

August 2015

QIAsymphony[®] DSP DNA Instructions for Use (Handbook)



192 (cat. no. 937236)



96 (cat. no. 937255)

Version 1



For in vitro diagnostic use

QIAsymphony DSP DNA Mini Kit

QIAsymphony DSP DNA Midi Kit



937236, 937255



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Intended Use

The QIASymphony® DSP DNA Mini Kit and QIASymphony DSP DNA Midi Kit utilize magnetic-particle technology for automated isolation and purification of DNA from biological specimens.

The products are intended to be used by professional users, such as technicians and physicians who are trained in molecular biological techniques.

The QIASymphony DSP DNA system is intended for in vitro diagnostic use.

Summary and Explanation

QIASymphony DSP DNA Kits are intended to be used only in combination with the QIASymphony SP. QIASymphony DSP DNA Kits provide reagents for fully automated and simultaneous purification of total DNA from human whole blood, buffy coat, tissues and FFPE tissues, as well as viral DNA from human whole blood. However, performance characteristics for every virus, tissue or FFPE tissue have not been established and must be validated by the user. Magnetic-particle technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. Purified nucleic acids are ready for direct use in downstream applications, such as amplification or other enzymatic reactions. The QIASymphony SP performs all steps of the purification procedure. Up to 96 samples, in batches of 24, are processed in a single run. Tissue and FFPE tissue protocols require manual sample pretreatment.

Principles of the Procedure

QIAasympphony technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles (Figure 1, below). 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, page 5). The user can choose between different elution volumes.

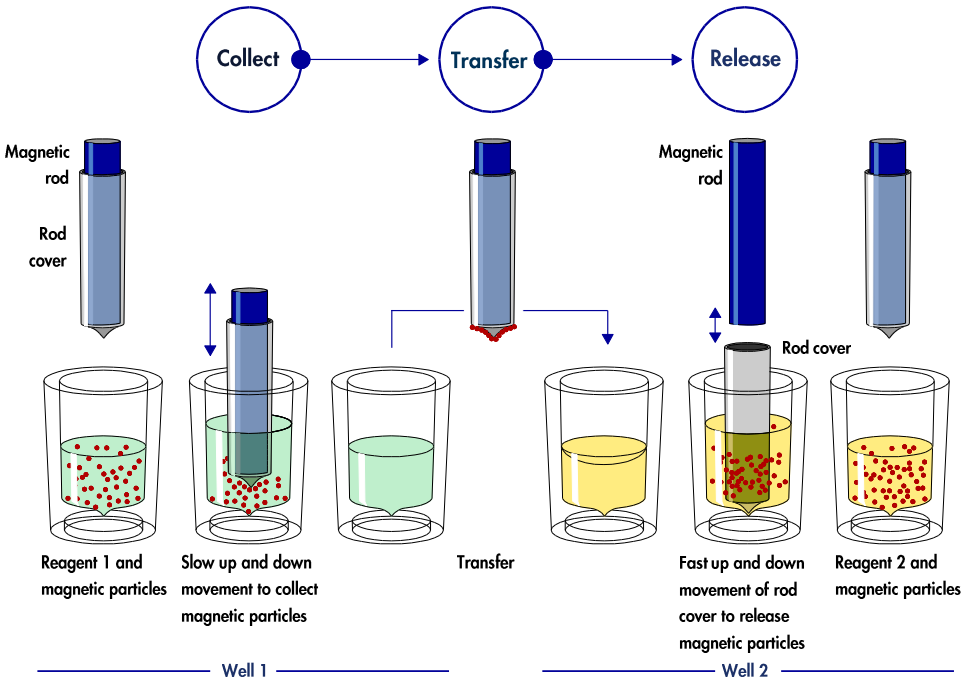
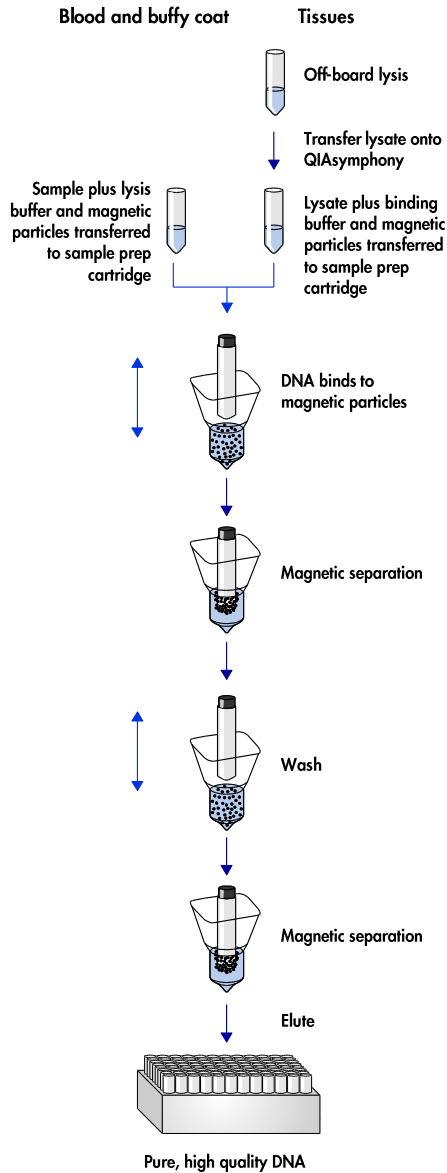


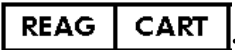

Figure 1. Schematic of the QIAasympphony SP principle. The QIAasympphony SP processes a sample containing magnetic particles as follows: A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another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

QIAasympy DSP DNA Procedure



Materials Provided

Kit contents

QIAsymphony DSP DNA Kit			Mini	Midi
Catalog number			937236	937255
Number of preps			192	96*
RC	Reagent Cartridge [†]		2	2
ER	Enzyme Rack		2	2
PL	Piercing Lid		2	2
ATE	Buffer ATE (20 ml) [‡]		20 ml	20 ml
RSS	Reuse Seal Set [§]		2	2
	Instructions for Use (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.

[¶] See page 23 for symbols list with definitions.

Materials Required but Not Provided

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) available from the product supplier.

- QIAsymphony SP
- 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 (e.g., 2 ml sample tubes with screw caps, Sarstedt® cat. no. 72.693, or without caps, Sarstedt cat. no. 72.608 or Sarstedt cat. no. 72.694). Compatible primary and secondary tube formats are listed at www.qiagen.com/goto/dspdnakits.
- Elution tubes or plates. Compatible elution tube and plate formats are listed at www.qiagen.com/goto/dspdnakits.
- Phosphate-buffered saline (PBS, may be required for diluting samples)
- Vortexer
- Optional: DNase-free RNase A (to minimize RNA content)
- For additional materials needed for Tissue and Virus Blood applications, please refer to protocol sheets at www.qiagen.com/goto/dspdnakits.

Warnings and Precautions

For in vitro diagnostic use.

Read all instructions carefully before using the kit.

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 (RC) 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 hazard and precautionary statements apply to components of QIASymphony DSP DNA Kits.

QSB1



Contains: Brij 58; guanidine thiocyanate; isopropanol. Danger! May be harmful if swallowed or in contact with skin. Causes severe skin burns and eye damage. May cause drowsiness or dizziness. Harmful to aquatic life with long lasting effects. Highly flammable liquid and vapor. Contact with acids liberates very toxic gas. Dispose of contents/container to an approved waste disposal plant. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF ON SKIN (or hair): Remove/take off immediately all contaminated clothing. Rinse skin with water/shower. Immediately call a POISON CENTER or doctor/physician. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Store in a well-ventilated place. Keep container tightly closed. Wear protective gloves/protective clothing/eye protection/face protection.

MBS

Warning! Causes mild skin irritation. If skin irritation occurs: Get medical advice/attention.

Proteinase K



Contains: Proteinase K. Danger! Causes mild skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Dispose of contents/container to an approved waste disposal plant. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. Wear respiratory protection.

QSL1



Contains: guanidine hydrochloride; maleic acid. Warning! May be harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. May cause an allergic skin reaction. If eye irritation persists: Get medical advice/attention. Take off contaminated clothing and wash it before reuse. Wear protective gloves/protective clothing/eye protection/face protection.

QSW1



Contains: ethanol; guanidine hydrochloride; lithium chloride. Warning! May be harmful if swallowed. Causes skin irritation. Causes serious eye irritation. Flammable liquid and vapor. Dispose of contents/container to an approved waste disposal plant. If eye irritation persists: Get medical advice/attention. Take off contaminated clothing and wash it before reuse. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Store in a well-ventilated place. Keep cool. Wear protective gloves/protective clothing/eye protection/face protection.

QSW2



Contains: ethanol. Danger! Causes serious eye irritation. Highly flammable liquid and vapor. Dispose of contents/container to an approved waste disposal plant. If eye irritation persists: Get medical advice/attention. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Store in a well-ventilated place. Keep cool. Wear protective gloves/protective clothing/eye protection/face protection.

Reagent Storage and Handling

QIASymphony DSP DNA Kits should be stored upright at room temperature (15–25°C). The magnetic particles in the reagent cartridges (RC) remain active when stored at this temperature. When stored properly, the kit is stable until the expiration date on the kit box.

Note: The label on the QIASymphony DSP DNA Kit box displays the expiration date of the kit. The result file documents the expiration dates for only the reagent cartridge (RC).

Kit components

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

Do not store reagent cartridges (RC) at temperatures below 15°C.

Partially used reagent cartridges (RC) can be stored for a maximum of 4 weeks, enabling cost-efficient reuse of reagents and more flexible sample processing. If a reagent cartridge (RC) is partially used, replace the cover of the trough containing the magnetic particles, and seal the reagent cartridge (RC) with the provided Reuse Seal Strips immediately after the end of the protocol run to avoid evaporation.

To avoid reagent evaporation, the reagent cartridge (RC) 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 (RC) is open and the required buffer volumes, potentially reducing the total number of sample preparations possible per cartridge.

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

Specimen Collection and Preparation

Prevent formation of foam in or on the samples. Depending on the starting material, sample pretreatment may be required.

Samples should be equilibrated to room temperature (15–25°C) before starting the run.

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/goto/dspdnakits.

Procedure

Automated purification on QIAasympphony SP

The QIAasympphony 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 manuals supplied with your instrument for operating instructions.

Note: Optional maintenance is not mandatory for instrument function, but is highly recommended to reduce 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/goto/dspdnakits.

Loading reagent cartridges (RC) into the “Reagents and Consumables” drawer

Reagents for purification of DNA are contained in an innovative reagent cartridge (RC) (Figure 2, page 13). Each trough of the reagent cartridge (RC) contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer. Partially used reagent cartridges (RC) can be reclosed with Reuse Seal Strips (RSS) for later reuse, which avoids generation of waste due to leftover reagents at the end of the purification procedure.

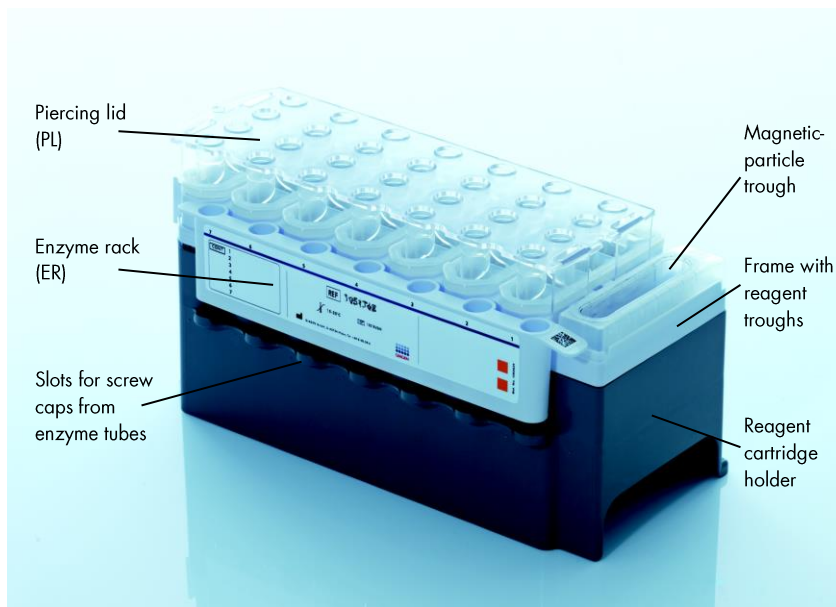


Figure 2. QIAasympphony reagent cartridge (RC). The reagent cartridge (RC) contains all reagents required for the protocol run.

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 (RC) into the reagent cartridge holder. Place the enzyme rack (ER) into the reagent cartridge holder. Before using a reagent cartridge (RC) for the first time, place the piercing lid (PL) on top of the reagent cartridge (RC) (Figure 2, above).

Note: The piercing lid (PL) is sharp. Take care when placing it onto the reagent cartridge (RC). Make sure to place the piercing lid (PL) onto the reagent cartridge (RC) 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, page 13), the reagent cartridge (RC) is subsequently loaded into the “Reagents and Consumables” drawer.

Partially used reagent cartridges (RC) can be stored until needed again, see “Reagent Storage and Handling” page 10.

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: Make sure that the covers of the unit boxes are removed before loading the unit boxes into the “Reagents and Consumables” drawer.

Note: Tips have filters to help prevent cross-contamination.

Tip rack slots on the QIASymphony SP 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 for 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/goto/dspdnakits. For plasticware ordering information, see page 30.

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: Make sure 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 user manuals provided with your instrument. Empty the tip bag after a maximum of 96 samples have been processed to avoid a tip jam.

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. Dispose of the liquid waste according to your local safety and environment regulations. Do not autoclave the filled waste bottle. Empty the waste bottle after a maximum of 96 samples have been processed.

Loading the “Eluate” drawer

Load the required elution rack into the “Eluate” drawer. As long-term storage of eluates in the “Eluate” drawer may lead to evaporation of eluates, the cooling position must be used. Only use “Elution slot 1” with the corresponding cooling adapter.

Inventory scan

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

Preparation of sample material

QIASymphony DSP DNA Kits are designed for automated purification of total DNA from human whole blood, buffy coat, tissues and formalin-fixed paraffin-embedded (FFPE) tissues, as well as viral DNA from human whole blood (Table 1, page 17).

Prevent formation of foam in or on the samples. Depending on the starting material, sample pretreatment may be required. Samples should be equilibrated to room temperature (15–25°C) before starting the run. Tissue and FFPE tissue protocols require manual sample pretreatment.

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/goto/dspdnakits.

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. 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 DSP DNA Kits copurify RNA and DNA if both are present in the sample. In order to minimize RNA content in the sample, add RNase A to the sample in the step indicated in the respective pretreatment protocol. For more information refer to protocol sheets at www.qiagen.com/goto/dspdnakits.

Storing DNA

Purified DNA can be stored at 2–8°C for up to 5 days. For long-term storage, store at –20°C or –80°C.

Table 1. Protocol overview

Sample	Sample volume (µl)	Elution volume (µl)	Kit	QIAasympyony SP protocol
Whole blood	200	50, 100, 200	Mini	Blood 200 DSP
	400	100, 200, 400	Midi	Blood 400 DSP
	1000	200, 400, 500	Midi	Blood 1000 DSP
Buffy coat	200	200, 300, 400	Mini	DNA Buffy Coat 200 DSP
	400	200, 400	Midi	DNA Buffy Coat 400 DSP
Virus blood	200	60, 85, 110, 165	Mini	VirusBlood200 DSP
Tissue	200	50, 100, 200,400	Mini	Tissue LC 200 DSP
	200	100, 200, 400	Mini	Tissue HC 200 DSP

Important points before starting

- Make sure that you are familiar with operating the QIAasympyony 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 “Principles of the Procedure” starting on page 4.
- Make you are familiar with the protocol sheet corresponding to the procedure you want to use (www.qiagen.com/goto/dspdnakits).
- 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.*

* Make sure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer’s instructions.

- Try to avoid vigorous shaking of the reagent cartridge (RC) otherwise foam may be generated, which can lead to liquid-level detection problems.

Things to do before starting

- Before starting the procedure, make sure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes before first use.
- Make sure that the piercing lid is placed on the reagent cartridge and the lid of the magnetic-particle trough has been removed or, if using a partially used reagent cartridge, make sure the Reuse Seal Strips have been removed.
- Make sure to open the enzyme tubes.
- 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 sample tubes compatible with a certain protocol, see the corresponding labware list (available at www.qiagen.com/goto/dspdnakits).
- For information about minimum sample volumes for samples in primary and secondary tubes for a certain protocol, see the corresponding labware list (available at www.qiagen.com/goto/dspdnakits). This information also indicates which tubes can be used for different protocols.

Protocol: Purification of DNA

The following is a general protocol for using QIASymphony DSP DNA Kits. Detailed information for each protocol, including volumes and tubes, is provided in protocol sheets that can be downloaded at www.qiagen.com/goto/dspdnakits.

1. Close all drawers and the hood.
2. Power 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 QIA Symphony SP.

3. Log on to the instrument.
4. Make sure the "Waste" drawer is properly prepared 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".

"Elution slot 1", with the corresponding cooling adapter, must be used.

When using a 96-well plate, make sure that the plate is in the correct orientation, as incorrect placement may cause sample mix-up in downstream analysis.

When using the Elution Microtubes CL rack, remove the bottom by twisting the rack until the bottom comes off.

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.

IMPORTANT: For VirusBlood200 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 and using an internal control, refer to the relevant protocol sheet (available at www.qiagen.com/goto/dspdnakits).

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)

Elution volume and output position

For VirusBlood200 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.

10. 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**.

11. Retrieve the elution rack containing the purified nucleic acids from the “Eluate” drawer.

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

We recommend removing the eluate plate from the “Eluate” drawer immediately after the run has finished. Depending on temperature and humidity, elution plates 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 plates containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean tube (see appendix, page 28).

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 tubes containing proteinase K 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 (RC), see “Reagent Storage and Handling”, page 10.

14. Discard used sample tubes 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 user manuals supplied with your instrument. Make sure to clean the tip guards regularly to minimize the risk of cross-contamination.

16. Close the instrument drawers and power OFF the QIAAsymphony SP.

Quality Control

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

Limitations

System performance has been established in performance evaluation studies purifying total DNA from human whole blood, buffy coat, tissues and FFPE tissues, as well as viral DNA from human whole blood.









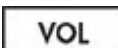



It is the user's responsibility to validate system performance for any procedures used in their laboratory that are not covered by the QIAGEN performance evaluation studies.




To minimize the risk of a negative impact on the diagnostic results, adequate controls for downstream applications should be used. For further validation, the guidelines of the International Conference on Harmonisation of Technical Requirements (ICH) in *ICH Q2 (R1) Validation of Analytical Procedures: Text and Methodology* are recommended.

Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

Symbols

The symbols in the following table are used in these instructions for use.

Symbol	Symbol definition
 Σ <N>	Contains reagents sufficient for <N> tests
	Use by
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Material number (i.e., component labeling)
	Components (i.e., a list of what is included)
	Number (i.e., vials, bottles)
Rn	R is the revision of the Instructions for Use (Handbook), n is the revision number
	Volume
	Temperature limitation
	Manufacturer
	Only for use with

Symbol	Symbol definition
EC REP	Authorized representative in the European Community
	Consult instructions for use
CONT	Contains (contents)
WELL	Well number (i. e., reagent cartridge well)
REAG CART	Reagent cartridge
ELU BUF	Elution buffer (Buffer ATE)
IPA	Isopropanol
PROTK	Proteinase K
GITC	Guanidine thiocyanate
GuHCL	Guanidine hydrochloride
EtOH	Ethanol
MALEIC ACID	Maleic acid
BRIJ 58	BRIJ 58
LiCl	Lithium chloride
GTIN	Global Trade Item Number
	Caution
	Sharp edge

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 or 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 the user manuals supplied with your instrument.
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Precipitate in reagent trough of opened cartridge

- | | |
|--------------------------------------|---|
| a) Buffer evaporation | Excessive evaporation may lead to increased salt concentration in buffers. Discard reagent cartridge (RC). Make sure to seal buffer troughs of a partially used reagent cartridge (RC) with Reuse Seal Strips when not being used for purification. |
| b) Storage of reagent cartridge (RC) | Storage of reagent cartridge (RC) below 15°C may lead to formation of precipitates. If necessary, remove the troughs containing Buffer QSL1 and QSB1 from the reagent cartridge (RC) and incubate in a water bath* at 37°C for 30 minutes with occasional shaking to dissolve precipitate. Make sure to replace the trough in the correct position. If the reagent cartridge (RC) is already pierced, make sure that the trough is reclosed with a Reuse Seal Strip and incubate the complete reagent cartridge (RC) in a water bath* at 37°C for 30 minutes with occasional shaking. |

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 minutes before use. |
|---|--|

* Make sure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Comments and suggestions

b) Frozen blood or buffy coat samples were not mixed properly after thawing	Thaw frozen blood or buffy coat samples with mild agitation to ensure thorough mixing.
c) Incomplete sample lysis	Before use, check that Buffer QSL1 and QSB1 do not contain precipitates. If necessary, remove the troughs containing Buffers QSL1 and QSB1 from the reagent cartridge (RC) and incubate in a water bath* for 30 minutes at 37°C with occasional shaking to dissolve precipitate. If the reagent cartridge (RC) is already pierced, make sure that the troughs are reclosed with Reuse Seal Strips, and incubate the complete reagent cartridge (RC) for 30 minutes 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.
e) Clogging of pipet tip due to insoluble material	Insoluble material was not removed from the sample prior to starting the QIAasympphony purification procedure. If required, use pretreatment procedures as described in the corresponding protocol sheets, for example, for viscous sample materials. Protocol sheets are available at www.qiagen.com/goto/dspdnakits .
f) Poor buffy coat preparation when using buffy coat protocol	Make sure that the leukocyte fraction is efficiently harvested.
g) Low leukocyte count in the whole blood sample used as starting material for buffy coat preparation	If using the buffy coat protocol, increase volume of whole blood used and keep the volume of leukocytes harvested constant.
h) Incomplete lysis of tissues	If the lysate contains insoluble material, extend the proteinase K incubation time.
i) Pellet was lost during FFPE pretreatment with xylene/ethanol	Carefully observe samples during pretreatment.

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 28).*
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* Make sure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Comments and suggestions

- | | |
|--|--|
| 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 28).* |
|--|--|

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 appendix, page 28).*

* Make sure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Appendix: Quantification and Determination of Purity of DNA

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 ($A_{260} = 1 = 50 \mu\text{g/ml}$).

Use Buffer ATE 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 29).

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 potential presence of background reading.

Use the following formula to calculate DNA concentration and yield: Concentration of DNA sample = $50 \mu\text{g/ml} \times (A_{260} - A_{320}) \times \text{dilution factor}$. Total amount of DNA purified = concentration \times volume of sample in milliliters.

In case magnetic particles were carried over in the eluate and might affect downstream application (e.g., 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).

If magnetic particles need to be removed, 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.

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 DSP 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.

Ordering Information

Product	Contents	Cat. no.
QIASymphony DSP DNA Mini Kit (192)	Includes 2 reagent cartridges and enzyme racks and accessories	937236
QIASymphony DSP DNA Midi Kit (96)	Includes 2 reagent cartridges and enzyme racks and accessories	937255
Related products		
Buffer ATL (4 x 50 ml)	4 x 50 ml Buffer ATL for use with QIASymphony Tissue protocols	939016
Deparaffinization Solution (1 x 50 ml)	1 x 50 ml Deparaffinization Solution for use with QIASymphony FFPE Tissue protocols	939018
Accessory Trough (10)	Accessory troughs for use with the QIASymphony SP	997012
Reagent Cartridge Holder (2)	Reagent cartridge holder for use with the QIASymphony SP	997008
Tube Insert, 2 ml, v2, sample carrier, Qsym	Secondary tube adapter (for 2 ml screw-cap tubes) for use with the QIASymphony tube carrier	9242083
Tube Insert, 11 mm, Revision, sample carrier, Qsym	Primary tube adapter (11 mm) for use with the QIASymphony tube carrier	9242057
Tube Insert, 13 mm, sample carrier, Qsym	Primary tube adapter (13 mm) for use with the QIASymphony tube carrier	9242058
Cooling Adapter, 2 ml, V2, Qsym	Cooling adapter for 2 ml screw-cap tubes. For use in the QIASymphony "Eluate" drawer	9020674

Cooling Adapter, EMT, v2, Qsym	Cooling adapter for EMT racks. For use in the QIA Symphony "Eluate" drawer	9020730
Sample Prep Cartridges, 8-well (336)	8-well sample prep cartridges for use with the QIA Symphony SP	997002
8-Rod Covers (144)	8-Rod Covers for use with the QIA Symphony SP	997004
Filter-Tips, 200 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube® and the QIA Symphony SP/AS	990332
Filter-Tips, 1500 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIA Symphony SP/AS	997024
Tip Disposal Bags (15)	Tip disposal bags for use with the QIA Symphony SP	9013395
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
Reuse Seal Set (20)	Reuse seal sets for sealing partly used QIA Symphony reagent cartridges	997006

Elution Microtubes CL (24 x 96)	Non-sterile polypropylene tubes (0.85 ml maximum capacity, less than 0.7 ml storage capacity, 0.4 ml elution capacity); 2304 in racks of 96; includes cap strips	19588
QIASymphony SP	QIASymphony sample prep module, 1 year warranty on parts and labor	9001297

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