number of samples	**24**	**96**	**24**	**96**
Reagent cartridges	1	1	1	1
Sample prep cartridges*	21	84	21	84
8-Rod Covers†	3	12	3	12
1500 µl tips‡§	72	288	72	288
200 µl tips‡§	24	96	24	96

* 28 sample prep cartridges/unit box.

† Twelve 8-Rod Covers/unit box.

‡ 32 tips/tip rack.

§ Number of required tips includes tips for 1 inventory scan per reagent cartridge.

Human whole blood and buffy coat

Purification of genomic DNA from human whole blood

Whole blood samples treated with EDTA, citrate, or heparin can be used, and may be either fresh or frozen. Frozen samples should be thawed quickly in a 37°C water bath with mild agitation to ensure thorough mixing and then equilibrated to room temperature (15–25°C) before beginning the procedure. Yield and quality of the purified DNA depend on the storage conditions of the blood. Fresher blood samples may yield better results. The volumes of whole blood that can be used are shown in Table 6 (page 19).

For short-term storage of up to 10 days, collect blood in tubes containing EDTA as an anticoagulant, and store at 2–8°C. However, for applications requiring maximum fragment size, such as Southern blotting, we recommend storage at 2–8°C for up to 3 days only, as low levels of DNA degradation will occur after this time.

For long-term storage (over 10 days), collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required), and store at –70°C.

Purification of viral DNA from human whole blood

For isolation of viral DNA, we recommend using whole blood samples treated with EDTA or citrate. Samples should be processed within 24 hours of collection. Store or transport samples at 2–25°C.

Using an internal control for purification of viral DNA

Using the QIAsymphony DNA Mini Kit and protocol for purification of viral DNA from human whole blood in combination with amplification systems that use an internal control may require the introduction of these internal controls into the purification procedure to monitor the efficiency of sample preparation and downstream assay.

Internal controls should be diluted in Buffer ATE. A total volume of 60 µl internal control–Buffer ATE mixture is added per sample. The amount of internal control added depends on the assay system and the elution volume chosen in the QIAsymphony SP protocol. Calculation and validation must be performed by the user. Refer to the instructions of the manufacturer of the downstream assay to determine the optimal concentration of internal control. Using a concentration other than that recommended may lead to invalid or incorrect results if the internal control is used for calculation of titers.

A mix of several internal controls can be used to analyze different parameters within a single eluate. Compatibility of different internal controls must be validated by the user.

When calculating the amount of internal control(s) to use as well as the titer of the processed sample, it is necessary to take into consideration the actual volume of elution solution that is used for each sample. Since small volumes of liquid are lost during transfer and contact with the magnetic particles, the initial volume of elution solution must be greater than the selected volume to ensure that the final eluate is of the correct volume. Table 3 provides the initial elution volumes to allow accurate calculation of internal controls and titer.

Table 3. Elution volumes for the QIAsymphony "Virus Blood 200" protocol

Selected elution volume (µl)*	Initial elution volume (µl)†
60	95
85	120
110	145
165	200

* The elution volume is selected in the touchscreen. This is the minimum accessible volume of the eluate in the final elution tube or plate.

† The initial volume of elution solution required to ensure that the minimum accessible volume of eluate corresponds to the selected volume.

Table 4 provides information for calculating the volume of the internal control–Buffer ATE mixture. We recommend preparing fresh mixtures for each run prior to use.

Table 4. Preparation of the internal control–Buffer ATE mixture

Selected elution volume (µl)	Initial elution volume (µl)	Volume internal control (µl)	Volume Buffer ATE (µl)	Final volume per sample (µl)
60	95	9.5	50.5	60
85	120	12.0	48.0	60
110	145	14.5	45.5	60
165	200	20.0	40.0	60

In this example, 0.1 µl internal control is used for 1 µl eluate.

Depending on the number of samples to be processed, we recommend using 2 ml tubes (Sarstedt, cat. nos. 72.693 and 72.608) or 14 ml 17 x 100 mm polystyrene, round-bottom tubes (Becton Dickinson, cat. no. 352051) for diluting the internal control, as described in Table 5. It is possible to split the volume into 2 or more tubes. Internal control mixture(s) must be placed in slot A of the "Sample" drawer.

Table 5. Calculating the volume of internal control mixture

Tube type	Calculation of internal control mixture volume per tube
Microtube 2 ml, PP (Sarstedt, cat. no. 12.693 or 12.608)*	(n x 60 µl) + 240 µl†
Tube 14 ml, 17 x 100 mm polystyrene round-bottom (Becton Dickinson, cat. no. 352051)	(n x 60 µl) + 480 µl‡

n = sample number

* Do not fill with more than 1.92 ml (corresponding to a maximum of 28 samples).

† 240 µl internal control mixture, which corresponds to 4 additional samples, is required.

‡ 480 µl internal control mixture, which corresponds to 8 additional samples, is required.

Assay Control Sets

Assay Control Sets are used with protocols, even when the protocol does not use an internal control. A default Assay Control Set is preinstalled for each protocol (see Table 6, page 19). When an internal control is used, it might be necessary to create an additional Assay Control Set as described in the *QIAsymphony Management Console User Guide*.

Note: When using the default "Virus Blood 200 *without* IC" Assay Control Set for protocols that do not use an internal control, the use of Buffer ATE is still required. Buffer ATE must be placed in slot A of the "Sample" drawer: the volume must include 60 µl per sample as well as an additional 240 µl or 480 µl (according to Table 5, page 17).

Buffy coat

Buffy coat is a leukocyte-enriched fraction of whole blood. The efficiency of leukocyte enrichment depends on the procedure used to prepare buffy coat and on the accuracy with which the buffy coat layer is extracted. Prepare buffy coat by centrifuging whole blood samples containing a standard anticoagulant (EDTA, citrate, or heparin) at 900–1100 x *g* for 10 minutes at room temperature (15–25°C). After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes. Approximately 1 ml leukocyte-containing fraction should be harvested from 10 ml centrifuged whole blood, which, on average, gives a 5–6x enrichment. To avoid overloading the DNA purification procedure, do not prepare buffy coat samples of >10x enrichment. If buffy coat samples are of >10x enrichment, dilute the samples to ≤10x enrichment with PBS or use less starting material in the DNA purification procedure.

Buffy coat samples may be used immediately or stored at –20°C for purification of DNA at a later date. Frozen samples should be thawed quickly in a 37°C water bath with mild agitation to ensure thorough mixing and then equilibrated to room temperature before beginning the procedure.

QIAsymphony DNA blood procedures

The volumes of blood and buffy coat that can be used for standard protocols are listed in Table 6, next page. Standard protocols are the default protocols that are installed on the instrument when purchased. An "Extended Protocol Package" providing the protocols indicated in Table 7, page 20, is available on request. For more information, contact QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Table 6. Sample and elution volumes for QIAsymphony DNA blood procedures

Sample volume (µl)	Elution (µl)	Kit	QIAsymphony SP protocol	Assay Control Set*
Human whole blood (genomic DNA purification)				
200	50, 100, 200	Mini	DNA Blood 200	Blood 200
400	100, 200, 400	Midi	DNA Blood 400	Blood 400
1000	200, 400, 500	Midi	DNA Blood 1000	Blood 1000
	200, 400, 500	Midi	DNA Blood 1000 FIX†	Blood 1000 FIX†
Human whole blood (viral DNA purification)				
200	60, 85, 110, 165	Mini	Virus Blood 200	Virus Blood 200 *without* IC
Buffy coat				
200	200, 300, 400	Mini	DNA Buffy Coat 200 FIX†	BC 200 FIX†
400	200, 400	Midi	DNA Buffy Coat 400 FIX†	BC 400 FIX†

* The term "Assay Control Set" is used in the user interface when choosing the protocol. For more information about Assay Control Sets, see the *QIAsymphony SP User Manual*.

† Liquid-level detection is not performed in the "FIX" versions, which ensures maximal sample usage. However, neither the presence of a sample nor the sample volume is checked. Tubes or wells must contain exactly 200 µl, 400 µl, or 1000 µl sample at the bottom of the tube or well. The QIAsymphony SP will not detect that less volume is loaded or that the sample is missing. Do not use volumes greater than 200 µl, 400 µl, or 1000 µl, since this may lead to errors during sample preparation.

Information about minimum sample volumes for samples in primary and secondary tubes is available at www.qiagen.com/QIAsymphony/Resources. This table also indicates which tubes can be used for the different protocols and which protocols provide clot detection. Please refer to this table before loading the samples onto the worktable to ensure that your samples will be processed correctly.

Table 7. Sample and elution volumes for the Extended Protocol Package

Sample volume (µl)	Elution (µl)	Kit	QIAsymphony SP protocol	Assay Control Set*
Whole blood				
200	50, 100, 200	Mini	DNA Blood 200 FIX	Blood 200 FIX†
400	100, 200, 400	Midi	DNA Blood 400 FIX	Blood 400 FIX†
Buffy coat				
200	200, 300, 400	Mini	DNA Buffy Coat 200	BC 200
400	100, 200, 400	Midi	DNA Buffy Coat 400	BC 400 FIX

* The term "Assay Control Set" is used in the user interface when choosing the protocol. For more information about Assay Control Sets, see the *QIAsymphony SP User Manual*.

† Liquid-level detection is not performed in the "FIX" versions, which ensures maximal sample usage. However, neither the presence of a sample nor the sample volume is checked. Tubes or wells must contain exactly 200 µl, 400 µl, or 1000 µl sample at the bottom of the tube or well. The QIAsymphony SP will not detect that less volume is loaded or that the sample is missing. Do not use volumes greater than 200 µl, 400 µl, or 1000 µl, since this may lead to errors during sample preparation.

Information about minimum sample volumes for samples in primary and secondary tubes is available at www.qiagen.com/QIAsymphony/Resources. This table also indicates which tubes can be used for the different protocols and which protocols provide clot detection. Please refer to this table before loading the samples onto the worktable to ensure that your samples will be processed correctly.

Tissues

Fresh, frozen, or stabilized tissue can be used for DNA purification. Yield and quality of DNA from tissue will depend on the tissue type, source, and storage conditions. Fresh tissue can be cut into small pieces and stored at –20°C or –80°C before processing. The recommended amounts of starting material for use in QIAsymphony DNA tissue procedures are shown in Table 8, page 21, and elution volumes are shown in Table 9, page 21. Tissues with high genomic DNA content and large numbers of cultured cells will give increased DNA yields when processed with the "DNA Tissue High Content" protocol. For samples with expected DNA yields less than 30 µg we recommend using the "DNA Tissue Low Content" protocol. Typical yields to be expected from different tissue types are shown in Table 10, page 23. If no information about the expected yield is available, we recommend starting with 25 mg sample material. Depending on the yield obtained, the sample size can be increased in subsequent preparations. Note that overloading preparations may cause carryover of magnetic particles into the eluate and compromise DNA purity.

Table 8. Sample sizes for QIAsymphony DNA tissue procedures

Sample type	Sample amount	QIAsymphony SP protocol	Assay Control Set*
Heart	25 mg	DNA Tissue Low Content	Tissue LC 200
Spleen	25 mg	DNA Tissue High Content	Tissue HC 200
Lung	25 mg	DNA Tissue Low Content	Tissue LC 200
Liver	25 mg	DNA Tissue High Content	Tissue HC 200
Kidney	25 mg	DNA Tissue Low Content	Tissue LC 200
Muscle	50 mg	DNA Tissue Low Content	Tissue LC 200
Mouse tail	0.8 cm	DNA Tissue Low Content	Tissue LC 200
Jurkat cells	2 x 10^6 1 x 10^7	DNA Tissue Low Content DNA Tissue High Content	Tissue LC 200 Tissue HC 200
Bacteria	4 x 10^9	DNA Tissue High Content	Tissue HC 200

* The term "Assay Control Set" is used in the user interface when choosing the protocol. For more information about Assay Control Sets, see the *QIAsymphony SP User Manual*.

Table 9. Elution volumes for QIAsymphony DNA tissue procedures

Protocol	Elution volume (µl)
DNA Tissue Low Content	50, 100, 200, 400
DNA Tissue High Content	100, 200, 400

Cultured cells

Both fresh and frozen cultured cells may be used. We recommend using 2 x 10^6 cells in the "DNA Tissue Low Content" protocol and up to 1 x 10^7 cells in the "DNA Tissue High Content" protocol. Frozen cell pellets should be resuspended in Buffer P1, as described in the protocol.

Bacteria

Both fresh and frozen bacterial cultures may be used. We recommend using up to 4 x 10^9 cells. Bacterial growth is usually measured as optical density (OD) of the bacterial culture using a spectrophotometer. However, OD readings strongly depend on the type of spectrophotometer used and the bacterial species measured. We therefore recommend calibrating the spectrophotometer used by correlating measured ODs to bacterial cell numbers. Frozen pellets should be resuspended in Buffer P1 (Gram-positive bacteria) or Buffer ATL (Gram-negative bacteria), as described in the pretreatment protocols.

Lysis with proteinase K

QIAsymphony DNA Kits contain proteinase K, which possesses a high specific activity that remains stable over a wide range of temperatures and pH values, with substantially increased activity at higher temperatures.

Quantification of DNA

Carryover of magnetic particles may affect the absorbance reading at 260 nm (A_{260}) of the purified DNA. The measured absorbance at 320 nm (A_{320}) should be subtracted from all absorbance readings. See "Quantification of DNA", page 40, for more information.

Note: For accurate quantification of DNA by absorbance at 260 nm, we recommend diluting the sample in elution buffer (Buffer ATE). Dilution of the sample in water may lead to inaccurate values. The elution buffer has a high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed. We therefore strongly recommend using elution buffer as a blank. Extra elution buffer to blank the spectrophotometer is provided in a separate bottle with QIAsymphony DNA Kits.

Yield of purified DNA

DNA yields depend on the sample type, number of nucleated cells in the sample, the quality of the starting material and the protocol used for isolation of DNA. Table 10, page 23, lists typical yields obtained from different sample volumes and types. Elution in smaller volumes increases the final DNA concentration in the eluate, but slightly reduces overall DNA yield. We recommend using an elution volume appropriate for the intended downstream application.

Table 10. Typical genomic DNA yields obtained from a range of sample types

Sample types	Sample size	Elution volume (µl)	Typical DNA yield (µg)
Whole blood*	200 µl	200	4–12
	400 µl	400	8–24
	1000 µl	500	15–45
Buffy coat†	200 µl	200	12–40
	400 µl	400	24–72
Spleen	25 mg	200	40–80
Liver	25 mg	200	25–50
Muscle	50 mg	200	5–15
Lung	25 mg	200	10–25
Kidney	25 mg	200	15–30
Rat tail	50 mg	200	20–40
Jurkat cells	1x 10^7 cells	200	60–80

* For donors with white blood cell counts of 4–11 x 10^6 cells/ml.

† For buffy coat 5–6x enrichment from blood with a white blood cell count of 4–11 x 10^6 cells/ml.

Storage and quality of purified DNA

Purified genomic DNA can be stored at –80°C, –20°C, or at 2–8°C.

Purified viral DNA can be stored at 2–8°C for 3 days before use in analysis and should be kept at –20°C or –80°C for long-term storage.

QIAsymphony DNA procedures yield pure DNA with A_{260}/A_{280} ratios of 1.7–1.9. Purified DNA is up to 50 kb in size, and is suitable for use in all downstream applications.

Co-purified RNA may increase A_{260}/A_{280} ratios to values of up to 2.2. Treat samples with RNase A according to the protocol if RNA-free DNA is required.

Protocol: Purification of DNA from Human Whole Blood and Buffy Coat

This protocol is for purification of total (genomic and mitochondrial) DNA from fresh or frozen human whole blood or buffy coat using the QIAsymphony SP and the QIAsymphony DNA Mini or Midi Kit. Blood sample volumes of 200 µl, 400 µl, or 1000 µl and buffy coat sample volumes of 200 µl or 400 µl can be processed.

Important points before starting

- Ensure that you are familiar with operating the QIAsymphony SP. Refer to the *QIAsymphony SP User Manual* for operating instructions.
- Before beginning the procedure, read "Important Notes" starting on page 12.
- Before using a reagent cartridge for the first time, check that Buffers QSL1 and QSB1 do not contain a precipitate. If necessary, remove the troughs containing Buffers QSL1 and QSB1 from the reagent cartridge and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the troughs in the correct positions. If the reagent cartridge is already pierced, make sure that the troughs are sealed with Reuse Seal Strips and incubate the complete reagent cartridge for 30 minutes at 37°C with occasional shaking in a water bath.
- Try to avoid vigorous shaking of the reagent cartridge otherwise foam may be generated, which can lead to liquid-level detection problems.
- For 200 µl samples, use the QIAsymphony DNA Mini Kit; for 400 µl and 1000 µl samples, use the QIAsymphony DNA Midi Kit.
- QIAsymphony DNA Kits copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample before starting the procedure. The final RNase A concentration should be 2 mg/ml (e.g., add 4 µl of a 100 mg/ml RNase A solution to a 200 µl sample).

Things to do before starting

- If using frozen blood samples, thaw up to 96 whole blood samples and equilibrate to room temperature (15–25°C, see page 15).
- If using fresh blood samples in primary tubes, mix the blood samples thoroughly (e.g., by inverting the tubes several times) before loading them onto the QIAsymphony SP.
- If using buffy coat samples prepare them according to the instructions on page 18.
- If using frozen buffy coat samples, thaw up to 96 buffy coat samples and equilibrate to room temperature (see page 18).
- Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes before first use.

- Before loading the reagent cartridge remove the cover from the trough containing the magnetic particles and open the enzyme tubes. Make sure that the piercing lid is placed on the reagent cartridge or, if using a partially used reagent cartridge, make sure the Reuse Seal Strips have been removed.
- If samples are bar coded, orient samples in the tube carrier so that the bar codes face the bar code reader at the left side of the QIAsymphony SP.
- Information about minimum sample volumes for samples in primary and secondary tubes is available at www.qiagen.com/QIAsymphony/Resources. Information about which tubes can be used for the different protocols is also provided here. Please refer to this information before loading the samples onto the worktable to ensure that your samples will be processed correctly.

Procedure

1. **Close all drawers and the hood.**
2. **Switch on the QIAsymphony SP, and wait until the "Sample Preparation" screen appears and the initialization procedure has finished.**

   The power switch is located at the bottom, left corner of the QIAsymphony SP.
3. **Log on to the instrument.**
4. **Ensure the "Waste" drawer is prepared properly, and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.**
5. **Load the required elution rack into the "Eluate" drawer.**

   Do not load a 96-well plate onto "Elution slot 4".

   If eluates should be cooled, use "Elution slot 1" with the corresponding cooling adapter.
6. **Load the required reagent cartridge(s) and consumables (see Table 1, page 14) into the "Reagents and Consumables" drawer, and perform an inventory scan of the "Reagents and Consumables" drawer.**
7. **Place the samples into the appropriate sample carrier, and load them into the "Sample" drawer.**

8. **Using the touchscreen, enter the required information for each batch of samples to be processed.**

   Enter the following information:

   - Sample information (depending on sample racks used)
   - Protocol to be run ("Assay Control Set"). Choose the appropriate Assay Control Set from the "DNA Blood" application
   - Elution volume and output position

   After information about the batch has been entered, the status changes from "LOADED" to "QUEUED". As soon as one batch is queued the "Run" button appears.

   The Assay Control Set provides information about internal controls, if applicable.

9. **Press the "Run" button to start the purification procedure.**

   All processing steps are fully automated. At the end of the protocol run, the status of the batch changes from "RUNNING" to "COMPLETED".

10. **Retrieve the elution rack containing the purified DNA from the "Eluate" drawer.**

    The DNA is ready to use or can be stored at 2–8°C, –20°C, or –80°C.

    In general, magnetic particles are not carried over into eluates. If carryover does occur, magnetic particles in eluates will not affect most downstream applications. If magnetic particles need to be removed before performing downstream applications, tubes or plates containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean tube (see appendix, page 40).

    If the "Eluate" drawer is closed when a batch is running (e.g., if elution racks which contain eluates are removed), the run will be paused and an inventory scan of the "Eluate" drawer will be performed. A message appears during the scan and must be closed (by pressing the "Close" button) before the run can be restarted.

    Result files are generated for each elution plate.

11. **If the reagent cartridge(s) is only partially used, seal it with the provided Reuse Seal Strips and close the enzyme tubes with screw caps immediately after the end of the protocol run to avoid evaporation.**

    Note: For more information about storage of partially used reagent cartridges, see "Storage", page 5.

12. **Discard used sample tubes, plates, and waste according to your local safety regulations.**

    See page 7 for safety information.

13. **Clean the QIAsymphony SP.**

    Follow the maintenance instructions in the *QIAsymphony SP User Manual*.

14. **Close the workstation drawers, and switch off the QIAsymphony SP.**

Protocol: Purification of Viral DNA from Human Whole Blood

This protocol is for purification of viral DNA from fresh human whole blood using the QIAsymphony SP and the QIAsymphony DNA Mini Kit. Viral DNA from released as well as from cell-associated viruses is co-purified with genomic DNA from blood cells. Blood sample volumes of 200 µl can be processed.

Important points before starting

- Ensure that you are familiar with operating the QIAsymphony SP. Refer to the *QIAsymphony SP User Manual* for operating instructions.
- Before beginning the procedure, read "Important Notes" starting on page 12.
- Before using a reagent cartridge for the first time, check that Buffers QSL1 and QSB1 do not contain a precipitate. If necessary, remove the troughs containing Buffers QSL1 and QSB1 from the reagent cartridge and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the troughs in the correct positions. If the reagent cartridge is already pierced, make sure that the troughs are sealed with Reuse Seal Strips and incubate the complete reagent cartridge for 30 minutes at 37°C with occasional shaking in a water bath.
- Try to avoid vigorous shaking of the reagent cartridge otherwise foam may be generated, which can lead to liquid-level detection problems.
- This protocol is for use with the QIAsymphony DNA Mini Kit.

Things to do before starting

- If using blood samples in primary tubes, mix the blood samples thoroughly (e.g., by inverting the tubes several times) before loading them onto the QIAsymphony SP.
- Prepare a mixture containing internal controls as described in "Using internal controls" starting on page 16.
- Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes before first use.
- Before loading the reagent cartridge, remove the cover from the trough containing the magnetic particles and open the enzyme tubes. Make sure that the piercing lid is placed on the reagent cartridge or, if using a partially used reagent cartridge, make sure the Reuse Seal Strips have been removed.
- If samples are bar coded, orient samples in the tube carrier so that the bar codes face the bar code reader at the left side of the QIAsymphony SP.

- Information about minimum sample volumes for samples in primary and secondary tubes is available at www.qiagen.com/QIAsymphony/Resources. Information about which tubes can be used for the different protocols is also provided here. Please refer to this information before loading the samples onto the worktable to ensure that your samples will be processed correctly.

Procedure

1. **Close all drawers and the hood.**
2. **Switch on the QIAsymphony SP, and wait until the "Sample Preparation" screen appears and the initialization procedure has finished.**

   The power switch is located at the bottom, left corner of the QIAsymphony SP
3. **Log on to the instrument.**
4. **Ensure the "Waste" drawer is prepared properly, and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.**
5. **Load the required elution rack into the "Eluate" drawer.**

   Do not load a 96-well plate onto "Elution slot 4".

   If eluates should be cooled, use "Elution slot 1" with the corresponding cooling adapter.
6. **Load the required reagent cartridge(s) and consumables (see Table 1, page 14) into the "Reagents and Consumables" drawer, and perform an inventory scan of the "Reagents and Consumables" drawer.**
7. **Place the samples into the appropriate sample carrier, and load them into the "Sample" drawer.**
8. **The tube(s) containing the internal control–Buffer ATE mixture should be placed in slot A of the "Sample" drawer.**

   For more information about preparing the mixture, see "Using an internal control" starting on page 16.
9. **Using the touchscreen, enter the required information for each batch of samples to be processed.**

   Enter the following information:

   - Sample information (depending on sample racks used)
   - Protocol to be run ("Assay Control Set"). Choose the appropriate Assay Control Set from the "Virus" application
   - Elution volume and output position

After information about the batch has been entered, the status changes from "LOADED" to "QUEUED". As soon as one batch is queued the "Run" button appears.

The Assay Control Set provides information about internal controls, if applicable.

10. **Press the "IC" button in the touchscreen, and assign the correct type of labware and internal control.**
11. **Press the "Run" button to start the purification procedure.**

    All processing steps are fully automated. At the end of the protocol run, the status of the batch changes from "RUNNING" to "COMPLETED".
12. **Retrieve the elution rack containing the purified nucleic acids from the "Eluate" drawer.**

    Purified viral DNA can be stored at 2–8°C for 3 days before use in analysis and should be kept at –20°C or –80°C for long-term storage.

    In general, magnetic particles are not carried over into eluates. If carryover does occur, magnetic particles in eluates will not affect most downstream applications.

    If magnetic particles need to be removed before performing downstream applications, tubes or plates containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean tube (see appendix, page 40).

    If the "Eluate" drawer is closed when a batch is running (e.g., if elution racks that contain eluates are removed), the run will be paused and an inventory scan of the "Eluate" drawer will be performed. A message appears during the scan and must be closed (by pressing the "Close" button) before the run can be restarted.

    Result files are generated for each elution plate.
13. **If a reagent cartridge is only partially used, seal it with the provided Reuse Seal Strips and close the enzyme tubes with screw caps immediately after the end of the protocol run to avoid evaporation.**

    Note: For more information about storage of partially used reagent cartridges, see "Storage", page 5.
14. **Discard used sample tubes, plates, and waste according to your local safety regulations.**

    See page 7 for safety information.
15. **Clean the QIAsymphony SP.**

    Follow the maintenance instructions in the *QIAsymphony SP User Manual*.
16. **Close the instrument drawers, and switch off the QIAsymphony SP.**

Protocol: Pretreatment of Tissues

This protocol is for purification of total (genomic and mitochondrial) DNA from tissue samples using the QIAsymphony SP and the QIAsymphony DNA Mini Kit.

Important point before starting

- QIAsymphony magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample before starting the procedure or in step 5. The final RNase A concentration should be 2 mg/ml (e.g., add 4 µl of a 100 mg/ml RNase A solution to a 200 µl sample).

Things to do before starting

- Check that Buffer ATL does not contain a white precipitate. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- Set a thermomixer or shaker–incubator to 56°C for use in step 4 of the procedure.

Procedure

1. **Transfer the tissue sample to a 2 ml microcentrifuge tube (not supplied).**
2. **Add 180 µl Buffer ATL.**
3. **Add 20 µl proteinase K, and mix by tapping the tube.**
4. **Place the tube in a thermomixer or shaker–incubator, and incubate at 56°C with shaking at 900 rpm until the tissue is completely lysed.**

   Lysis time varies depending on the tissue type processed. For most tissues, lysis is completed within 3 h. Overnight lysis is possible and does not affect the preparation.
5. **If RNA-free DNA is required, proceed with step 5a, otherwise proceed directly to step 5b.**

5a. **Add 4 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature (15–25°C). Proceed with step 6.**

5b. **No RNase A treatment is required. Proceed with step 6.**

6. **Homogenize the sample by pipetting up and down several times.**

   If pieces of insoluble material are still present, centrifuge at 3000 x *g* for 1 min.
7. **Carefully transfer the supernatant to sample tubes or plates that are compatible with the sample carrier of the QIAsymphony SP.**

   For a full list of compatible vessels, see www.qiagen.com/QIAsymphony/Resources. We recommend use of 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 42).
8. **Continue with the protocol "Purification of DNA from Tissues, Cultured Cells, and Bacterial Cultures" (page 34).**

Protocol: Pretreatment of Cultured Cells

This protocol is for purification of total (genomic and mitochondrial) DNA from cultured cells using the QIAsymphony SP and the QIAsymphony DNA Mini Kit.

Important point before starting

- QIAsymphony magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample before starting the procedure or in step 5. The final RNase A concentration should be 2 mg/ml (e.g., add 4 µl of a 100 mg/ml RNase A solution to a 200 µl sample).

Things to do before starting

- Set a thermomixer or shaker–incubator to 56°C for use in step 4 of the procedure.

Procedure

1. **Centrifuge a maximum 1 x 10^7 cells at 300 x *g* for 5 min at room temperature (15–25°C). Remove and discard the supernatant, taking care not to disturb the cell pellet.**

   The cell pellet can be stored at –20°C or –70°C for future use or can be used immediately. Up to 2 x 10^6 cells can be used in the "DNA Tissue Low Content" protocol. For 2 x 10^6 to 1 x 10^7 cells, use the "DNA Tissue High Content" protocol.

2. **Resuspend the pellet in 180 µl Buffer P1 and transfer the sample to a 2 ml microcentrifuge tube (not supplied).**
3. **Add 20 µl proteinase K, and mix by tapping the tube.**
4. **Place the sample in a thermomixer or shaker–incubator, and incubate at 56°C with shaking at 900 rpm for 30 min to 2 h.**

   Lysis time depends on the type of cells and the cell number.

5. **If RNA-free DNA is required, proceed with step 5a, otherwise proceed directly to step 5b.**

5a. **Add 4 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature. Proceed with step 6.**

5b. **No RNase A treatment is required. Proceed with step 6.**

6. **Carefully transfer the entire lysate to sample tubes or plates that are compatible with the sample carrier of the QIAsymphony SP.**

   For a full list of compatible vessels, see www.qiagen.com/QIAsymphony/Resources. We recommend use of 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 42).

7. **Continue with the protocol "Purification of DNA from Tissues, Cultured Cells, and Bacterial Cultures" (page 34).**

Protocol: Pretreatment of Gram-Negative Bacteria

This protocol is for purification of total DNA from Gram-negative bacteria using the QIAsymphony SP and the QIAsymphony DNA Mini Kit.

Important points before starting

- Check that Buffer ATL does not contain a white precipitate. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- QIAsymphony magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample before starting the procedure or in step 5. The final RNase A concentration should be 2 mg/ml (e.g., add 4 µl of a 100 mg/ml RNase A solution to a 200 µl sample).

Things to do before starting

- Set a thermomixer or shaker–incubator to 56°C for use in step 4 of the procedure.

Procedure

1. **Harvest a maximum of 4 x 10^9 cells by centrifugation for 10 min at 5000 x *g* at room temperature (15–25°C). Remove and discard the supernatant, taking care not to disturb the bacterial pellet.**

   The cell pellet can be stored at –20°C or –70°C for future use or can be used immediately.

2. **Resuspend the bacterial pellet in 180 µl Buffer ATL and transfer the sample to a 2 ml microcentrifuge tube (not supplied).**
3. **Add 20 µl proteinase K, and mix by tapping the tube.**
4. **Place the sample in a thermomixer or shaker–incubator, and incubate at 56°C with shaking at 900 rpm for 30 min to 2 h.**

   Lysis time depends on the type of cells and the cell number.

5. **If RNA-free DNA is required, proceed with step 5a, otherwise proceed directly to step 5b.**

5a. **Add 4 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature. Proceed with step 6.**

5b. **No RNase A treatment is required. Proceed with step 6.**

6. **Carefully transfer the entire lysate to sample tubes or plates that are compatible with the sample carrier of the QIAsymphony SP.**

   For a full list of compatible vessels, see www.qiagen.com/QIAsymphony/Resources. We recommend use of 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 42).

7. **Continue with the protocol "Purification of DNA from Tissues, Cultured Cells, and Bacterial Cultures" (page 34).**

Protocol: Pretreatment of Gram-Positive Bacteria

This protocol is for purification of total DNA from Gram-positive bacteria using the QIAsymphony SP and the QIAsymphony DNA Mini Kit.

Important point before starting

- QIAsymphony magnetic particles copurify RNA and DNA if both are present in the sample. If RNA-free DNA is required, add RNase A to the sample before lysates are loaded to the instrument or in step 7. The final RNase A concentration should be 2 mg/ml (e.g., add 4 µl of a 100 mg/ml RNase A solution to a 200 µl sample).

Things to do before starting

- Set a thermomixer or shaker–incubator to 37°C for use in step 4 of the procedure and to 56°C for use in step 6 of the procedure.

Procedure

1. **Harvest a maximum of 4 x 10^9 cells by centrifugation for 10 min at 5000 x *g* at room temperature (15–25°C). Remove and discard the supernatant, taking care not to disturb the bacterial pellet.**

   The cell pellet can be stored at –20°C or –70°C for future use or be used immediately.
2. **Resuspend the bacterial pellet in 160 µl Buffer P1 and transfer the sample to a 2 ml microcentrifuge tube (not supplied).**
3. **Add 20 µl lysozyme (100 mg/ml), and mix by tapping the tube.**
4. **Place the sample in a thermomixer or shaker–incubator, and incubate at 37°C with shaking at 900 rpm for 30 min to 2 h.**

   Lysis time depends on the type of cells and the cell number.
5. **Add 20 µl proteinase K, and mix by tapping the tube.**
6. **Incubate at 56°C with shaking at 900 rpm for additional 30 min.**
7. **If RNA-free DNA is required, proceed with step 7a, otherwise proceed directly to step 7b.**

7a. **Add 4 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature. Proceed with step 8.**

7b. **No RNase A treatment is required. Proceed with step 8.**

8. **Carefully transfer the entire lysate to sample tubes or plates that are compatible with the sample carrier of the QIAsymphony SP.**

   For a full list of compatible vessels, see www.qiagen.com/QIAsymphony/Resources. We recommend use of 2 ml tubes (e.g. Sarstedt, cat. no. 72.693 or 72.608) or S-Blocks (see ordering information, page 42).
9. **Continue with the protocol "Purification of DNA from Tissues, Cultured Cells, and Bacterial Cultures" (page 34).**

Protocol: Purification of DNA from Tissues, Cultured Cells, and Bacterial Cultures

Important points before starting

- Ensure that you are familiar with operating the QIAsymphony SP. Refer to the *QIAsymphony SP User Manual* for operating instructions.
- Before beginning the procedure, read "Important Notes" starting on page 12.
- Before using a reagent cartridge for the first time, check that Buffers QSL1 and QSB1 do not contain a precipitate. If necessary, remove the troughs containing Buffers QSL1 and QSB1 from the reagent cartridge and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the troughs in the correct positions. If the reagent cartridge is already pierced, make sure that the troughs are sealed with Reuse Seal Strips and incubate the complete reagent cartridge for 30 minutes at 37°C with occasional shaking in a water bath.
- Try to avoid vigorous shaking the of reagent cartridge otherwise foam may be generated, which can lead to liquid-level detection problems.
- Check that Buffer ATL does not contain a white precipitate. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- If processing tissues with low DNA content (e.g., heart, lung, muscle, or mouse tail), select the "DNA Tissue Low Content" protocol; if processing tissues with high DNA content (e.g., spleen or liver), select the "DNA Tissue High Content" protocol. The "DNA Tissue Low Content" protocol is suited for purification of up to 30 µg of DNA. If the sample is expected to have a higher DNA content, the "DNA Tissue High Content" should be used. If no RNase treatment is performed, note that co-purification of RNA results in reduced DNA binding capacity.

Things to do before starting

- Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes before first use.
- Before loading the reagent cartridge remove the cover from the trough containing the magnetic particles and open the enzyme tubes. Make sure that the piercing lid is placed on the reagent cartridge or, if using a partially used reagent cartridge, make sure the Reuse Seal Strips have been removed.
- If samples are bar coded, orient samples in the tube carrier so that the bar codes face the bar code reader at the left side of the QIAsymphony SP.

Procedure

1. **Close all drawers and the hood.**
2. **Switch on the QIAsymphony SP and wait until the "Sample Preparation" screen appears and the initialization procedure has finished.**

   The power switch is located at the bottom, left corner of the QIAsymphony SP.
3. **Log on to the instrument.**
4. **Ensure the "Waste" drawer is prepared properly, and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.**
5. **Load the required elution rack into the "Eluate" drawer.**

   Do not load a 96-well plate onto "Elution slot 4".

   If eluates should be cooled, use "Elution slot 1" with the corresponding cooling adapter.
6. **Load the required reagent cartridge(s) and consumables (see Table 1, page 14) into the "Reagents and Consumables" drawer, and perform an inventory scan of the "Reagents and Consumables" drawer.**
7. **Place the samples into the appropriate sample carrier, and load them into the "Sample" drawer.**
8. **Using the touchscreen, enter the required information for each batch of samples to be processed.**

   Enter the following information:

   - Sample information (depending on sample racks used)
   - Protocol to be run ("Assay Control Set"). Choose the appropriate Assay Control Set from the "DNA Tissue" application
   - Elution volume and output position

   After information about the batch has been entered, the status changes from "LOADED" to "QUEUED". As soon as one batch is queued the "Run" button appears.

   The Assay Control Set provides information about internal controls, if applicable.
9. **Press the "Run" button to start processing.**

   All processing steps are fully automated. At the end of the protocol run, the status of the batch changes from "RUNNING" to "COMPLETED".

10. **Retrieve the elution rack containing the purified DNA from the "Eluate" drawer.**

    The DNA is ready to use or can be stored at 2–8°C, –20°C, or –80°C.

    In general, magnetic particles are not carried over into eluates. If carryover does occur, magnetic particles in eluates will not affect most downstream applications. If magnetic particles need to be removed before performing downstream applications, tubes or plates containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean tube (see appendix, page 40).

    If the "Eluate" drawer is closed when a batch is running (e.g., if elution racks that contain eluates are removed), the run will be paused and an inventory scan of the "Eluate" drawer will be performed. A message appears during the scan and must be closed (by pressing the "Close" button) before the run can be restarted.

    Result files are generated for each elution plate.

11. **If the reagent cartridge(s) is only partially used, seal it with the provided Reuse Seal Strips and close the enzyme tubes with screw caps immediately after the end of the protocol run to avoid evaporation.**

    For more information about storage of partially used reagent cartridges, see "Storage", page 5.

12. **Discard used sample tubes, plates, and waste according to your local safety regulations.**

    See page 7 for safety information.

13. **Clean the QIAsymphony SP.**

    Follow the maintenance instructions in the *QIAsymphony SP User Manual*.

14. **Close the workstation drawers, and switch off the QIAsymphony SP.**

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
General handling		
	Error message displayed in the touchscreen	If an error message is displayed during a protocol run, refer to "Troubleshooting" in the *QIAsymphony SP User Manual*.
Precipitate in reagent trough of opened cartridge		
a)	Buffer evaporation	Excessive evaporation can lead to increased salt concentration in buffers. Discard reagent cartridge. Make sure to seal buffer troughs of a partially used reagent cartridge with Reuse Seal Strips when not being used for DNA purification.
b)	Storage of reagent cartridge	Storage of reagent cartridge under 15°C may lead to formation of precipitates. If necessary, remove the troughs containing Buffers QSL1 and QSB1 from the reagent cartridge and incubate for 30 min at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the trough in the correct position. If the reagent cartridge is already pierced, make sure that the trough is reclosed with a Reuse Seal Strip and incubate the complete reagent cartridge for 30 min at 37°C with occasional shaking in a water bath.
Low DNA yield		
a)	Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 min before use.
b)	Frozen blood or buffy coat samples were not mixed properly after thawing	Thaw frozen blood samples quickly in a 37°C water bath with mild agitation to ensure thorough mixing.

		Comments and suggestions
c)	Incomplete sample lysis	Before use, check that Buffers QSL1 and QSB1 do not contain precipitates. If necessary, remove the troughs containing Buffers QSL1 and QSB1 from the reagent cartridge and incubate for 30 min at 37°C with occasional shaking to dissolve precipitate. If the reagent cartridge is already pierced, make sure that the trough is reclosed with a Reuse Seal Strip, and incubate the complete reagent cartridge for 30 min at 37°C with occasional shaking in a water bath.
d)	Incomplete digestion of tissue samples	Ensure that the tissue is completely digested by extending the time of incubation with proteinase K.
d)	Clogging of pipet tip due to insoluble material	Insoluble material, such as undigested cartilage, was not removed from the digested sample prior to starting the QIAsymphony DNA purification procedure. To remove insoluble material, centrifuge the sample at 300 x *g* for 1 min, as indicated in the protocol, and transfer the supernatant to a new sample tube.
e)	Clogging of pipet tip due to sample overload	Reduce the sample input volume.
f)	Poor buffy coat preparation when using the buffy coat protocol	Ensure that the leukocyte fraction is efficiently harvested.
g)	Low leukocyte count in the whole blood sample	If using the buffy coat protocol, increase volume of whole blood used and keep the volume of leukocytes harvested constant.
h)	Incomplete lysis of cultured cells or bacteria	If the lysate is viscous, extend the proteinase K incubation time.

DNA does not perform well in downstream applications

a)	Insufficient DNA used in downstream application	Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see the appendix, page 40).
b)	Excess DNA used in downstream application	Excess DNA can inhibit some enzymatic reactions. Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see the appendix, page 40).
c)	Degraded DNA obtained from tissue samples	Too much sample might have been used. For most sample types, 25 mg tissue is sufficient for a reaction volume of 200 µl.

A_{260}/A_{280} ratio for purified DNA is low

	Absorbance reading at 320 nm was not subtracted from the absorbance readings at 260 nm and 280 nm	To correct for the presence of magnetic particles in the eluate, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm (see the appendix, page 40).

Appendix: Handling, Quantification, and Determination of Purity of DNA

Storage of DNA

Purified genomic DNA can be stored at –80°C, –20°C, or at 2–8°C.

Purified viral DNA can be stored at 2–8°C for 3 days before use in analysis and should be kept at –20°C or –80°C for long-term storage.

Quantification of DNA

The concentration of DNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. An absorbance of 1 unit at 260 nm corresponds to 50 µg of DNA per milliliter (A_{260} = 1 → 50 µg/ml). Use water to dilute the samples and to calibrate the spectrophotometer. The ratio between the absorbance values at 260 nm and 280 nm gives an estimate of DNA purity (see "Purity of DNA" on page 41). Carryover of magnetic particles in the eluate may affect the A_{260} reading. If the purified DNA is to be analyzed by fluorescent capillary sequencing, the tube containing the eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see below).

To quantify DNA purified using QIAsymphony DNA procedures:

- Apply the tube containing the DNA to a suitable magnetic separator (e.g., QIAGEN 12-Tube Magnet, cat. no. 36912) until the magnetic particles are separated. If DNA is in microplates, apply the microplate to a suitable magnetic separator (e.g., QIAGEN 96-Well Magnet Type A, cat. no. 36915) until the magnetic particles are separated.
- If a suitable magnetic separator is not available, centrifuge the tube containing the DNA for 1 minute at full speed in a microcentrifuge to pellet any remaining magnetic particles.
- Once separation is complete, carefully withdraw 10–50 µl of purified DNA and dilute to a final volume of 100 µl in water.
- Measure the absorbance at 320, 280, and 260 nm. Subtract the absorbance reading obtained at 320 nm from the readings obtained at 260 and 280 nm to correct for the presence of magnetic particles.

Concentration of DNA sample = 50 µg/ml x ($A_{260} – A_{320}$) x dilution factor

Total amount of DNA purified = concentration x volume of sample in milliliters

Note: For accurate quantification of DNA by absorbance at 260 nm, we recommend diluting the sample in the corresponding elution buffer. Dilution of the sample in water may lead to inaccurate values. Elution buffer has high absorbance at 220 nm, which can lead to high background absorbance levels if the spectrophotometer is not properly zeroed. Extra elution buffer to blank the spectrophotometer is provided in a separate bottle with QIAsymphony DNA Kits.

Purity of DNA

Purity is determined by calculating the ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm; i.e., $(A_{260} - A_{320})/(A_{280} - A_{320})$. Pure DNA has an A_{260}/A_{280} ratio of 1.7–1.9.

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.

Ordering Information

Product	Contents	Cat. no.
QIAsymphony DNA Mini Kit (192)	For up to 192 preps of 200 µl each: Includes 2 reagent cartridges and enzyme racks and accessories	931236
QIAsymphony DNA Midi Kit (96)	For 96 preps of 1000 µl each: Includes 2 reagent cartridges and enzyme racks and accessories	931255
Related products		
Accessory Trough (10)	For use with the QIAsymphony SP	997012
Reagent Cartridge Holder (2)	For use with the QIAsymphony SP	997008
Sample Carrier, plate, Qsym	Plate carrier for sample input. For use with the QIAsymphony SP	9017660
Tube Insert, 11 mm, sample carrier, Qsym	Primary tube adapter (11 mm) for use with the QIAsymphony tube carrier	9241033
Tube Insert, 13 mm, sample carrier, Qsym	Primary tube adapter (13 mm) for use with the QIAsymphony tube carrier	9241034
Tube Insert, 2 ml, sample carrier, Qsym	Secondary tube adapter (for 2 ml screw-cap tubes) for use with the QIAsymphony tube carrier	9241032
Cooling Adapter, tubes, 2 ml, Qsym	Cooling adapter for 2 ml screw-cap tubes for use in the QIAsymphony "Eluate" drawer	9018088
Cooling Adapter, EMT, Qsym	Cooling adapter for EMT racks for use in the QIAsymphony "Eluate" drawer	9018086
Cooling Adapter, MTP, RB, Qsym	Cooling adapter for round-bottom microtiter plates (MTP) for use in the QIAsymphony "Eluate" drawer	9018085
Cooling Adapter, PCR, Qsym	Cooling adapter for PCR plates for use in the QIAsymphony "Eluate" drawer	9018087
Adapter, tubes, 2 ml, Qsym	Adapter for 2 ml screw-cap tubes for use in the QIAsymphony "Eluate" drawer	9018577

Ordering Information

Product	Contents	Cat. no.
Sample Prep Cartridges, 8-well (336)	8-well sample prep cartridges for use with the QIAsymphony SP	997002
8-Rod Covers (144)	8-Rod Covers for use with the QIAsymphony SP	997004
Filter-Tips, 200 µl (1024)	Sterile, Disposable Filter-Tips, racked; (8 x 128)	990332
Filter-Tips, 1500 µl (1024)	Sterile, Disposable Filter-Tips, racked; (8 x 128)	997024
Tip Disposal Bags (15)	For use with the QIAsymphony SP	9013395
Buffer ATL (200 ml)	200 ml Tissue Lysis Buffer for 1000 preps	19076
Buffer P1 (500 ml)	500 ml Resuspension Buffer (RNase A not included)	19051
RNase A (17,500 U)	2.5 ml (100 mg/ml; 7000 units/ml solution)	19101
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912
96-Well Magnet Type A	Magnet for separating magnetic particles in wells of 96-well plates, 2 x 96-Well Microplates FB	36915
S-Blocks (24)	96-well blocks with 2.2 ml wells, 24 per case	19585

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