March 2015

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

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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

QIAGEN sets standards in:

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

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

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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 6 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.

When stored properly, the kit is stable until the expiration date on the kit box.

Partially used reagent cartridges can be stored for a maximum of 2 weeks, enabling cost-efficient use 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.

Avoid exposure of the reagent cartridges to UV light (e.g., used for decontamination) as exposure may cause accelerated aging of the reagent cartridges and buffers.

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.

Warnings and Precautions

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.

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.

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.

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. 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.

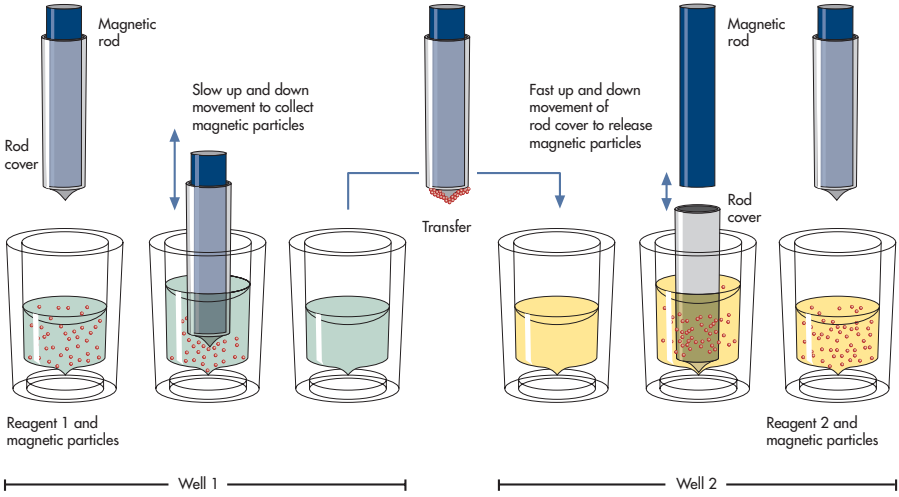
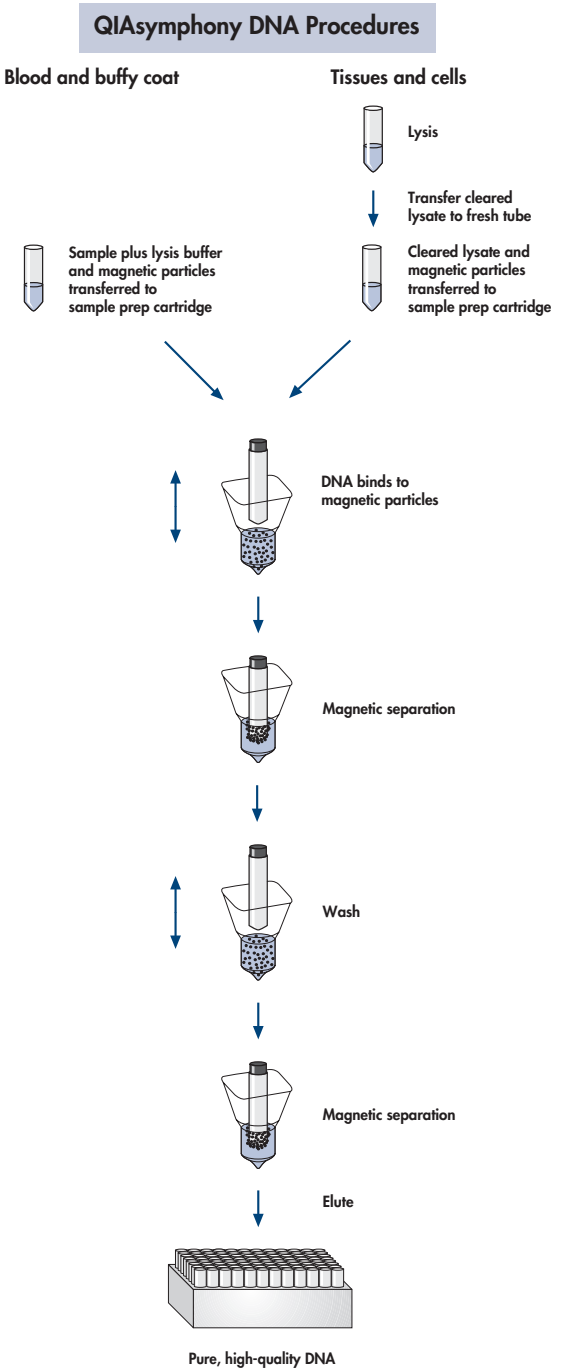


Figure 1. Schematic of the QIAsymphony SP principle. The QIAsymphony SP processes a sample containing magnetic particles as follows: A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another well 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
Blood and buffy coat
Tissues and cells
Lysis
Transfer cleared lysate to fresh tube
Sample plus lysis buffer and magnetic particles transferred to sample prep cartridge
Cleared lysate and magnetic particles transferred to sample prep cartridge
DNA binds to magnetic particles
Magnetic separation
Wash
Magnetic separation
Elute
Pure, high-quality DNA

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 racks (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/QIAsymphonyDNAKits. Labware lists are available under the Resources tab in this page.
- Elution tubes or racks. Compatible elution tube and rack formats are listed at www.qiagen.com/QIAsymphonyDNAKits. Labware lists are available under the Resources tab in this page.
- 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)

- For using internal controls: 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 user manual supplied with your instrument for operating instructions.

We recommend following the maintenance instructions given in the user manual to reduce the risk of contamination.

The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at www.qiagen.com/QIAsymphonyDNAKits. Labware lists are available under the Resources tab in this page.

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 closed with Reuse Seal Strips for later use, 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 4.

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.

Note: Ensure that the covers of the unit boxes are removed before loading the unit boxes into the "Reagents and Consumables" drawer.

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, or unit boxes with sample prep cartridges or 8-Rod Covers before starting another protocol run. The QIAsymphony SP can use partially used tip racks and unit boxes.

For the consumables required, see the relevant protocol sheet available at www.qiagen.com/QIAsymphonyDNAKits. Click on the Resources tab. For ordering information, see page 26.

Loading the "Waste" drawer

Used sample prep cartridges and 8-Rod Covers are placed 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 discarding 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 user manual supplied with your instrument.

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.

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.

Using FIX labware

Using liquid–level detection (LLD) for sample transfer allows the use of primary and secondary tubes. However, this requires certain dead volumes in the respective tubes.

In order to minimize dead volumes, secondary tubes should be used without liquid–level detection. Specific FIX labware is available (e.g. SAR_**FIX**_#72.694 T2.0 ScrewSkirt) which can also be selected on the touchscreen of the QIAsymphony SP. This tube/rack type imposes aspiration restrictions. The sample is aspirated at a particular height in the tube that is defined by the volume of sample to be transferred. Therefore it is essential to make sure that the volume listed in the labware list is used.

Sample tubes that can be used with or without liquid–level detection and required sample volumes are listed at www.qiagen.com/QIAsymphonyDNAKits. Labware lists are available under the Resources tab in this page.

Tubes that are using liquid–level detection and tubes that are not using liquid–level detection can be processed within one batch/run.

Preparation of sample material

QIAsymphony DNA Kits are suitable for use with a wide range of sample types, including human whole blood and buffy coat, tissues, cultured cells, and bacterial cultures. Depending on the starting material, sample pretreatment may be required. Samples should be equilibrated to room temperature (15–25°C) before starting the run. Prevent formation of foam in or on the samples.

For more information about the automated procedure (including information about sample tubes that can be used with specific protocols) and specific sample pretreatments, see the relevant protocol sheet available at www.qiagen.com/QIAsymphonyDNAKits. Click on the Resources tab.

Using an internal control for purification of viral DNA

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

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.

Internal controls should be diluted in Buffer ATE. A total volume of 60 µl internal control–Buffer ATE mixture is added per sample.

When calculating the amount of internal control(s) and 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. Small volumes of liquid are lost during transfer and through contact with the magnetic particles. Because of this dead volume, the initial volume of elution buffers that is aspirated by the QIAsymphony is greater than the volume selected by the operator. This ensures that the actual final volume matches the selected final volume.

The minimum volume of internal control–Buffer ATE mixture must include sufficient additional volume to take into account liquid loss due to pipetting and evaporation. We recommend preparing fresh mixtures for each run just before use.

The Virus Blood protocol sheet provides detailed information on compatible tube formats, the initial elution volume, and the minimum volume of internal control–Buffer ATE mixture.

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. 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 Manual*.

Note: When using the default "Virus Blood 200 *default* 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.

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. Enzyme activity is substantially increased 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 24, 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.

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 1 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.

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 ml of a 100 mg/ml RNase A solution to a 200 ml sample).

Table 1. 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 up to 24 h 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: General Purification Protocol

The following is a general protocol for using QIAsymphony DNA Kits. Detailed information for each protocol, including volumes and tubes, is provided in protocol sheets that can be downloaded at www.qiagen.com/QIAsymphonyDNAKits. Click on the Resources tab.

Important points before starting

- Ensure that you are familiar with operating the QIAsymphony SP. Refer to the user manuals supplied with your instrument for operating instructions.
- Optional maintenance is not mandatory for instrument function, but is highly recommended to reduce risk of contamination.
- Before beginning the procedure, read "Important Notes" starting on page 11.
- Ensure you are familiar with the protocol sheet corresponding to the procedure you want to use (available by clicking on the Resources tab at www.qiagen.com/QIAsymphonyDNAKits).
- 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.

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.
- For information about compatible sample tube and minimum sample volumes for samples in primary and secondary tubes, see the corresponding protocol sheet at www.qiagen.com/QIAsymphonyDNAKits. Click on the Resources tab.

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 loaded 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.

   When using a 96-well plate, make sure that the A1 well of the plate is on the top left corner to avoid sample mixup in downstream analysis.
6. **Load the required reagent cartridge(s) and consumables into the "Reagents and Consumables" drawer.**
7. **Perform an inventory scan of the "Reagents and Consumables" drawer.**
8. **Place the samples into the appropriate sample carrier, and load them into the "Sample" drawer.**
9. **For Virus Blood applications: 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, refer to the relevant protocol sheet and see "Using an internal control for purification of viral DNA" (page 14).
10. **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 (i.e., "Assay Control Set")
    - Elution volume and output position
    - For Virus Blood applications: Tube(s) containing internal control(s)

    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.

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.**

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

    For Virus Blood applications: For short-term storage of up to 24 h, we recommend storing purified nucleic acids at 2–8°C. For long-term storage of over 24 h, we recommend storing purified nucleic acids at –20°C.

    We recommend removing the elution rack from the "Eluate" drawer immediately after the run has finished. Depending on temperature and humidity, elution racks left in the QIAsymphony SP after the run is completed may experience condensation or evaporation.

    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 racks containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean tube (see appendix, page 24.)

    If the "Eluate" drawer is opened 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 when the drawer is closed.

    Result files are generated for each elution rack.

13. **If a reagent cartridge is only partially used, seal it with the provided Reuse Seal Strips and close tubes containing proteinase K 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 4.

14. **Discard used sample tubes, racks, and waste according to your local safety regulations.**

    See page 6 for safety information.

15. **Clean the QIAsymphony SP.**

    Follow the maintenance instructions in the user manuals supplied with your instrument. Clean the tip guards regularly to minimize the risk of cross-contamination.

16. **Close the instrument 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 user manual supplied with your instrument.
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 cartridges below 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 closed 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 closed 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 24).
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 24).
c)	Degraded DNA obtained from tissue samples	Too much sample might have been used. Overloading with too much sample may lead to insufficient lysis and therefore insufficient inactivation of potential DNAses. For recommended sample sizes, refer to the protocol sheet at www.qiagen.com/QIAsymphonyDNAKits. Click on the Resources tab.

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 24).

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 up to 24 hours 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). The ratio between the absorbance values at 260 nm and 280 nm gives an estimate of DNA purity (see "Purity of DNA" on page 25). 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 effects of background absorbance.

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 millilitres

Carryover of magnetic particles in the eluate may affect the A_{260} reading. If the purified DNA is to be analyzed e.g., 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:

- Apply the tube containing the DNA to a suitable magnetic separator (e.g., 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., 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 the purified DNA and transfer to a new tube or rack.

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. Evaporation of eluates potentially increases the risk of impact on the measurement especially when low amounts of eluates are used undiluted. 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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