June 2012

BioSprint® 96 One-For-All Vet Handbook

For purification of viral nucleic acids and bacterial DNA from animal

- whole blood
- serum
- plasma
- other body fluids
- tissue homogenates
- swabs and washes

using the BioSprint 96 workstation

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.

Contents

Kit Contents 4

Storage 4

Intended Use 5

Safety Information 5

Quality Control 6

Introduction 7

Principle and procedure 7

Description of BioSprint 96 protocols 10

Equipment and Reagents to Be Supplied by User 11

Important Notes 12

Starting material 12

Animal whole blood 13

Animal tissues 13

Animal serum, plasma, other body fluids, swabs, and washes 14

Yields of nucleic acids 15

Storing nucleic acids 15

Using carrier RNA and internal controls 15

Preparing reagents 16

Handling RNA 17

Protocols

■ **Purification of Viral Nucleic Acids and Bacterial DNA from Animal Whole Blood** 18

■ **Purification of Viral Nucleic Acids and Bacterial DNA from Animal Tissue Homogenates, Serum, Plasma, Other Body Fluids, Swabs, and Washes** 22

Troubleshooting Guide 26

References 29

Ordering Information 30

Kit Contents

BioSprint 96 One-For-All Vet Kit	**(384)**
Catalog no.	**947057**
Number of preps	**384**
Buffer AL*	3 x 33 ml
Buffer RLT*	4 x 45 ml
QIAGEN® Proteinase K	3 x 6 ml
Carrier RNA (poly A)	4 x 310 µg
MagAttract® Suspension G†	13 ml
Buffer AW1*‡ (concentrate)	2 x 121 ml
Buffer RPE (concentrate)‡	2 x 65 ml
Buffer AVE†	125 ml
Large 96-Rod Cover	4
96-Well Microplate MP	8
S-Block	16
Reagent Container, 100 ml	4
Quick-Start Guide	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 5 for safety information.

† Contains sodium azide as a preservative.

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

Storage

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

Lyophilized carrier RNA is stable for up to 1 year when stored at room temperature. Carrier RNA should only be dissolved in Buffer AVE or in an internal control solution (if used and dissolved in an appropriate buffer). Dissolved carrier RNA should be immediately frozen at –20°C or added to the Buffer AL or Buffer RLT mixture as described in the sample preparation protocols starting on page 18. These solutions should be prepared fresh, and are stable at room temperature for up to 48 hours. Unused carrier RNA dissolved in Buffer AVE should be frozen in aliquots at –20°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

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

Intended Use

The BioSprint 96 One-For-All Vet Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

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

Buffer AL and Buffer AW1 contain guanidine hydrochloride and Buffer RLT contains guanidine thiocyanate, 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. If liquid containing potentially infectious agents is spilt on the BioSprint 96 workstation, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite, followed by water.

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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of BioSprint 96 One-For-All Vet Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The procedures described in this handbook are for rapid purification of viral RNA and DNA as well as bacterial DNA from a broad range of veterinary samples such as whole blood, serum, plasma, tissues, swabs, washes, and other sample types using the BioSprint 96 workstation (see "Starting material" on page 12). However, performance is not guaranteed for every combination of sample type and pathogen species and must be validated by the user.

BioSprint 96 technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. The purified nucleic acids are ready for use in downstream applications, such as amplification or other enzymatic reactions.

MagMAX™ Express-96 Magnetic Particle Processor (Invitrogen) and KingFisher® 96 (Thermo Fischer Scientific, Inc.) users can also use the BioSprint 96 One-For-All Vet Kit on these instruments by simply following the protocols on pages 18 and 22. The appropriate KingFisher software protocol is available from QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Principle and procedure

The BioSprint 96 One-For-All Vet Kit uses MagAttract magnetic-particle technology for nucleic acid purification. This technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles (Figure 1, page 8).

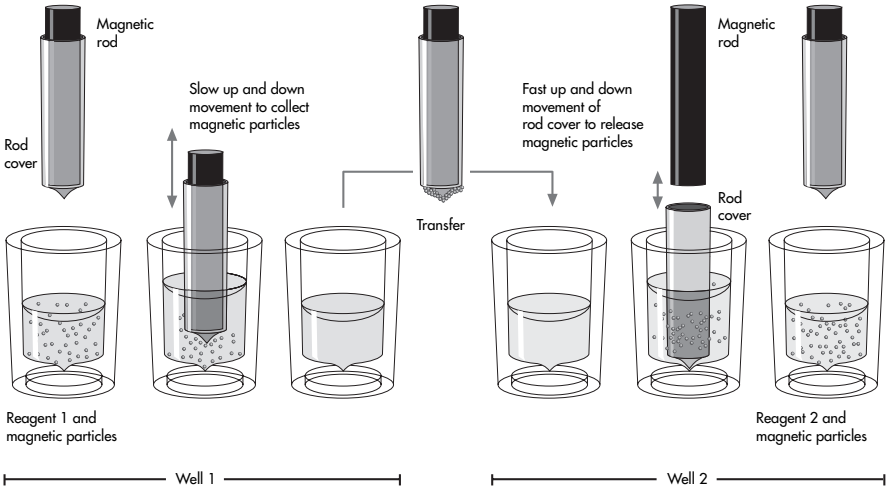
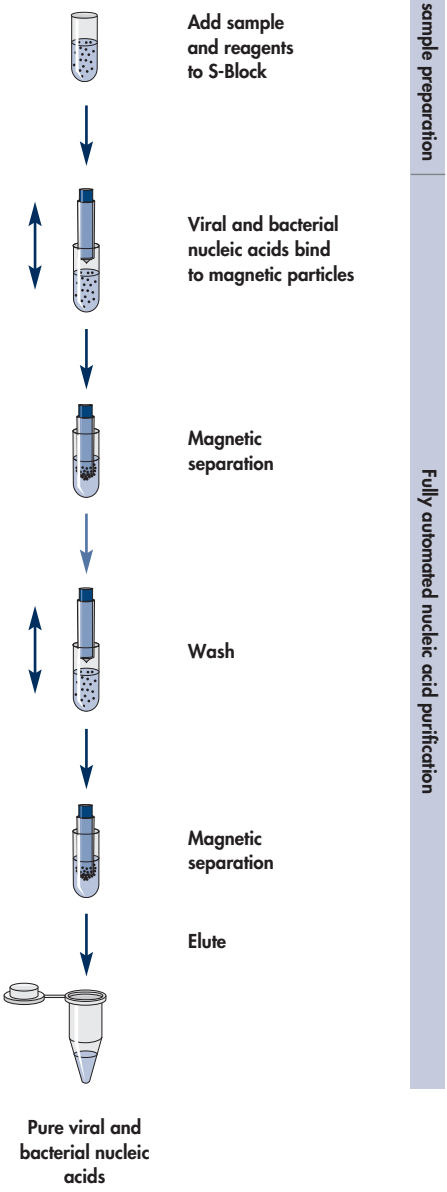


Figure 1. Schematic of the BioSprint 96 principle. The BioSprint 96 processes a sample containing magnetic particles as follows: A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another well and the magnetic particles are released. The BioSprint 96 uses a magnetic head containing an array of 96 magnetic rods, and can therefore process up to 96 samples simultaneously. Steps 1 and 2 are repeated several times during sample processing.

The purification procedure is designed to ensure convenient and reproducible handling of potentially infectious samples (see flowchart, page 9).

Depending on the starting material, samples can be lysed in a single step in the presence of chaotropic salts and proteinase K, releasing nucleic acids to bind to the silica surface of the MagAttract magnetic particles. DNA and RNA bound to the magnetic particles are then efficiently washed. Two different wash buffers are used, followed by an air drying step, which considerably improves the purity of the nucleic acids. High-quality nucleic acids are eluted in Buffer AVE. Nucleic acid yields depend on sample type and sample storage.

BioSprint 96 One-For-All Vet Procedure



Description of BioSprint 96 protocols

The following application protocols are preinstalled on the BioSprint 96 workstation and are to be used in combination with the BioSprint 96 One-For-All Vet Kit:

The **BS96 Vet Blood 200** protocol is optimized for efficient purification of viral nucleic acids and bacterial DNA from up to 200 µl animal whole blood. Purification time is approximately 24 minutes, not including upfront handling steps for prefilling S-Blocks and 96-well microplates. The lysis and binding solution used in the procedure is Buffer AL, which contains the chaotropic salt guanidine hydrochloride. The procedure includes 4 wash steps to efficiently remove proteins, nucleases, and other impurities found in whole blood samples.

The **BS96 Vet 100** protocol is optimized for efficient purification of viral nucleic acids and bacterial DNA from up to 100 µl samples of:

- Animal tissue homogenates
- Serum
- Plasma
- Other body fluids such as cerebrospinal fluid (CSF), synovial fluid, peritoneal fluid
- Media from nasal, pharyngeal, and cloacal swabs
- Washes such as bronchial alveolar lavage (BAL)

Depending on the sample type, a pretreatment may be necessary. Purification time is approximately 24 minutes, not including upfront handling steps for prefilling S-Blocks and 96-well microplates. The lysis and binding solution used in the procedure is Buffer RLT, which contains the chaotropic salt guanidine thiocyanate. The procedure includes 3 wash steps.

Equipment and Reagents to Be Supplied by User

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

- BioSprint 96 workstation (cat. no. 9000852)
- Magnetic head for use with Large 96-Rod Covers (supplied with the BioSprint 96)
- Pipettors and disposable pipet tips with aerosol barriers (20–1000 µl)
- Multichannel pipettor and disposable 1000 µl pipet tips with aerosol barriers (e.g., Finnpipette® Digital and Finntip® Filters from Thermo Electron, see www.thermo.com)*
- Multidispenser (e.g., Finnpipette Stepper from Thermo Electron see www.thermo.com)*
- Ethanol (96–100%)†
- Isopropanol
- Phosphate-buffered saline (PBS), may be required for diluting samples
- Optional for tissue samples: β-mercaptoethanol (β-ME) or dithiothreitol (DTT); see "Important Notes" (page 12)
- Vortexer
- Soft cloth or tissue and 70% ethanol or other disinfectant to clean the BioSprint 96 worktable

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

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

Important Notes

Starting material

The amounts of starting material for use with the BioSprint 96 One-For-All Vet Kit are listed in Table 1.

Table 1. Sample volumes used in BioSprint 96 One-For-All Vet protocols

Sample	Maximum amount of sample (µl)	BioSprint 96 protocol
Whole blood*	200	BS96 Vet Blood 200 (page 18)
Tissue homogenates from <10 mg tissue	100	BS96 Vet 100 (page 22)
Serum or plasma	100	BS96 Vet 100 (page 22)
Body fluids (e.g., aspirates such as CSF, synovial, and peritoneal)	100	BS96 Vet 100 (page 22)
Media from swabs (e.g., pharyngeal, tracheal, or cloacal)†	100	BS96 Vet 100 (page 22)
Washes (e.g., BAL)	100	BS96 Vet 100 (page 22)

* We recommend using 50–200 µl animal blood containing nonnucleated erythrocytes. If necessary, the volume of animal blood used can be reduced and the sample volume adjusted to 200 µl with PBS. For blood samples containing nucleated erythrocytes (e.g., samples from bird and fish), use less than 50 µl blood and adjust the sample volume to 200 µl with PBS.

† See "Animal serum, plasma, other body fluids, swabs, and washes" on page 14.

The protocols in this handbook are optimized for purification of viral and bacterial nucleic acids from easy-to-lyse sample types of low to moderate complexity. The BioSprint 96 One-For-All Vet protocol combines efficient lysis and binding in a single step, enabling quick, straight-forward sample processing. For sample types of higher complexity, such as tissue, and for certain difficult-to-lyse pathogens, such as Gram-positive bacteria, specialized disruption and/or lysis pretreatments may be necessary. The user should determine appropriate upfront pretreatments for such materials. General information about recommended sample types and pretreatments is given in the following sections. If you need further information, contact QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Animal whole blood

Blood samples treated with EDTA, citrate, or heparin as anticoagulant can be used for nucleic acid purification. Samples can be either fresh or frozen, provided that they have not been frozen and thawed more than once. After collection and centrifugation, whole blood samples can be stored at 2–8°C for up to 6 hours. For longer storage, we recommend freezing aliquots at –20°C or –80°C.

Frozen blood must not be thawed more than once before processing. Repeated freeze–thawing leads to denaturation and precipitation of proteins, resulting in a potential reduction in viral titers and, therefore, reduced yields of viral nucleic acids.

We recommend using 50–200 µl blood containing nonnucleated erythrocytes. If necessary, the volume of blood used can be reduced and the sample volume adjusted to 200 µl with PBS. For blood samples containing nucleated erythrocytes (e.g., samples from bird and fish), use less than 50 µl blood and adjust the sample volume to 200 µl with PBS.

Up to 200 µl whole blood can be processed with the "BS96 Vet Blood 200" protocol (see "Purification of Viral Nucleic Acids and Bacterial DNA from Animal Whole Blood" on page 18).

Animal tissues

Prior to processing on the BioSprint 96, it is necessary to disrupt and homogenize animal tissue samples. Disruption and homogenization can be carried out using a rotor–stator homogenizer, such as the QIAGEN TissueRuptor (cat. no. 9001271), or a bead mill, such as the QIAGEN TissueLyser II (cat. no. 85300). The TissueLyser LT (cat. no. 85600) can be used to disrupt up to 12 samples in parallel. A supplementary protocol for simultaneous disruption of up to 96 tissue samples using the TissueLyser can be obtained by contacting QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Samples can be submerged in any aqueous solution (e.g., PBS) for sample disruption and homogenization. Be sure the volume is sufficient to fully cover the samples.

Note: For optimal results, we recommend disrupting and homogenizing samples in Buffer RLT (see ordering information, page 30).

After disruption and homogenization, we strongly recommend centrifuging tissue homogenates, especially those from fibrous tissues, for 2 minutes at maximum speed (e.g., 12,000 x *g* in a bench-top microcentrifuge) to ensure any residual solid pieces are removed.

Note: Solid pieces remaining in the homogenate may aggregate with the MagAttract magnetic particles, which could decrease nucleic acid yield.

Up to 100 µl supernatant from the homogenized tissue sample can be used with the "BS96 Vet 100" protocol (see "Purification of Viral Nucleic Acids and Bacterial DNA from Animal Tissue Homogenates, Serum, Plasma, Other Body Fluids, Swabs, and Washes" on page 22).

Addition of β-mercaptoethanol (β-ME) to Buffer RLT may increase the efficiency of lysis of tissue samples by disturbing disulfide bridges in the tissue matrix. Prepare 10 µl β-ME per 1 ml Buffer RLT in a fume hood and wear appropriate protective clothing. The mixture can be stored at room temperature (15–25°C) for up to 1 month.

Alternatively, dithiothreitol (DTT) can be used for lysis. Prepare a 1 M DTT stock solution in water and use immediately or freeze in single-use aliquots. Add 40 µl 1 M DTT per 1 ml Buffer RLT. The mixture can be stored at room temperature for up to 1 month.

Optional protocol for large-volume tissue homogenates

If large amounts of tissue (up to 25 mg) are required, tissue can be disrupted and homogenized in at least 400 µl Buffer RLT. A larger volume of buffer may be used if necessary for efficient lysis. Use 400 µl of the homogenate for purification with the "BS96 Vet 100" protocol.

For these samples only: Do not add Buffer RLT when preparing the Buffer RLT mixture (see Table 4 on page 23), as it has already been added to the lysate. The other components of the mixture (isopropanol, MagAttract Suspension G, and carrier RNA) are added as described in the protocol.

Buffer RLT must be purchased separately (see ordering information, page 30) to ensure sufficient volumes when processing large-volume tissue samples. More information concerning pretreatment of tissue is available from QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Animal serum, plasma, other body fluids, swabs, and washes

Frozen plasma or serum must not be thawed more than once before processing. Repeated freeze–thawing leads to denaturation and precipitation of proteins, resulting in a potential reduction in viral titers and, therefore, reduced yields of viral nucleic acids.

We recommend storing swabs in transport media; for example, viral transport media (VTM) or brain–heart infusion broth (BHI). Remove the swab and squeeze out the liquid by pressing the swab against the inside of the storage tube. We recommend centrifuging the swab media briefly to ensure any residual solid materials are removed.

Note: Solid pieces remaining in the homogenate may aggregate with the MagAttract magnetic particles, which could decrease nucleic acid yield.

Up to 100 µl serum, plasma, other body fluid, swab media supernatant, or wash fluid can be processed with the "BS96 Vet 100" protocol (see "Purification of Viral Nucleic Acids and Bacterial DNA from Animal Tissue Homogenates, Serum, Plasma, Other Body Fluids, Swabs, and Washes" on page 22).

Yields of nucleic acids

Depending on the sample type, the yields of viral and bacterial nucleic acids obtained are normally below 1 µg and therefore difficult to quantify using a spectrophotometer. In addition, eluates prepared with carrier RNA may contain much more carrier RNA than target nucleic acids. Furthermore, these protocols recover total nucleic acids. Therefore, cellular DNA and RNA will be copurified from any cells in the sample along with viral nucleic acids and bacterial DNA. We recommend using quantitative amplification methods to determine yields.

Storing nucleic acids

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

Using carrier RNA and internal controls

Carrier RNA and internal controls may be used with the BioSprint 96 One-For-All Vet Kit. We recommend adding carrier RNA (see below). Use of an internal control is optional (see below), depending on the amplification system used. If both are required, the carrier RNA and internal control are added to the Buffer AL or Buffer RLT mixture (see protocols starting on page 18). The Buffer AL and Buffer RLT mixtures should be prepared fresh, and are stable at room temperature (15–25°C) for up to 48 hours.

Adding carrier RNA

We recommend using carrier RNA as it enhances binding of viral nucleic acids and bacterial DNA to the silica surface of the magnetic particles, which is especially important when the target molecules are low abundance. In addition, an excess of carrier RNA reduces the chances of viral RNA degradation in the rare event that RNases are not denatured by the chaotropic salts and detergents in the lysis buffers. Not using carrier RNA may decrease the recovery of viral nucleic acids.

Carrier RNA is added to the Buffer AL or Buffer RLT mixture (see protocols starting on page 18) so that approximately 2.7 µg carrier RNA is present in a sample. To obtain the highest levels of sensitivity in amplification reactions, it may be necessary to adjust the amount of carrier RNA solution added.

Adding an internal control

Using the BioSprint 96 One-For-All Vet Kit in combination with amplification systems that use an internal control may require introduction of these internal controls during the purification procedure to monitor the efficiency of sample preparation and downstream assay.

The amount of internal control added depends on the assay system and the elution volume chosen in the BioSprint 96 protocol. Calculation and validation must be performed by the user. Refer to the manufacturer's instructions to determine the optimal concentration of internal control. Using a concentration other than that recommended may reduce amplification efficiency, resulting in incorrect results if the internal control is used for calculation of titers.

Internal control is added to the Buffer AL or Buffer RLT mixture (see Table 2 on page 19 and Table 4 on page 23).

Preparing reagents

Carrier RNA stock solution

Add 310 µl Buffer AVE to the tube containing 310 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl. Dissolved carrier RNA should be immediately added to the Buffer AL or Buffer RLT mixture (see protocols starting on page 18). This solution should be prepared fresh, and is stable at room temperature (15–25°C) for up to 48 hours. Unused carrier RNA dissolved in Buffer AVE should be frozen in aliquots at –20°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

Note: Carrier RNA does not dissolve in Buffer AL or Buffer RLT and must first be dissolved in Buffer AVE before addition to the Buffer AL or Buffer RLT mixture.

Proteinase K

The BioSprint 96 One-For-All Vet Kit contains ready-to-use proteinase K supplied in a specially formulated storage buffer. The activity of the proteinase K solution is 600 mAU/ml (40 mAU/mg protein) and has been chosen to provide optimal results.

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

Buffer AL

Mix Buffer AL thoroughly by shaking before use. Buffer AL is stable for up to 1 year when stored at room temperature (15–25°C).

Note: Do not add carrier RNA directly to Buffer AL.

Buffer RLT

Mix Buffer RLT thoroughly by shaking before use. Buffer RLT is stable for up to 1 year when stored at room temperature (15–25°C).

Note: Do not add carrier RNA directly to Buffer RLT.

MagAttract Suspension G

To ensure that the magnetic silica particles are fully resuspended, MagAttract Suspension G must be shaken and vortexed before use. Before the first use, shake the bottle, and vortex for 3 minutes. Before subsequent uses, shake the bottle and vortex for 1 minute.

Buffer AW1

Buffer AW1 is supplied as a concentrate. Add 160 ml ethanol (96–100%) to the bottle containing 121 ml Buffer AW1 concentrate. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW1 can be stored at room temperature (15–25°C) for up to 1 year.

Note: Always mix reconstituted Buffer AW1 before use by shaking the bottle 5 times.

Buffer RPE

Buffer RPE is supplied as a concentrate. Add 260 ml ethanol (96–100%) to the bottle containing 65 ml Buffer RPE concentrate. Tick the check box on the bottle to indicate that ethanol has been added. Store reconstituted Buffer RPE at room temperature (15–25°C).

Note: Always mix reconstituted Buffer RPE before use by shaking the bottle 5 times.

Handling Buffer AVE

Buffer AVE is RNase-free upon delivery. It contains sodium azide, an antimicrobial agent that prevents growth of RNase-producing organisms. However, as this buffer does not contain any RNase-degrading chemicals, it will not actively inhibit RNases introduced by inappropriate handling. When handling Buffer AVE, take extreme care to avoid contamination with RNases.

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.

Protocol: Purification of Viral Nucleic Acids and Bacterial DNA from Animal Whole Blood

This protocol is for purification of viral nucleic acids and bacterial DNA from animal whole blood using the BioSprint 96 workstation and the BioSprint 96 One-For-All Vet Kit with the "BS96 Vet Blood 200" protocol. Sample volumes can be up to 200 µl.

Important points before starting

- Ensure that you are familiar with correct operation of the BioSprint 96. Refer to the *BioSprint 96 User Manual* for operating instructions.
- Before beginning the procedure, read "Important Notes" (page 12).
- Check that Buffer AW1, Buffer RPE, and carrier RNA have been prepared according to the instructions in "Preparing reagents" (page 16).
- Check that Buffer AL does not contain a white precipitate. If necessary, incubate Buffer AL for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
- The 96-rod covers are supplied either as packets of 2, or as packets of 1 inserted into an S-Block. If using a new packet of 2, store the second 96-rod cover on another S-Block or plate. Care should be taken to not bend the 96-rod covers.

Things to do before starting

- Thaw and equilibrate up to 96 whole blood samples at room temperature (15–25°C).
- If the volume of blood sample is less than 200 µl, add PBS to a final volume of 200 µl.
- Prepare the Buffer AL mixture according to Table 2 on the next page for use in step 3 of the procedure. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time, and for 1 minute before subsequent use.

  Note: Prepare a volume of the Buffer AL mixture that is 20% greater than that required for the total number of sample purifications to be performed; 400 µl mixture is required per sample (see step 3 of the procedure).

  For a full run of 96 samples, we recommend preparing the mixture directly in the bottle of Buffer AL provided with the kit. Add the appropriate amounts of isopropanol, MagAttract Suspension G, and redissolved carrier RNA as indicated to the bottle containing 23 ml of Buffer AL. For runs with fewer samples, see Table 2 on the next page for guidance.

Table 2. Preparation of Buffer AL mixture

	Number of samples*		
Reagent	**1**	**48**	**96†**
Buffer AL	200 µl	11.5 ml	23 ml
Isopropanol	200 µl	11.5 ml	23 ml
MagAttract Suspension G	25 µl	1.5 ml	3 ml
Carrier RNA (1 µg/µl)	Approximately 2.7 µl	155 µl	310 µl

* The volume prepared is 120% of the required volume to compensate for pipetting error and possible evaporation.

† When preparing a mixture for 96 samples, we recommend mixing the reagents directly in the bottle of Buffer AL provided with the kit.

Procedure

1. **Pipet 40 µl proteinase K into the bottom of a well of an S-Block. Add 200 µl sample to the proteinase K. If processing smaller volumes, adjust the volume to 200 µl with PBS.**

   Record the wells into which you load the samples.

2. **Vortex or shake the mixture containing Buffer AL, isopropanol, MagAttract Suspension G, and carrier RNA thoroughly for 30 s.**

   For pipetting with multichannel pipets, pour the mixture into a Reagent Container, 100 ml, provided with the kit.

3. **Add 400 µl Buffer AL mixture to each sample in the S-Block.**

   In general, it is not necessary to vortex the mixture during dispensing of 96 aliquots if working without interruption. If dispensing takes longer than 3 min per 96-well plate, we recommend sealing the reagent container tightly and vortexing carefully to ensure that MagAttract Suspension G remains fully resuspended.

4. **Prepare 4 additional S-Blocks (slots 2–5) and two 96-well microplates (slots 6 and 7) according to Table 3 on the next page. The S-Blocks and microplates are loaded onto the worktable in step 8.**

   In each plate or block, the number of wells filled with buffer should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1–H1 to A6–H6 of each plate or block).

Table 3. BioSprint 96 worktable setup and reagent volumes

Slot	Loading message	Format	Item to add	Volume per well (µl)
7	Load Rod Cover	96-well microplate MP	Large 96-Rod Cover	–
6	Load Elution	96-well microplate MP	Buffer AVE	75–200
5	Load Wash 4	S-Block	Buffer RPE	500
4	Load Wash 3	S-Block	Buffer RPE	500
3	Load Wash 2	S-Block	Buffer AW1	500
2	Load Wash 1	S-Block	Buffer AW1	700
1	Load Lysate	S-Block	Lysate*	640

* Includes 40 µl proteinase K, 200 µl sample, and 400 µl Buffer AL mixture.

5. **Switch on the BioSprint 96 at the power switch.**
6. **Slide open the front door of the protective cover.**
7. **Select the "BS96 Vet Blood 200" protocol using the ▲ and ▼ keys. Press "Start" to start the protocol run.**
8. **The LCD displays a message asking you to load slot 7 of the worktable with the 96-rod cover (see Table 3 above). After loading slot 7, press "Start". The worktable rotates and a new message appears, asking you to load slot 6 with the elution plate. Load slot 6 and press "Start" again. Continue this process of pressing "Start" and loading a particular slot until all slots are loaded.**

   Each slot is labeled with a number. Load each plate or block so that well A1 is aligned with the slot label (i.e., well A1 faces inward).
9. **Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.**

   See the *BioSprint 96 User Manual* for safety information.
10. **Press "Start" to start sample processing.**

11. **After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press "Start" after removing each plate or block. The first item to be removed contains the purified samples.**

    Carryover of magnetic particles in eluates does not affect most downstream applications. Magnetic-particle carryover can be minimized by placing the microplate containing eluates in a suitable magnet and transferring the eluates to a clean microplate (see "Carryover of magnetic particles" on page 27).

12. **Press "Stop" after all plates and blocks are removed.**
13. **Discard the used plates, blocks, and 96-rod cover according to your local safety regulations.**

    See page 5 for safety information.

14. **Switch off the BioSprint 96 at the power switch.**
15. **Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.**

    Do not use bleach as disinfectant. See page 5 for safety information.

Protocol: Purification of Viral Nucleic Acids and Bacterial DNA from Animal Tissue Homogenates, Serum, Plasma, Other Body Fluids, Swabs, and Washes

This protocol is for purification of viral nucleic acids and bacterial DNA from tissue homogenates, serum, plasma, other body fluids, swab medium, or wash fluid using the BioSprint 96 workstation and the BioSprint 96 One-For-All Vet Kit with the "BS96 Vet 100" protocol. Sample volumes can be up to 100 µl.

Important points before starting

- Ensure that you are familiar with correct operation of the BioSprint 96. Refer to the *BioSprint 96 User Manual* for operating instructions.
- Before beginning the procedure, read "Important Notes" (page 12).
- Check that Buffer AW1, Buffer RPE, and carrier RNA have been prepared according to the instructions in "Preparing reagents" (page 16).
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming and then place at room temperature (15–25°C).
- The 96-rod covers are supplied either as packets of 2, or as packets of 1 inserted into an S-Block. If using a new packet of 2, store the second 96-rod cover on another S-Block or plate. Care should be taken to not bend 96-rod covers.

Things to do before starting

- Prepare the Buffer RLT mixture according to Table 4 on the next page for use in step 3 of the procedure. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time, and for 1 minute before subsequent use.

  Note: Prepare a volume of the Buffer RLT mixture that is 20% greater than that required for the total number of sample purifications to be performed; 600 µl mixture is required per sample (see step 6 of the procedure).

  For a full run of 96 samples, we recommend preparing the mixture directly in the bottle of Buffer RLT provided with the kit. Add the appropriate amounts of isopropanol, MagAttract Suspension G, and redissolved carrier RNA as indicated to the bottle containing 35 ml of Buffer RLT. For runs with fewer samples, see Table 4 on the next page for guidance.

Table 4. Preparation of Buffer RLT mixture

	Number of samples*		
Reagent	**1**	**48**	**96†**
Buffer RLT	300 µl	17.5 ml	35 ml
Isopropanol	300 µl	17.5 ml	35 ml
MagAttract Suspension G	25 µl	1.5 ml	3 ml
Carrier RNA (1 µg/µl)	Approximately 2.7 µl	155 µl	310 µl

* The volume prepared is 120% of the required volume to compensate for pipetting error and possible evaporation.

† When preparing mixture for 96 samples, we recommend mixing the reagents directly in the bottle of Buffer RLT provided with the kit.

Procedure

1. **Pipet 40 µl proteinase K into the bottom of a well of an S-Block. Add 100 µl sample to the proteinase K. If processing smaller volumes, adjust the volume to 100 µl with PBS.**

   Record the wells into which you load the samples.

2. **Vortex or shake the mixture containing Buffer RLT, isopropanol, MagAttract Suspension G, and carrier RNA thoroughly for 30 s.**

   For pipetting with multichannel pipets, pour the mix into a Reagent Container, 100 ml, provided with the kit.

3. **Add 600 µl Buffer RLT mixture to each sample in the S-Block.**

   In general, it is not necessary to vortex the mixture during dispensing of 96 aliquots if working without interruption. If dispensing takes longer than 3 min per 96-well plate, we recommend sealing the reagent container tightly and vortexing carefully to ensure that MagAttract Suspension G remains fully resuspended.

4. **Prepare 3 additional S-Blocks (slots 2–4) and two 96-well microplates (slots 5 and 6) according to Table 5 on the next page. The S-Blocks and microplates are loaded onto the worktable in step 8.**

   In each plate or block, the number of wells filled with buffer should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1–H1 to A6–H6 of each plate or block).

Table 5. BioSprint 96 worktable setup and reagent volumes

Slot	Loading message	Format	Item to add	Volume per well (µl)
6	Load Rod Cover	96-well microplate MP	Large 96-Rod Cover	–
5	Load Elution	96-well microplate MP	Buffer AVE	75–200
4	Load Wash 3	S-Block	Buffer RPE	500
3	Load Wash 2	S-Block	Buffer RPE	500
2	Load Wash 1	S-Block	Buffer AW1	700
1	Load Lysate	S-Block	Lysate*	740

* Includes 40 µl proteinase K, 100 µl sample, and 600 µl Buffer RLT mixture.

5. **Switch on the BioSprint 96 at the power switch.**
6. **Slide open the front door of the protective cover.**
7. **Select the "BS96 Vet 100" protocol using the ▲ and ▼ keys on the BioSprint 96 workstation. Press "Start" to start the protocol run.**
8. **The LCD displays a message asking you to load slot 6 of the worktable with the 96-rod cover (see Table 5 above). After loading slot 6, press "Start". The worktable rotates and a new message appears, asking you to load slot 5 with the elution plate. Load slot 5 and press "Start" again. Continue this process of pressing "Start" and loading a particular slot until all slots are loaded.**

   Each slot is labeled with a number. Load each plate or block so that well A1 is aligned with the slot label (i.e., well A1 faces inward).
9. **Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.**

   See the *BioSprint 96 User Manual* for safety information.
10. **Press "Start" to start sample processing.**
11. **After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press "Start" after removing each plate or block. The first item to be removed contains the purified samples.**

    Carryover of magnetic particles in eluates does not affect most downstream applications. Magnetic-particle carryover can be minimized by placing the microplate containing eluates in a suitable magnet and transferring the eluates to a clean microplate (see "Carryover of magnetic particles" on page 27).

12. **Press "Stop" after all plates and blocks are removed.**
13. **Discard the used plates, blocks, and 96-rod cover according to your local safety regulations.**

    See page 5 for safety information.
14. **Switch off the BioSprint 96 at the power switch.**
15. **Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.**

    Do not use bleach as disinfectant. See page 5 for safety information.

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
Low yield of viral DNA and RNA		
a)	MagAttract Suspension G not completely resuspended	Ensure that MagAttract Suspension G is fully resuspended before adding to Buffer AL or Buffer RLT mixture. Vortex for at least 3 min before the first use, and for 1 min before subsequent uses.
b)	Buffer AL or Buffer RLT mixture prepared incorrectly	Ensure that Buffer AL or Buffer RLT mixture was prepared with the correct volumes of additional reagents as indicated on the buffer bottle or according to the tables in the protocols (pages 18 and 23). Repeat the DNA purification procedure with new samples.
c)	Buffer AW1 or Buffer RPE prepared incorrectly	Check that Buffer AW1 or Buffer RPE concentrate was diluted with the correct volume of ethanol, as indicated on the bottle. Use 96–100% ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the DNA purification procedure with new samples.
d)	Reagents loaded onto worktable in wrong order	Ensure that all reagents are loaded onto the BioSprint 96 worktable in the correct order. Repeat the DNA purification procedure with new samples.
e)	Frozen samples not mixed properly after thawing	Thaw frozen samples quickly in a 37°C water bath with mild agitation to ensure thorough mixing.

		Comments and suggestions
f)	Insufficient sample lysis	Proteinase K was stored at elevated temperatures for too long. Repeat the DNA purification procedure using new samples and fresh proteinase K (see storage recommendations on page 4). For difficult-to-lyse pathogens and/or complex sample material, an optional incubation step can be performed after step 1 of the protocol procedure (see pages 19 and 23). In this case, mix the sample thoroughly after addition of proteinase K and incubate for 10 min at 56°C. Proceed with step 2.
DNA or RNA does not perform well in downstream applications		
a)	Little or no DNA or RNA in the eluate	See "Low yield of viral DNA and RNA" (previous page) for possible reasons. Increase the amount of eluate added to the reaction, if possible.
b)	Carryover of magnetic particles	Carryover of magnetic particles in eluates does not affect most downstream applications. Magnetic-particle carryover can be minimized by placing the microplate containing eluates in a suitable magnet (e.g., 96-Well Magnet Type A or 12-Tube Magnet; see ordering information, page 30) for 1 min, and transferring the eluates to a clean microplate. If a suitable magnet is not available, centrifuge the microplate containing eluates at full speed for 1 min to pellet any remaining magnetic particles, and transfer the supernatants to a clean microplate.
c)	Too much eluate in the amplification reaction	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly.

		Comments and suggestions
d)	RNA degraded	RNA may have been degraded by RNases in the original samples. Ensure that the samples are processed immediately after collection or removal from storage. Repeat the purification protocol with new samples.
e)	Nucleic acids in samples already degraded prior to purification	Samples were frozen and thawed more than once or stored at room temperature (15–25°C) for too long. Always use fresh samples or samples thawed only once. Repeat the purification protocol with new samples.
f)	Carrier RNA not added to Buffer AL or Buffer RLT mixture	Reconstitute carrier RNA in Buffer AVE and mix with an appropriate volume of the Buffer AL or Buffer RLT mixture as described in "Using carrier RNA and internal controls" (page 15). Repeat the purification protocol with new samples.
g)	Too much or too little carrier RNA in the eluate	Determine the maximum amount of carrier RNA suitable for your amplification reaction. Adjust the concentration of carrier RNA solution added to the Buffer AL or Buffer RLT mixture accordingly.
h)	Degraded carrier RNA	Carrier RNA reconstituted in Buffer AVE was not stored at –20°C or underwent multiple freeze–thaw cycles. Alternatively, the Buffer AL or Buffer RLT mixture was stored for over 48 h at room temperature. Prepare a new tube of carrier RNA dissolved in Buffer AVE and store appropriately. Repeat the purification procedure with new samples.
i)	PCR inhibition	Some sample types (e.g., animal whole blood, feces) may contain high amounts of PCR inhibiting substances. Removal of inhibitors might not be complete without special treatment. Reduce the amount of sample input or/and the amount of eluate added to the amplification reaction. Subject feces samples to special PCR-inhibitor reducing pretreatments, as described in the *QIAamp® DNA Stool Handbook*.

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.
BioSprint 96 One-For-All Vet Kit (384)	For 384 preps: Large 96-Rod Covers, 96-Well Microplates MP, S-Blocks, MagAttract Suspension G, Buffers and Reagents	947057
Buffer ATL (200 ml)	200 ml Tissue Lysis Buffer for 1000 preps	19076
QIAGEN Proteinase K (2 ml)	2 ml (>600 mAU/ml, solution)	19131
QIAGEN Proteinase K (10 ml)	10 ml (>600 mAU/ml, solution)	19133
Buffer AE (240 ml)	Buffers and reagents for use with QIAGEN products. Contents: 240 ml Elution Buffer for 1000 preps	19077
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570
Accessories		
Large 96-Rod Cover (16)	16 x Large 96-Rod Covers for use with the BioSprint 96	1031668
96-Well Microplates MP (20)	96-well microplates, 20 per case	1031656
S-Blocks (24)	96-well blocks with 2.2 ml square wells, for collecting wash and lysis fractions from 96-well plates. Contents: 96-well blocks with 2.2 ml wells, 24 per case	19585
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
96-Well Microplates FB (24)	96-well microplates with flat-bottom wells, 24 per case, for use with the 96-Well Magnet	36985

Ordering Information

Product	Contents	Cat. no.
QIAGEN 96-Well Centrifugation System		
Centrifuge 4–16 (120 V, 50/60 Hz)	Universal laboratory centrifuge with brushless motor	81310
Centrifuge 4–16K (220–240 V, 50/60 Hz)	Refrigerated universal laboratory centrifuge with brushless motor	81410
Plate Rotor 2 x 96	Rotor for 2 QIAGEN 96-well plates, for use with QIAGEN Centrifuges	81031
Related products		
BioSprint 96 DNA Blood Kit — for rapid purification of DNA from cells, tissue, blood, buffy coat, buccal swabs, and dried blood spots using the BioSprint 96 workstation		
BioSprint 96 DNA Blood Kit (48)*	For 48 preps: Large 96-Rod Covers, 96-Well Microplates MP, S-Blocks, MagAttract Suspension G, Buffers and Reagents	940054
BioSprint 96 DNA Plant Kit — for rapid purification of total DNA from plant tissue using the BioSprint 96 workstation		
BioSprint 96 DNA Plant Kit (576)*	For 576 preps: Large 96-Rod Covers, 96-Well Microplates MP, S-Blocks, MagAttract Suspension G, Buffers and Reagents	941557
Solutions for low-throughput testing		
QIAamp MinElute Virus Spin Kit (50)	For 50 minipreps: 50 QIAamp MinElute Columns, QIAGEN Protease, Carrier RNA, Buffers, Collection Tubes (2 ml)	57704

* Other kit sizes are available; see www.qiagen.com.

Ordering Information

Product	Contents	Cat. no.
QIAcube® (110 V)* QIAcube (230 V)†	Robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-column kits, 1 year warranty on parts and labor‡	9001292* 9001293†
Starter Pack, QIAcube	Pack includes: reagent bottle racks (3); rack labeling strips (8); 200 µl Filter-Tips (1024); 1000 µl Filter-Tips (1024); 1000 µl Filter-Tips, wide-bore (1024); 30 ml reagent bottles (18); rotor adapters (240); rotor adapter holder; 1.5 ml elution tubes (240)	990395
Filter-Tips, 200 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube and the QIAsymphony® SP	990332
Filter-Tips, 1000 µl (1024)	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube	990352
Rotor Adapters (10 x 24)	For 240 preps: 240 Disposable Rotor Adapters and 240 Elution Tubes (1.5 ml); for use with the QIAcube	990394

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

* US, Canada, and Japan.

† Rest of world.

‡ Agreements for comprehensive service coverage are available; please inquire.

Notes

Notes

Trademarks:

QIAGEN®, QIAamp®, QIAcube®, QIAsymphony®, BioSprint®, MagAttract® (QIAGEN Group); Finnpipette®, Finntip® (Thermo Electron Oy); MagMAX™ (Ambion, Inc.); KingFisher® (Thermo Fischer Scientific, Inc.).

Limited License Agreement for BioSprint 96 One-For-All Vet Kit

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

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.qiagen.com.

www.qiagen.com

Australia ▪ techservice-au@qiagen.com

Austria ▪ techservice-at@qiagen.com

Belgium ▪ techservice-bnl@qiagen.com

Brazil ▪ suportetecnico.brasil@qiagen.com

Canada ▪ techservice-ca@qiagen.com

China ▪ techservice-cn@qiagen.com

Denmark ▪ techservice-nordic@qiagen.com

Finland ▪ techservice-nordic@qiagen.com

France ▪ techservice-fr@qiagen.com

Germany ▪ techservice-de@qiagen.com

Hong Kong ▪ techservice-hk@qiagen.com

India ▪ techservice-india@qiagen.com

Ireland ▪ techservice-uk@qiagen.com

Italy ▪ techservice-it@qiagen.com

Japan ▪ techservice-jp@qiagen.com

Korea (South) ▪ techservice-kr@qiagen.com

Luxembourg ▪ techservice-bnl@qiagen.com

Mexico ▪ techservice-mx@qiagen.com

The Netherlands ▪ techservice-bnl@qiagen.com

Norway ▪ techservice-nordic@qiagen.com

Singapore ▪ techservice-sg@qiagen.com

Sweden ▪ techservice-nordic@qiagen.com

Switzerland ▪ techservice-ch@qiagen.com

UK ▪ techservice-uk@qiagen.com

USA ▪ techservice-us@qiagen.com

QIAGEN

1072928 06/2012

Sample & Assay Technologies