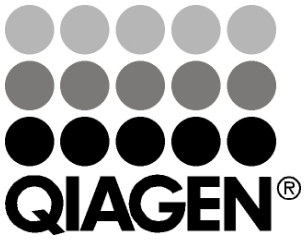
August 2011

QIAsymphony® Certal Handbook

QIAsymphony Certal Residual DNA Kit
QIAsymphony Certal Vaccine NA Kit

For purification of residual host cell DNA and viral NA from bioprocess purification buffer, cell culture supernatant samples, and vaccine preparations 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.

Contents

Kit Contents

QIAsymphony Certal Residual DNA Kit	
Catalog no.	**931855**
Number of preps*	**96**
QIAsymphony Certal Cartridge†	2
Enzyme Rack	2
Piercing Lid	2
Buffer AVE (20ml)‡	2
Buffer AVE (2ml)‡	2
Buffer CA‡	1
Carrier RNA	2 x 1350 µg
Reuse Seal Set§	2
Handbook	1

* Number of preps depends on the sample volume and protocol used. Up to 96 samples can be prepared with sample volumes of 500 µl using the QIAsymphony Certal Residual DNA Kit (resDNA1000 protocol).

† Contains guanidine salts. Not compatible with disinfectants containing bleach. See page 8 for safety information.

‡ Contains sodium azide as a preservative.

§ A Reuse Seal Set contains 8 Reuse Seal Strips.

Kit Contents

QIAsymphony Certal Vaccine NA Kit	
Catalog no.	**931955**
Number of preps*	**96**
QIAsymphony Certal Cartridge†	2
Enzyme Rack	2
Piercing Lid	2
Buffer AVE (20ml)‡	2
Buffer AVE (2ml)‡	2
Buffer CB	1
Carrier RNA	2 x 1350 µg
Reuse Seal Set§	2
Handbook	1

* Number of preps depends on the sample volume and protocol used. Up to 96 samples can be prepared with sample volumes of 500 µl using the QIAsymphony Certal Vaccine NA Kit (vacNA1000 protocol).

† Contains guanidine salts. Not compatible with disinfectants containing bleach. See page 8 for safety information.

‡ Contains sodium azide as a preservative.

§ A Reuse Seal Set contains 8 Reuse Seal Strips.

Storage

QIAsymphony Certal Kits should be stored at room temperature (15–25°C) with the exception of buffer CA. Buffer CA should be stored at 2–8°C. Do not store reagent cartridges at temperatures below 15°C.

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

When stored properly, a 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 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 Use Limitations

QIAsymphony Certal 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.

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 the QIAsymphony Certal 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 the QIAsymphony Certal Kits 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 the QIAsymphony Certal Kits.

QSL2

Contains guanidine thiocyanate: harmful. Risk and safety phrases:* R20/21/22-32, S13-26-36/37/39-46

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

* R11: Highly flammable; R22: Harmful if swallowed; R20/21/22: Harmful by inhalation, in contact with skin, and if swallowed; R32: Contact with acids liberates very toxic gas; R36: Irritating to eyes; R36/38: Irritating to eyes and skin; R36/37/38: Irritating to eyes, respiratory system, and skin; R42/43: May cause sensitization by inhalation and skin contact; 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; 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: Irritating to eyes and respiratory system; R36/38: Irritating to eyes and skin; S36/37/39: Wear suitable protective clothing, gloves, and eye/face protection; S46: If swallowed, seek medical advice immediately and show container or label.

QSW5

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

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

* R11: Highly flammable; R22: Harmful if swallowed; R20/21/22: Harmful by inhalation, in contact with skin, and if swallowed; R32: Contact with acids liberates very toxic gas; R36: Irritating to eyes; R36/38: Irritating to eyes and skin; R36/37/38: Irritating to eyes, respiratory system, and skin; R42/43: May cause sensitization by inhalation and skin contact; 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; 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: Irritating to eyes and respiratory system; R36/38: Irritating to eyes and skin; S36/37/39: Wear suitable protective clothing, gloves, and eye/face protection; S46: If swallowed, seek medical advice immediately and show container or label.

Introduction

QIAsymphony Certal Kits are intended to be used only in combination with the QIAsymphony SP. QIAsymphony Certal Kits provide reagents for fully automated and simultaneous purification of nucleic acids from samples generated during production and purification of cell-culture based biotechnology-derived biologics, biopharmaceutical protein drugs (e.g., monoclonal antibodies), or from virus-propagating cell lines for vaccine production. Samples include cell-culture supernatants or fermentation broths after removal of cells, bioprocess purification buffers from chromatography purification procedures, or fractions from vaccine purification (e.g., after inactivation).

When working with biopharmaceuticals, it is essential to evaluate the final material to ensure that it is free of contaminating substances, such as residual DNA from the host cell. Quantitative data is needed to ensure a safe, high quality product. QIAGEN's integrated system for molecular testing — the QIAsymphony RGQ — delivers optimized and ready-to-use solutions for automated purification and quantification of residual host cell DNA and viral nucleic acids. This includes sample preparation on the QIAsymphony SP using QIAsymphony Certal Kits, assay setup using Certal Detection Kits, and detection on the Rotor-Gene® Q PCR cycler. This workflow supports the generation of sensitive and reliable results, regardless of whether the starting material is derived from bioprocess purification buffer, cell culture supernatant samples, or vaccine preparations.

The QIAsymphony Certal Residual DNA Kit is intended for the extraction of residual host cell DNA and viral nucleic acids from biologics (e.g., monoclonal antibodies) purified using chromatography procedures. A proprietary buffer (buffer CA) is used to equilibrate matrix effects caused by chromatography reagents, thereby improving the recovery of the nucleic acids from bioprocess samples.

The QIAsymphony Certal Vaccine NA Kit is intended for the extraction of viral nucleic acids from cell-based vaccine production. A proprietary buffer (buffer CB) is used to equilibrate for matrix effects caused by chemical or physical treatment during inactivation and purification of the propagated virus from vaccine production.

No pre-conditioning with either buffer CA or buffer CB is necessary for the extraction of host-cell DNA or viral nucleic acids from cell-culture supernatant or fermentation broth.

Examples of purification buffers used in chromatography procedures are:

- Phosphate-based buffers with high salt concentration (e.g., up to 1 M NaCl)
- Acidic glycine or citrate buffers with low pH (e.g., pH 3)
- Tris-based buffers with different pH and salt concentrations

Proven, performance-leading magnetic-particle technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. The purified nucleic acids are ready for direct use in downstream applications, such as amplification or other detection reactions (e.g., Threshold assay). The QIAsymphony SP performs all steps of the purification procedure. Up to 96 samples, in batches of up to 24, are processed in a single run.*

For downstream detection, the QIAsymphony Certal Kits are compatible with the Certal CHO Detection Kit and the Certal Vero Detection Kit, enabling highly sensitive detection of residual genomic DNA from CHO and Vero cell lines using quantitative PCR. We recommend the Rotor-Gene Q for high-precision results; for details, visit www.qiagen.com/goto/Rotor-GeneQ.

Principle and procedure

QIAsymphony technology combines the speed and efficiency of silica-based nucleic acid 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. The user can choose between different elution volumes (60 µl, 85 µl, and 110 µl), depending on the protocol.

* Number of preps depends on the sample volume and protocol used. Up to 96 samples can be prepared with sample volumes of 500 µl using the QIAsymphony Certal Residual DNA Kit (resDNA1000 protocol) or the QIAsymphony Certal Vaccine NA Kit (vacNA1000 protocol).

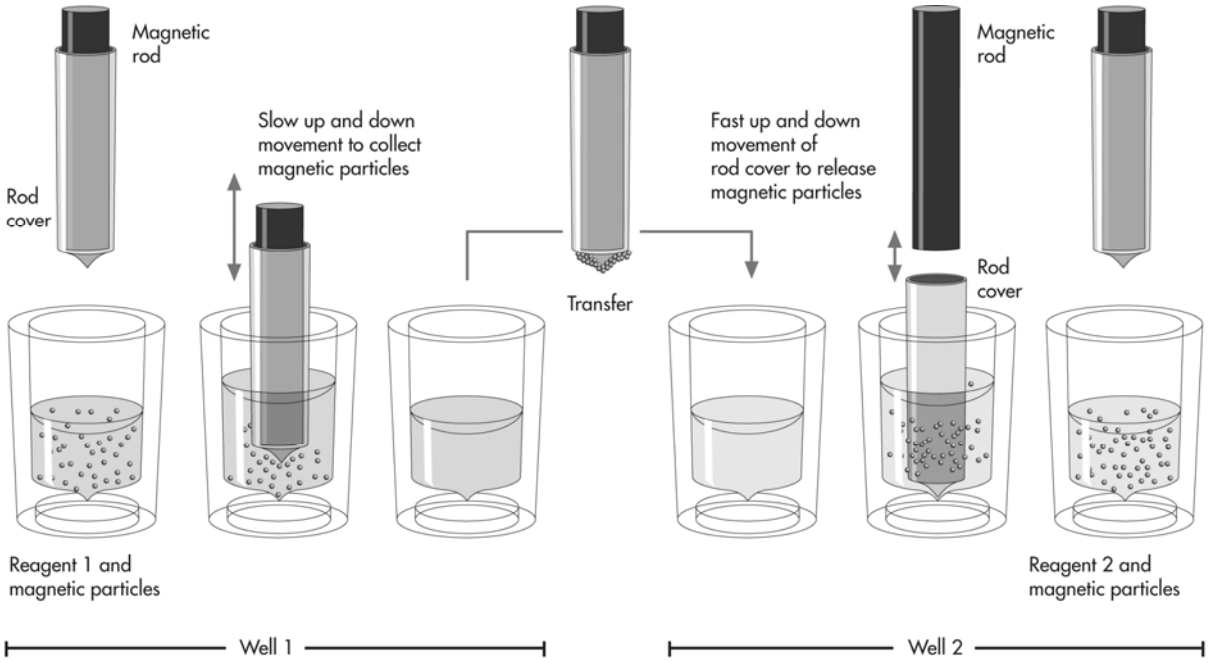
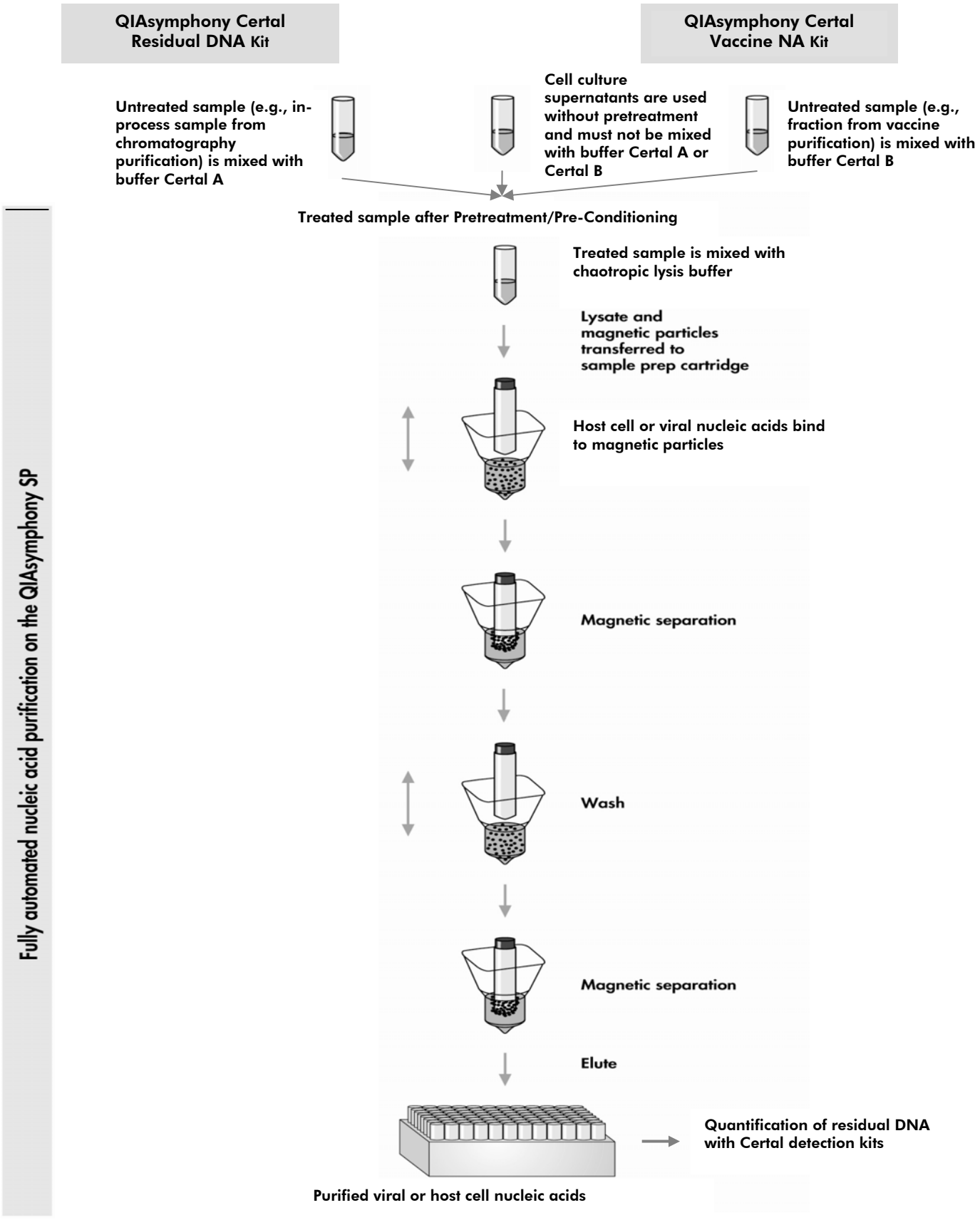


Figure 1. Schematic of the QIAsymphony SP principle. The QIAsymphony SP processes a sample containing magnetic particles as follows: A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another well 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 Certal Residual DNA Kit
QIAsymphony Certal Vaccine NA Kit
Untreated sample (e.g., in-process sample from chromatography purification) is mixed with buffer Certal A
Cell culture supernatants are used without pretreatment and must not be mixed with buffer Certal A or Certal B
Untreated sample (e.g., fraction from vaccine purification) is mixed with buffer Certal B
Treated sample after Pretreatment/Pre-Conditioning
Treated sample is mixed with chaotropic lysis buffer
Lysate and magnetic particles transferred to sample prep cartridge
Host cell or viral nucleic acids bind to magnetic particles
Magnetic separation
Wash
Magnetic separation
Elute
Quantification of residual DNA with Certal detection kits
Purified viral or host cell nucleic acids
Fully automated nucleic acid purification on the QIAsymphony SP

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.

- 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, www.sarstedt.com). Compatible primary and secondary tube and plate formats are listed at www.qiagen.com/goto/certal
- Elution tubes or plates: Compatible elution tube and plate formats are listed at www.qiagen.com/goto/certal
- Vortexer
- Centrifuge

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)

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 nucleic acids from the "Eluate" drawer after processing. Refer to the *QIAsymphony SP/AS User Manuals* for operating instructions.

Loading reagent cartridges into the "Reagents and Consumables" drawer

Reagents for purification of nucleic acids 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.

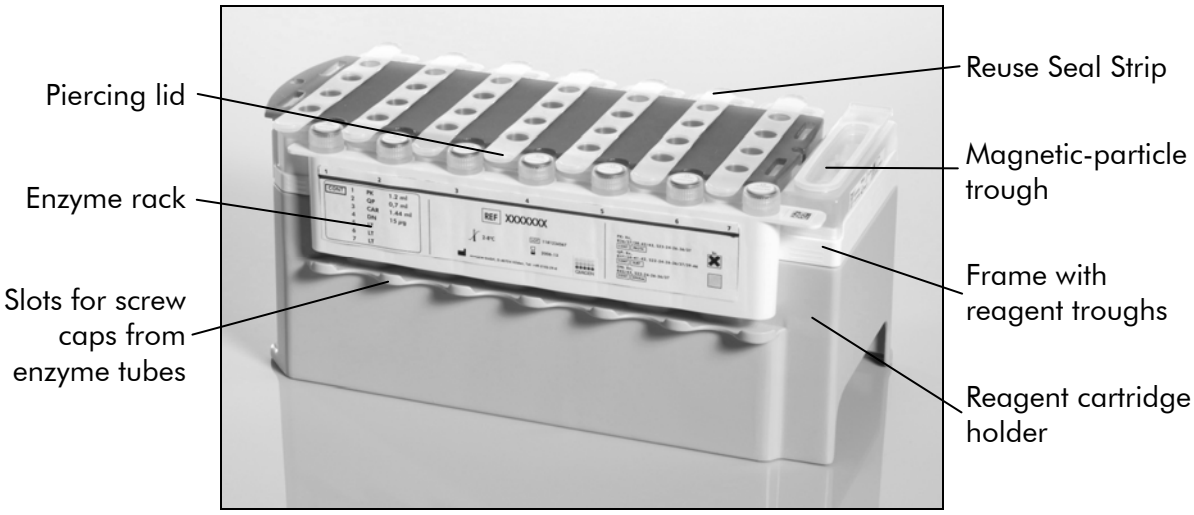


Figure 2. QIAsymphony reagent cartridge. The reagent cartridge contains all reagents required for the protocol run.

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.

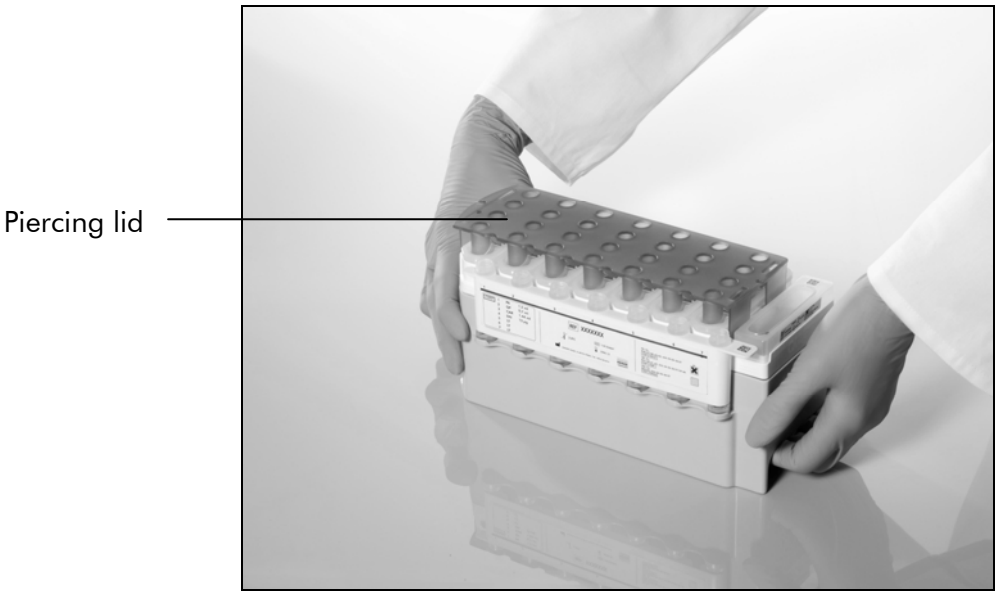


Figure 3. Easy worktable setup with reagent cartridges.

Partially used reagent cartridges can be stored until needed again (see "Storage", page 6).

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 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 manually 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/certal. Click on the User Support tab. For plasticware ordering information, see page 29.

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/AS User Manuals*.

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.

Preparation of sample material

QIAsymphony Certal Kits are suitable for use with samples that contain bioprocess purification buffers used in chromatography purification procedures (ion exchange chromatography or Protein A/G affinity chromatography), cell-culture supernatants or fermentation broth after removal of cells, or fractions from vaccine purification (e.g., after inactivation). Depending on the starting material, sample pre-conditioning is required and samples have to be mixed with buffer CA or buffer CB in a ratio of 1:1. The amount of starting material that can be used is limited by the binding capacity of the magnetic particles and by the amount of contaminants in the sample that may interfere with the procedure.

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/certal. Click on the User Support tab.

Preparing carrier RNA–Buffer AVE mixtures

The provided carrier RNA has to be used with these kits. If carrier RNA is not added, recovery of nucleic acids may be significantly reduced.

Carrier RNA serves two purposes during the purification procedure. First, it enhances binding of nucleic acids to the silica surface of the magnetic particles. Second, the addition of large amounts of carrier RNA reduces the chances of RNA degradation in the rare event that RNases are not denatured by the chaotropic salts and detergent in the lysis buffer.

To prepare a carrier RNA stock solution, add 1350 µl Buffer AVE (provided in 2 ml vials) to the tube containing 1350 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store at –20°C. Do not freeze and thaw the aliquots more than 2 times.

Calculating the amount of carrier RNA

Purification protocols are optimized for different concentrations of carrier RNA. The relevant protocol sheet provides information on preparation of carrier RNA–Buffer AVE mixtures.

Calculating the volume of carrier RNA mixture per tube

The minimum volume of carrier RNA–Buffer AVE mixture must include sufficient additional volume to take into account liquid loss due to pipetting and evaporation. Compatible tube formats including minimum volume of carrier RNA–Buffer AVE mixtures are listed at www.qiagen.com/goto/certal.

Tubes containing carrier RNA–Buffer AVE mixtures are placed in a tube carrier. The tube carrier containing the carrier RNA–Buffer AVE mixture(s) must be placed in slot A of the sample drawer. Up to 8 tubes of the mixture can be used per batch and up to 24 tubes can be used per run of 4 batches.

If less carrier RNA has been shown to be better for your amplification system, adjust the volume of carrier RNA accordingly. The use of a different concentration of carrier RNA must be validated for each particular sample type and downstream assay.

If no carrier RNA is used, the mixture loaded into slot A must contain Buffer AVE only (120 µl Buffer AVE per sample).

Using an internal control

Using internal controls to monitor the efficiency of sample preparation with the QIAsymphony Certal Kits requires the controls to be added with the carrier RNA–Buffer AVE mixture. The total volume of the internal control–carrier RNA mixture remains 120 µl.

The amount of internal control added depends on the assay system and the elution volume chosen within the QIAsymphony SP protocol. Calculation and validation must be performed by the user. Refer to the manufacturer's instructions for the downstream assay to determine the optimal concentration of internal control. Using a concentration other than that recommended may lead to incorrect results, especially if the internal control is used for calculation of titers.

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

When calculating the amount of internal control 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 amounts of liquid are lost during transfer and contact with the magnetic particles, the initial volume of elution solution must be larger than the selected volume to ensure that the final eluate is of the correct volume.

The relevant protocol sheet provides information for calculating the volume of internal control mixture according to the type of tube used.

Assay Control Sets

Assay Control Sets are used for the resDNA1000 and vacNA1000 protocols, even when no internal controls are used. A default Assay Control Set is preinstalled for each protocol. When an internal control is used, it may be necessary to create an additional Assay Control Set as described in the *QIAsymphony Management Console User Manual*.

Note: When using the default Assay Control Sets designed for working without internal control, the use of carrier RNA is still required.

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and only minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure.

Yields of nucleic acids

Eluates prepared with carrier RNA may contain much more carrier RNA than target nucleic acids. We recommend using quantitative amplification methods to determine yields. For sensitive quantification of either CHO or Vero genomic DNA, the Certal CHO Detection Kit or Certal Vero Detection Kit can be used. For alternative host cell lines, any suitable quantitative PCR assay can be used.

An appropriate assay (e.g., real-time PCR) is required for quantitation, and to determine the recovery rate of extracted nucleic acids from a sample with unknown concentration. To determine the nucleic acid content of a sample, set up a standard curve using a defined template. A suitable standard curve would be a range of defined concentrations (e.g., five 10-fold dilutions) of genomic DNA purified from the cultured production cell line [CHO etc.] with QIAamp® DNA Mini Kit (cat. no. 51304).

For the best comparison of the unknown sample with the standards, we recommend using the same volume for the standard curve as the eluate fraction in the assay setup.

Note: The initial elution volume (e.g., 95 µl, 120 µl, or 145 µl) must be used for calculation of the total nucleic acid content in an unknown sample.

The following equation can be used to convert the value determined using the standard curve into the total nucleic acid amount of an unknown sample:

$$\textit{Total amount NA (pg)} = \frac{\textit{Result (pg)}^{*} \times \textit{Elution volume (µl)}^{\dagger}}{\textit{Assay volume (µl)}^{\ddagger}}$$

* Amount of nucleic acid determined by the standard curve

† Initial elution volume (see relevant protocol sheet)

‡ Volume from the final elution fraction used in the assay

Storing nucleic acids

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

Protocol: General Purification Protocol

This protocol is a general protocol for using QIAsymphony Certal Kits. Detailed information for each protocol, including volumes and tubes, is provided in protocol sheets that can be downloaded at www.qiagen.com/goto/certal. Click on the User Support tab.

Important points before starting

- Ensure that you are familiar with operating the QIAsymphony SP. Refer to the *QIAsymphony SP/AS User Manuals* for operating instructions
- Before beginning the procedure, read "Important Notes" starting on page 15.
- Ensure you are familiar with the protocol sheet corresponding to the procedure you want to use (available from User Support at www.qiagen.com/goto/certal).
- Before using a reagent cartridge for the first time, check that Buffers QSL2 and QSB1 do not contain a precipitate. If necessary, remove the troughs containing Buffers QSL2 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

- Prepare a mixture containing carrier RNA and sample preparation internal controls (optional) as described in the relevant protocol sheet.
- 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 starting the procedure, be sure that the enzyme rack containing proteinase K has been equilibrated to room temperature (15–25°C).

- 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 treated samples are bar coded, orient treated samples in the tube carrier so that the bar codes face the bar code reader at the left side of the QIAsymphony SP.
- When using secondary tube formats we recommend 2 ml Sarstedt tubes (cat. nos. 72.693 or 72.608). For information about sample tubes compatible with a certain protocol, see the corresponding protocol sheet (available from User Support at www.qiagen.com/goto/certal).
- For information about minimum sample volumes for samples in primary and secondary tubes for a certain protocol, see the corresponding protocol sheet (available from User Support at www.qiagen.com/goto/certal). This information also indicates which tubes can be used for the different protocols.

Procedure

Untreated sample pre-conditioning

1. **Pipet 500 µl of the untreated sample into a 1.5 ml tube (not provided).**

   Note: Cell culture supernatants or fermentation broth samples do not require pretreatment. Add 1000 µl of sample to a 2 ml Sarstedt tube (not provided) and place the sample into the tube carrier.
2. **Add 500 µl buffer CA or buffer CB to 500 µl of the untreated sample in a ratio of 1:1. Mix by vortexing vigorously for 10 s.**
3. **Centrifuge the tube in a microcentrifuge at full speed for 1 min and transfer the samples to a 2 ml Sarstedt tube (not provided).**
4. **Place the treated sample into the tube carrier.**

Nucleic acid purification

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 onto 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 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. **Place tube(s) containing the internal control–carrier RNA–Buffer AVE mixture into the tube carrier and load it into slot A of the "Sample" drawer.**

   For more information about preparing the mixture, see the relevant protocol sheet and "Using an internal control", starting on page 19.
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

   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 "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.**

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

    For sensitive quantification of CHO or Vero residual DNA in purified nucleic acid, we recommend using the Certal CHO Detection Kit or the Certal Vero Detection Kit.

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.

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.

12. **If a reagent cartridge is only partially used, seal it with the provided Reuse Seal Strips and close tubes containing magnetic particles 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 6.

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

    See page 8 for safety information.

14. **Clean the QIAsymphony SP.**

    Follow the maintenance instructions in the *QIAsymphony SP/AS User Manuals*.

15. **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 *QIAsymphony SP/AS User Manuals*.
Precipitate in reagent bottles	
Precipitate in buffer	Storage of buffer CB may lead to formation of precipitates. If necessary incubate for 30 min at 37°C with occasional shaking to dissolve precipitate.
Precipitate in reagent trough of opened cartridge	
a) Buffer evaporation	Excessive evaporation may lead to increased salt concentration or decreased alcohol concentrations 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 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 QSL2 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 troughs in the correct positions. If the reagent cartridge is already pierced, make sure that the troughs are reclosed with Reuse Seal Strips and incubate the complete reagent cartridge for 30 min at 37°C with occasional shaking in a water bath.
Low yield of nucleic acids	

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 samples were not mixed properly after thawing	Thaw frozen samples quickly in a 37°C water bath with mild agitation to ensure thorough mixing.
c)	Carrier RNA not added	Reconstitute carrier RNA in Buffer AVE and mix with appropriate volume of Buffer AVE as described in "Using carrier RNA and internal controls", starting on page 18. Repeat the purification procedure with new samples.
d)	Degraded carrier RNA	Carrier RNA reconstituted in Buffer AVE was stored at temperatures above –20°C. Prepare a new tube of carrier RNA dissolved in Buffer AVE (and internal control) and store appropriately. Repeat the purification procedure with new samples.
e)	Degraded nucleic acids	1) Samples were stored incorrectly or subjected to too many freeze–thaw cycles. Repeat the purification procedure with new samples. 2) Samples were treated with agents leading to/inducing strand breaks, size reduction, or cross-links of nucleic acids. Such treated samples can lead to negative results when evaluated by polymerase chain reaction. Repeat the purification procedure with new samples.
f)	Incomplete sample lysis	Before use, check that Buffer QSL2 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 troughs are reclosed with Reuse Seal Strips, and incubate the complete reagent cartridge for 30 min at 37°C with occasional shaking in a water bath.

g)	Clogging of pipet tip due to insoluble material	Insoluble material, such as cell debris, was not removed from the sample prior to starting the QIAsymphony purification procedure. To remove insoluble material for bioprocess sample or vaccine applications, centrifuge the sample at 12,000 x *g* for 2 min, and transfer the supernatant to a new 2 ml Sarstedt sample tube (Sarstedt, cat. nos. 72.693 or 72.608).

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 Certal Residual DNA Kit	Includes 2 reagent cartridges and enzyme racks and accessories	931855
QIAsymphony Certal Vaccine NA Kit	Includes 2 reagent cartridges and enzyme racks and accessories	931955
Related products		
Certal CHO Detection Kit (100)	Reagents and controls for 100 x 25 µl reactions: 1.25ml Certal Residual DNA PCR Master Mix, 100µl Uracil-N-Glycosylase (1U/µl), Internal Control DNA and Assay, CHO DNA Control and CHO-Assay, 210 µl High ROX dye solution, 210 µl ROX dye solution, 3ml Nucleic Acid Dilution Buffer, 1.9ml TE Buffer	211822
Certal Vero Detection Kit (100)	Reagents and controls for 100 x 25 µl reactions: 1.25ml Certal Residual DNA PCR Master Mix, 100µl Uracil-N-Glycosylase (1U/µl), Internal Control DNA and Assay, Vero DNA Control and Vero-Assay, 210 µl High ROX dye solution, 210 µl ROX dye solution, 3ml Nucleic Acid Dilution Buffer, 1.9ml TE Buffer	211842
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
Reagent Cartridge Holder (2)	Reagent cartridge holder for use with the QIAsymphony SP	997008
Accessory Trough (10)	Accessory troughs for use with the QIAsymphony SP	997012
Tip Disposal Bags (15)	Tip disposal bags for use with the QIAsymphony SP	9013395

Product	Contents	Cat. no.
Sample Carrier, plate, Qsym	Plate carrier for sample input for use with the QIAsymphony SP	9017660
Cooling Adapter, MTP, RB, Qsym	Cooling adapter for round-bottom microplates (MTP). For use in the QIAsymphony "Eluate" drawer	9018085
Cooling Adapter, EMT, Qsym	Cooling adapter for EMT racks. For use in the QIAsymphony "Eluate" drawer	9018086
Cooling Adapter, PCR, Qsym	Cooling adapter for PCR plates. For use in the QIAsymphony "Eluate" drawer	9018087
Cooling Adapter, tubes, 2 ml, Qsym	Cooling adapter for 2 ml screw-cap tubes. For use in the QIAsymphony "Eluate" drawer	9018088
Tube Insert, 2 ml, sample carrier, Qsym	Secondary tube adapter (for 2 ml screw-cap tubes) for use with the QIAsymphony tube carrier	9241032
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
Adapter, tubes, 2 ml, Qsym	Adapter for 2 ml screw-cap tubes. For use in the QIAsymphony "Eluate" drawer	9018577
Filter-Tips, 200 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube and the QIAsymphony SP	990332
Filter-Tips, 1500 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAsymphony SP	997024
Reuse Seal Set (20)	Reuse seal sets for sealing partly used QIAsymphony reagent cartridges	997006
Elution Microtubes CL (24 x 96)	Nonsterile 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

Product	Contents	Cat. no.
QIAsymphony SP	QIAsymphony sample prep module, 1-year warranty on parts and labor	9001297

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