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MagAttract® 96 *cador*® Pathogen Kit Handbook

For automated purification of viral RNA and DNA and bacterial DNA from animal samples using the BioSprint® 96 or equivalent workstation

Sample to Insight

QIAGEN

Contents

Kit Contents

MagAttract 96 *cador* Pathogen Kit	**(384)**
Catalog no.	**947457**
Number of preps	**384**
Buffer VXL*	2 x 30 ml
Buffer ACB*† (concentrate)	2 x 60 ml
QIAGEN Proteinase K	2 x 6 ml
Carrier RNA (poly A)	2 x 310 µg
MagAttract Suspension G§	13 ml
Buffer AW1*‡ (concentrate)	151 ml
Buffer AW2‡ (concentrate)	2 x 54 ml
Buffer AVE§	125 ml
Large 96-Rod Cover	4
S-Block	20
96-Well Microplate MP	4
Quick-Start Protocol	1

* CAUTION: Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 6 for safety information.

† Before using for the first time, add isopropanol as indicated on the bottle to obtain a working solution.

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

§ CAUTION: Contains sodium azide as a preservative.

Storage

All buffers and reagents are stable until the expiration date on the kit box at room temperature (15–25°C) without affecting performance.

Lyophilized carrier RNA can be stored at room temperature until the expiration date stated on the kit box. For use, lyophilized carrier RNA should be dissolved in Buffer AVE and then added to Buffer VXL mixture, as described in "Preparing reagents and instrument", on page 20. Carrier RNA/Buffer AVE/Buffer VXL mix solution should be prepared fresh. Unused carrier RNA dissolved in Buffer AVE should be immediately frozen in aliquots at –30 to –15°C. Do not subject aliquots of carrier RNA to more than 3 freeze–thaw cycles.

QIAGEN Proteinase K can be stored at room temperature (15–25°C). To store for extended periods of time, or if the ambient temperature often exceeds 25°C, we recommend storing at 2–8°C.

Intended Use

The MagAttract 96 *cador* Pathogen Kit is intended for the automated extraction of pathogen nucleic acids (viral RNA and DNA, and bacterial DNA) from animal whole blood, serum, plasma, other body fluids, swabs, washes, and tissue homogenate using the BioSprint 96 workstation.

For laboratory use. 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 VXL and Buffer AW1 contain guanidine hydrochloride and Buffer ACB contains guanidine thiocyanate, which can form highly reactive compounds if combined with bleach.

If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of MagAttract 96 *cador* Pathogen Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The MagAttract 96 *cador* Pathogen Kit enables the rapid purification of viral RNA and DNA, as well as bacterial DNA, from a broad range of animal sample types (see Table 1 on page 12) using the BioSprint 96 workstation (see "Starting material" on page 16). 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 Standard Magnetic Particle Processor (Life Technologies Corporation) and KingFisher™ Flex Magnetic Particle Processor (Thermo Fischer Scientific, Inc.) users can also use the MagAttract 96 *cador* Pathogen Kit on these instruments, by simply following the protocols on pages 23 through 26. The appropriate software protocol is available from QIAGEN Technical Services.

Principle and procedure

The MagAttract 96 *cador* Pathogen 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).

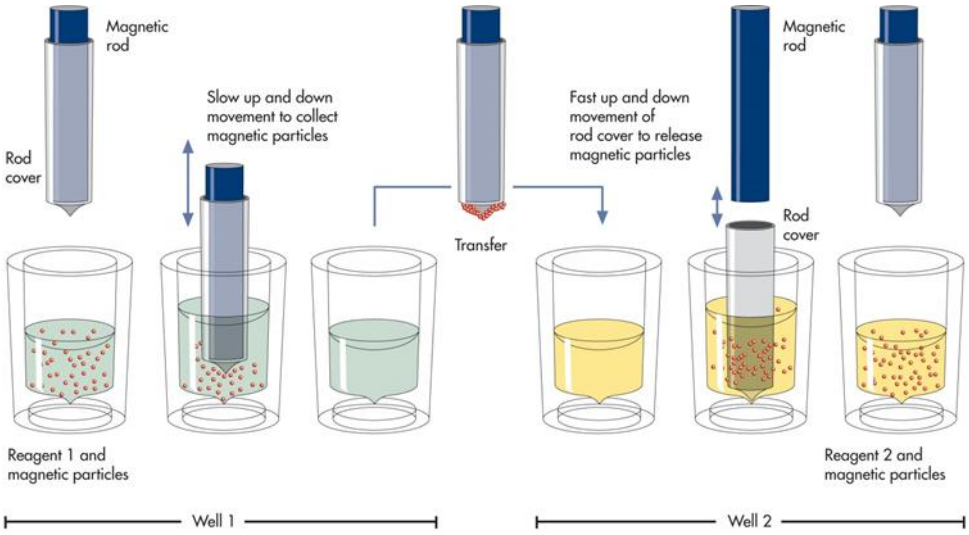


Figure 1. Schematic of the BioSprint 96 principle. The BioSprint 96 processes a sample containing magnetic particles, as follows: Step 1) A magnetic rod, protected by a rod cover, enters a well (see Well 1 in the figure) containing the sample and attracts the magnetic particles. Step 2) The magnetic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

The purification procedure is designed to ensure convenient, reproducible handling of potentially infectious samples (see flowchart, page 10).

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. Three 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.

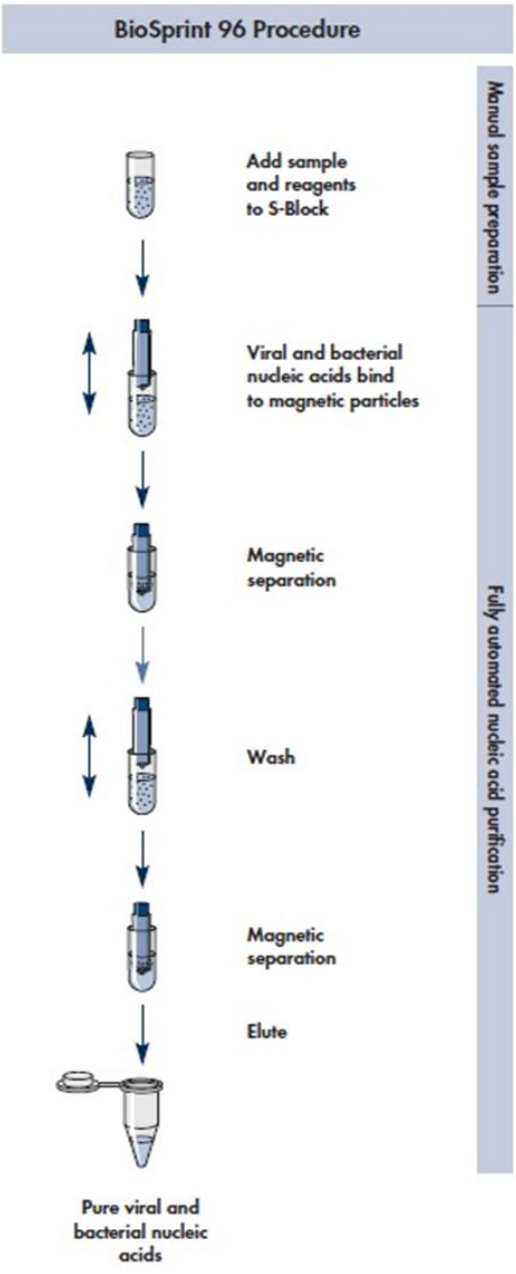
Description of BioSprint 96 protocols

There are two types of protocols in this handbook. In the first protocol, samples undergo nucleic acid purification directly, whereas in the second protocol, samples require pretreatment before nucleic acid purification.

For samples that require pretreatment, Table 1 on page 12 provides an overview of which pretreatment protocols are suited to different starting material and pathogen combinations.

- Nucleic Acid Purification Protocol (page 11)
- Pretreatment Protocols (pages 27–35)

Sample purification time is approximately 34 minutes, not including upfront handling steps for prefilling S-Blocks or 96-well microplates. The lysis and binding solutions used in the procedure are Buffer VXL and Buffer ACB, which contain the chaotropic salt guanidine thiocyanate. The procedure includes 3 wash steps.


BioSprint 96 Procedure
Manual sample preparation
Fully automated nucleic acid purification
Add sample and reagents to S-Block
Viral and bacterial nucleic acids bind to magnetic particles
Magnetic separation
Wash
Magnetic separation
Elute
Pure viral and bacterial nucleic acids

Nucleic acid purification protocol

The "Purification of Pathogen Nucleic acids from Fluid Samples" protocol (page 23) is optimized for purification of viral RNA and DNA, and the DNA of easy-to-lyse bacteria from up to 200 µl of fluid material. Suitable starting materials for direct processing using this method include:

- Whole blood
- Serum
- Plasma
- Oral fluid
- Body cavity fluids (e.g., peritoneal, synovial, cerebrospinal)
- Liquid extracts from swabs (e.g., nasal, pharyngeal, and cloacal* swabs)
- Wash fluids (e.g., from bronchoalveolar lavages)
- Other fluids, such as urine or feces suspensions*

Pretreatments

The various pretreatments included in this handbook are optimized for specific combinations of starting material and target pathogens. The choice of pretreatment depends on the workflow focus, and is to be followed by nucleic acid purification.

Table 1 on page 12 summarizes the pretreatments and their applications.

Some of the pretreatments may require additional components (see pages 14–15).

* The processing of samples with a high inhibitor content, such as urine and feces, may require a reduction in sample input volume or further measurements. For further pretreatment recommendations, contact QIAGEN Technical Services.

Table 1. Pretreatment protocols for fluid and tissue samples

Sample	Target	Pretreatment	Page
Fluids (e.g., whole blood, serum, plasma, swab or wash fluid, pretreated tissue)	Viral RNA and DNA, DNA of easy-to-lyse bacteria*	-	23
Whole blood or pretreated tissue	DNA of difficult-to-lyse bacteria*	Pretreatment B1 for difficult-to-lyse bacteria in whole blood or pretreated tissue	27
Serum, plasma, swabs, washes, body cavity fluids, urine	DNA of difficult-to-lyse bacteria*	Pretreatment B2 for difficult-to-lyse bacteria in body fluids†	29
Tissue (e.g., liver, spleen, kidney, lymph node)	Viral RNA and DNA‡	Pretreatment T1 Mechanical disruption of tissue	31
	Viral DNA§, bacterial DNA¶	Pretreatment T2 Enzymatic digestion of tissue	32
Feces	Viral RNA and DNA‡	Pretreatment F1 Non-lysing suspension method	34
	Bacterial DNA* and viral DNA	Pretreatment F2 Lysing suspension method	35

* Gram-positive bacteria are difficult to lyse due to their rigid cell wall. Many Gram-negative bacteria are easy to lyse, but some are not and will also benefit from Pretreatment B1 or B2 (see pages 27 and 29, respectively).

† Not suitable for whole blood.

‡ Not suitable for bacterial DNA due to centrifugation step (see page 31).

§ Not suitable for viral RNA as the lysis conditions do not sufficiently conserve RNA integrity.

¶ For difficult-to-lyse bacteria, use Pretreatment B1 (page 27).

For further pretreatment recommendations, contact QIAGEN Technical Services.

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.

For all protocols:

- BioSprint 96 workstation (cat. no. 9000852) or equivalent
- "BS96 *cador v2*" protocol for BioSprint96 workstation or equivalent
- Magnetic head for use with Large 96-Rod Covers (supplied with the BioSprint 96 or equivalent)
- Pipettors and disposable pipette tips with aerosol barriers (20–1000 µl)
- Multichannel pipettor and disposable 1000 µl pipette tips with aerosol barriers (e.g., Finnpipette® Digital Pipettes and Finntip® Filter Pipette Tips from Thermo Electron Oy Corporation, see www.thermo.com) *
- Multidispenser (e.g., Finnpipette Stepper Pipette from Thermo Electron Oy Corporation, see www.thermo.com)*
- Ethanol (96–100%)†
- Isopropanol
- Phosphate-buffered saline (PBS), may be required for diluting samples
- 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.

Pretreatment B1 – for difficult-to-lyse bacteria in whole blood or pretreated tissue

- Vortexer with Microtube Foam Insert (Scientific Industries, cat. no. 504-0234-00) or TurboMix Attachment (Scientific Industries, cat. no. SI-0564); or FastPrep®-24 (MP Biomedicals, cat. no. 6004500), or TissueLyser II (QIAGEN, cat. No. 85300) with a TissueLyser II Adapter Set 2 x 24 (QIAGEN, cat. no. 69982) or 2 x 96 (QIAGEN, cat. no. 69984), or TissueLyser LT (QIAGEN, cat. no. 85600) with the TissueLyser LT Adapter for 12 tubes (QIAGEN, cat. no. 69980)*
- Pathogen Lysis Tubes L (QIAGEN, cat. no. 19092) containing 50 Pathogen Lysis Tubes with glass beads and 1 vial Reagent DX (QIAGEN, cat. no. 19088) for bead-beating of bacteria
- Buffer ATL (QIAGEN, cat. no. 19076)

Pretreatment B2 – for difficult-to-lyse bacteria in body fluids

- Vortexer with Microtube Foam Insert (Scientific Industries, cat. no. 504-0234-00) or TurboMix Attachment (Scientific Industries, cat. no. SI-0564); or FastPrep-24 (MP Biomedicals, cat. no. 6004500), or TissueLyser II (QIAGEN, cat. no. 85300) with a TissueLyser II Adapter Set 2 x 24 (QIAGEN, cat. no. 69982) or 2 x 96 (QIAGEN, cat. no. 69984), or TissueLyser LT (QIAGEN, cat. no. 85600) with the TissueLyser LT Adapter for 12 tubes (QIAGEN, cat. no. 69980)*
- Pathogen Lysis Tubes L (QIAGEN, cat. no. 19092) or S (QIAGEN, cat. no. 19091) containing 50 Pathogen Lysis Tubes with glass beads and 1 vial Reagent DX (QIAGEN, cat. no. 19088) for bead-beating of bacteria
- Buffer ATL (QIAGEN, cat. no. 19076)

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

Pretreatment T1 – mechanical disruption of tissue

- TissueLyser II (QIAGEN, cat. no. 85300) with a TissueLyser II Adapter Set 2 x 24 (QIAGEN, cat. no. 69982), or TissueLyser LT (QIAGEN, cat. no. 85600) with the TissueLyser LT Adapter for 12 tubes (QIAGEN, cat. no. 69980), or FastPrep-24 (MP Biomedicals, cat. no. 6004500) or other bead-mill homogenizer*

  Note: A vortexer with Microtube Foam Insert (Scientific Industries, cat. no. 504-0234-00) can also be used
- 5 mm Stainless Steel Beads (QIAGEN, cat. no. 69989)
- PBS, pH 7.2 (50 mM potassium phosphate, 150 mM NaCl) or NaCl 0.9%

Pretreatment T2 – enzymatic digestion of tissue

- Thermoshaker suitable for 2 ml collection tubes
- Buffer ATL (QIAGEN, cat. no. 19076)

Pretreatment F1 – isolation of viral nucleic acids from feces

- Distilled water or RNase-Free Water (QIAGEN, cat. no. 129112)

Pretreatment F2 – isolation of bacterial and viral DNA from feces

- Buffer ASL (QIAGEN, cat. no. 19082)

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

Important Notes

Starting material

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 MagAttract 96 *cador* Pathogen protocol includes a special step that combines efficient lysis and binding in a single step, enabling quick, straight-forward sample processing. For sample types of higher complexity, such as tissue, feces and certain difficult-to-lyse pathogens, such as Gram-positive bacteria, specialized disruption and/or lysis pretreatments may be necessary. The user should determine appropriate pretreatments in advance, for such materials. General information about recommended sample types and pretreatments is given in the following sections. For further information, contact QIAGEN Technical Services.

Highly viscous fluids may require treatment to reduce their viscosity, to allow for efficient extraction of pathogen nucleic acids. Please contact QIAGEN Technical Services for recommendations.

Avoid repeated thawing and freezing of samples, since this may reduce nucleic acid yield and quality.

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 freeze-thawed more than once. Freeze-thawing more than once can lead to denaturation and precipitation of proteins, resulting in potential reduction in viral titers, and therefore, reduced yields of viral nucleic acids. 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 –30 to –15°C or –70°C.

We recommend using 50–200 µl blood containing non-nucleated erythrocytes. However, highly elevated cell counts due to inflammatory or neoplastic diseases may strongly increase the host nucleic acid content of a sample. In this case, reduction of sample input to 50 µl may improve results in downstream assays, particularly in RT-PCR. If using less than 200 µl blood, adjust the sample volume to 200 µl with PBS or 0.9% NaCl.

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 or 0.9% NaCl.

Animal serum, plasma, other body fluids, swab, and wash specimens

Frozen plasma or serum must not be thawed more than once before processing.

Up to 200 µl serum, plasma, other body fluid, swab media supernatant, or wash fluid can be processed.

The processing of samples with very high inhibitor contents, such as urine or fecal suspensions, may require a reduction in sample input volume and/or an extra pretreatment to remove inhibitors. To reduce the input volume, use 25–50 µl of the sample and adjust the volume to 200 µl with PBS or 0.9% NaCl.

For extraction of bacterial DNA, the input volume can be increased to more than 200 µl, e.g., 1.5 ml for increased sensitivity of bacterial detection. Gram-negative bacteria in cell-free fluids can be concentrated by centrifugation of higher volumes. Resuspend pellets in PBS and use 200 µl as starting volume. See Pretreatment B2 (page 29) for extraction of DNA from difficult-to-lyse bacteria.

Animal tissues

When working with tissue samples, mechanical or enzymatic disruption of the tissue structure is the prerequisite for liberation of cells, subsequent release of nucleic acids, and membrane permeability of the material.

Different tissue types can vary widely with regard to texture and rigidity, cell types, and content of host nucleic acids and inhibitory substances. In addition, the localization of pathogen nucleic acids in the tissue may vary depending on tissue type, pathogen, and stage of infection. Additional pretreatments for tissue samples are available at QIAGEN technical service, including a rapid protocol and recommendations for difficult tissues.

Up to 25 mg of fresh or frozen tissue can be used as a starting amount. For tissues with a very high number of cells for a given mass of tissue, such as spleen, a reduced amount of starting material (5–10 mg) should be used.

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

Yields of nucleic acids

For samples containing a low amount of cells (e.g., serum and plasma), the yield of viral nucleic acids obtained can be below 1 µg and is therefore difficult to quantify using a spectrophotometer. In addition, eluates prepared with carrier RNA may contain much more carrier RNA than target nucleic acids. The MagAttract 96 *cador* Kit recovers total nucleic acids. Therefore, cellular DNA and RNA will be co-purified from any cells in the sample along with viral RNA and DNA, and bacterial DNA, and cannot be distinguished using spectrophotometric measurements. We recommend using quantitative amplification methods such as quantitative real-time PCR or real-time RT-PCR to determine pathogen nucleic acid yields.

Using carrier RNA and internal controls

Carrier RNA

We recommend adding carrier RNA to fluids containing low amounts of cells such as serum, plasma, swab media, and wash fluid. This enhances adsorption of viral RNA and DNA to the magnetic particles, which is especially important when the target molecules are not abundant. 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 buffer. Not using carrier RNA may decrease the recovery of viral nucleic acids.

Internal control

Use of an internal control, such as the QIAGEN Internal Control (to be used with QuantiFast® Pathogen +IC Kits, see page 41 for ordering information), is optional, depending on the amplification system of choice. If the MagAttract 96 *cador* Pathogen Kit is used in combination with amplification systems that employ an internal control, introduction of these internal controls may be required during the purification procedure, to monitor the efficiency of sample preparation and downstream assay.

Add unprotected internal control nucleic acids (e.g., plasmid DNA or in vitro transcribed RNA) to VXL mixture only. Do not add these internal control nucleic acids directly to the sample.

The amount of internal control added depends on the assay system and the elution volume. Evaluation of the correct amount of internal control nucleic acid must be performed by the user. Refer to the manufacturer's instructions to determine the optimal concentration of internal control.

Storing nucleic acids

For short-term storage of up to 24 hours, we recommend storing the purified viral RNA and DNA at 2–8°C. For storage longer than 24 hours, we recommend storing purified nucleic acids at –30 to –15°C, or even –70°C in the case of RNA.

Handling RNA

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. Care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure.

Preparing reagents

Carrier RNA stock solution

For use, lyophilized carrier RNA should first be dissolved in Buffer AVE. Add 310 µl Buffer AVE to the tube containing 310 µg lyophilized carrier RNA to obtain a stock solution of 1 µg/µl. Add this solution to Buffer VXL mixture as in Table 2 on page 24. Unused carrier

RNA dissolved in Buffer AVE should be frozen in aliquots at –30 to –15°C. Aliquots of carrier RNA should not be subjected to more than 3 freeze–thaw cycles.

QIAGEN Proteinase K

The MagAttract 96 *cador* Pathogen Kit contains ready-to-use proteinase K supplied in a specially formulated storage buffer. The activity of the QIAGEN proteinase K solution is 600 mAU/ml.

QIAGEN 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 QIAGEN proteinase K at 2–8°C.

Buffer ACB

Buffer ACB is supplied as a concentrate. Before using for the first time, add isopropanol (100%) as indicated on the bottle. Tick the check box on the bottle label to indicate that isopropanol has been added. Mix well after adding isopropanol.

MagAttract Suspension G

Shake the bottle containing MagAttract Suspension B and vortex for 3 minutes (before first use) or 1 minute (before subsequent uses) to ensure that the magnetic silica particles are fully resuspended.

Buffer AW1

Buffer AW1 is supplied as a concentrate. Before using for the first time, add ethanol (96–100%) as indicated on the bottle. 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. Mix well after adding ethanol.

Buffer AW2

Buffer AW2 is supplied as a concentrate. Before using for the first time add ethanol (96–100%) as indicated on the bottle. Tick the check box on the bottle label to indicate that ethanol has been added. Mix well after adding ethanol.

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. Follow general precautions for working with RNA, such as frequent change of gloves and keeping tubes closed whenever possible.

Protocol: Purification of Pathogen Nucleic Acids from Fluid Samples

This protocol is for the purification of viral RNA and DNA, and the DNA of easy-to-lyse bacteria from fluid samples or pretreated tissue samples using the BioSprint 96 workstation and the MagAttract 96 *cador* Pathogen Kit with the "BS96 *cador v2*" protocol.

Important points before starting

- Ensure that you are familiar with the correct operation of the BioSprint 96 workstation. Refer to the *BioSprint 96 User Manual* for operating instructions.
- Before beginning the procedure, read "Important Notes" (page 16).
- Check that Buffer ACB, Buffer AW1, Buffer AW2, and carrier RNA have been prepared according to the instructions in "Preparing reagents" (page 20).
- Check that Buffer VXL or Buffer ACB does not contain a white precipitate. If necessary, incubate Buffer VXL or Buffer ACB 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 samples at room temperature (15–25°C).
- If the volume of blood sample is less than 200 µl, add PBS or 0.9% NaCl to a final volume of 200 µl.
- Prepare the Buffer VXL mixture according to Table 2 on page 24, 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 or 1 minute before subsequent uses.

Note: Prepare a volume of the Buffer VXL mixture that is 25% greater than that required for the total number of sample purifications to be performed; 500 µl mixture is required per sample (see step 3 of the procedure).

Note: Do not add proteinase K directly to the Buffer VXL mixture! This can cause clogs or precipitates. Follow the procedure as described below (pipetting proteinase K into the wells, followed by sample and then Buffer VXL mixture).

Table 2. Preparation of Buffer VXL mixture

	Number of samples*		
Reagent	**1**	**48**	**96**
Buffer VXL	100 µl	4.8 ml	9.6 ml
Buffer ACB	400 µl	19.2 ml	38.4 ml
MagAttract Suspension G	25 µl	1.2 ml	2.4 ml
Carrier RNA (1 µg/µl)	1 µl	48 µl	96 µl

* The volume prepared is 105% of the required volume to compensate for pipetting error and possible evaporation. Excess buffer should be discarded.

Procedure

1. Pipet 20 µl proteinase K into the bottom of a well of an S-Block. Add 200 µl sample to the proteinase K. If processing lower 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 VXL, Buffer ACB, MagAttract Suspension G and carrier RNA, thoroughly, for 30 s.

   Note: Do not add proteinase K directly to this Buffer VXL mixture!

3. Add 500 µl Buffer VXL mixture to each sample in the S-Block.

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

4. Prepare 4 additional S-Blocks (slots 2–6) and one additional 96-Well Microplate MP, according to Table 3. The S-Blocks and microplates are loaded onto the worktable in step 8.

   In each S-Block or microplate, 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 microplate or S-block (e.g., if processing 48 samples, fill wells A1–H1 to A6–H6 of each microplate or S-block).

Table 3. BioSprint 96 worktable setup and reagent volumes

Slot	Loading message	Format	Item to add	Volume per well (µl)
6	Load Rod Cover	S-Block	Large 96-Rod Cover	—
5	Load Elution	96-Well Microplate MP	Buffer AVE	100
4	Load Wash 3	S-Block	Ethanol (96–100%)	750
3	Load Wash 2	S-Block	Buffer AW2	700
2	Load Wash 1	S-Block	Buffer AW1	700
1	Load Lysate	S-Block	Lysate*	720

* Includes 20 µl proteinase K, 200 µl sample and 500 µl Buffer VXL 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 *cador v2*" protocol using the the ▲ and ▼ keys. 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 3 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 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 have been processed, remove the plates and blocks as instructed by the display of the BioSprint 96 workstation. 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 37).

12. Press "Stop" after all plates and blocks are removed.

13. Discard the used blocks and 96-rod cover, according to your local safety regulations.

    See page 6 for safety information.

14. Switch off the BioSprint 96 workstation 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 a disinfectant. See page 6 for safety information.

Sample Pretreatment Protocols

Pretreatment B1 — for Difficult-to-Lyse Bacteria in Whole Blood or Pretreated Tissue

This pretreatment is for the extraction of DNA of difficult-to-lyse bacteria from whole blood or pretreated tissue.

Important points before starting

- Buffer ATL and Pathogen Lysis Tubes L (including Reagent DX) must be ordered separately (for ordering information, see page 39).
- Buffer ATL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.

Important points before starting

- Before use, add 100 µl Reagent DX to 15 ml Buffer ATL. If smaller amounts are needed, transfer 1.5 ml of Buffer ATL into a sterile 2 ml vial and add 10 µl Reagent DX. Mix well after addition of Reagent DX. After preparation, the mixture is stable for 6 months at room temperature (15–25°C).

Procedure

1. Add 100 µl Buffer ATL (containing Reagent DX) into a fresh Pathogen Lysis Tube.
2. Add 400 µl blood or other sample fluids.

   If using less starting material, adjust the volume to 400 µl with PBS or 0.9% NaCl.
3. Place the Pathogen Lysis Tube on a vortexer with a Microtube Foam Insert and vortex for 10 min at maximum speed.

Alternatively, the Pathogen Lysis Tube may be processed on a TissueLyser LT for 10 min at 50 Hz, or on a FastPrep-24 by applying a velocity of 6.5 m/s for two 45 s periods with a 5 min resting time in between.

4. Remove the Pathogen Lysis Tube from the vortexer and briefly centrifuge the tube to remove drops from the inside of the lid.

   Use 200 µl of the supernatant as starting material for the protocol "Purification of Pathogen Nucleic Acids from Fluid Samples", on page 23.

Pretreatment B2 — for Difficult-to-Lyse Bacteria in Body Fluids

This pretreatment is for the extraction of bacterial DNA of difficult-to-lyse bacteria from cell-free fluids, such as serum.

Important points before starting

- Buffer ATL and Pathogen Lysis Tubes L or S (including Reagent DX) must be ordered separately (for ordering information, see page 39).

  Note: Evaluate whether L or S tubes are suitable depending on the bacterial target.
- Buffer ATL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.

Things to do before starting

- Before use, add 100 µl Reagent DX to 15 ml Buffer ATL. If smaller amounts are needed, transfer 1.5 ml of Buffer ATL into a sterile 2 ml vial and add 10 µl Reagent DX. Mix well after addition of Reagent DX. After preparation, the mixture is stable for 6 months at room temperature (15–25°C).

Procedure

1. Add up to 1.5 ml fluid sample to the Pathogen Lysis tube and centrifuge the tube for 5 min at maximum speed (>14,000 x g).
2. Remove and discard the supernatant, using a pipette. Repeat steps 1 and 2, if necessary.
3. Add 500 µl Buffer ATL (containing Reagent DX) and resuspend the pellet.
4. Place the Pathogen Lysis Tube on a vortexer with a Microtube Foam Insert and vortex for 10 min at maximum speed.

   Alternatively, the Pathogen Lysis Tube may be processed on a TissueLyser LT for 10 min at 50 Hz, or on a FastPrep-24 by applying a velocity of 6.5 m/s for two 45 s periods with a 5 min resting time in between.

5. Remove the Pathogen Lysis Tube from the vortexer and briefly centrifuge the tube to remove drops from the inside of the lid.

   Use 200 µl of the supernatant as starting material for the protocol “Purification of Pathogen Nucleic Acids from Fluid Samples”, on page 23.

Pretreatment T1 — Mechanical Disruption of Tissue

This pretreatment is for the extraction of viral RNA and DNA from most types of tissue. It is not suitable for bacterial DNA due to the centrifugation step.

Important point before starting

- Stainless steel beads must be ordered separately (for ordering information, see page 39).

Procedure

1. Place up to 25 mg tissue in 2 ml microcentrifuge tubes, each containing 1 stainless steel bead (5 mm mean diameter).

   For tissues with a very high cell count for a given mass of tissue (e.g., spleen), a reduced amount of starting material (5–10 mg) should be used.

   If working with fibrous tissue, cutting the tissue into smaller pieces before starting will improve disruption efficiency.
2. Add 300 µl PBS or 0.9% NaCl solution to each tube.
3. Place the tubes in the TissueLyser II Adapter Set.
4. Operate the TissueLyser II for 2 min at 25 Hz.

   Optional: If working with fiber-rich tissue, disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser II are now outermost, and reassemble the adapter set. Operate the TissueLyser II for a further 2 min at 25 Hz.
5. Disassemble the adapter set. Centrifuge the samples at 14,000 x g for 2 min at room temperature (15–25°C).
6. Use 200 µl of the supernatant from step 5 as starting material for the protocol "Purification of Pathogen Nucleic Acids from Fluid Samples", page 23.

   For fiber-rich tissues, complete disruption may not always be possible. Ensure that no solid particles are transferred to the purification protocol.

Pretreatment T2 — Enzymatic Digestion of Tissue

This pretreatment is for the extraction of bacterial and viral DNA from most types of tissue. It is not suitable for viral RNA because the lysis conditions do not sufficiently conserve RNA integrity.

Important point before starting

- Buffer ATL must be ordered separately (for ordering information, see page 39).

Things to do before starting

- Buffer ATL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.
- Heat a thermomixer block, shaking water bath, or rocking platform to 56°C for use in step 3 of the pretreatment protocol.

Procedure

1. Cut up to 25 mg tissue into small pieces and place in a 2 ml microcentrifuge tube. Add 180 µl Buffer ATL.

   For tissues with a very high cell count for a given mass of tissue (e.g., spleen), a reduced amount of starting material (5–10 mg) should be used. We recommend cutting the tissue into small pieces for efficient lysis.

2. Add 20 µl proteinase K. Close the cap and mix thoroughly by vortexing. Briefly centrifuge the tube to collect any solution from the lid.

   Note: When samples, pretreated as described above, are used in the subsequent nucleic acid purification protocol ("Purification of Pathogen Nucleic Acids from Fluid Samples" on page 23), carrier RNA and proteinase K can be omitted from the VXL mixture. In this case, substitute the volumes of carrier RNA and proteinase K with PBS or water.

3. Incubate at 56°C, with constant agitation, until the tissue is completely lysed.

Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h. For your convenience, overnight lysis is possible, but should be evaluated for specific sample types.

After incubation, the lysate may appear viscous, but should not be gelatinous. If a substantial gelatinous pellet remains after incubation and vortexing, extend incubation time at 56°C for proteinase K digestion and/or increase amount of proteinase K to 40 µl. Reduce the amount of starting material in future preparations of this tissue type.

If no thermomixer, shaking water bath, or rocking platform is available, incubate in a heating block or water bath and vortex occasionally during incubation, to disperse the sample.

4. Optional for viral DNA or DNA of easy-to-lyse bacteria; not suitable for difficult-to-lyse bacteria: If solid tissue or debris remains in the tubes after lysis, add 50 µl Buffer ATL. Mix by vortexing and centrifuge at 6,000 x g (8,000 rpm) for 1 min. Use 200 µl of the supernatant in step 5.
5. Use 200 µl lysate as starting material for step 5a or 5b.

   IMPORTANT: Ensure that no solid particles are transferred to the next protocol.

5a. For isolation of viral DNA or DNA from easy-to-lyse bacteria, proceed directly with the protocol "Purification of Pathogen Nucleic Acids from Fluid Samples" (page 23).
Note: Do not use proteinase K in step 1 of the purification protocol.

5b. For isolation of DNA from difficult-to-lyse bacteria, proceed with Pretreatment B1 (page 27).

Pretreatment F1 — for Isolation of Viral Nucleic Acids from Feces

This pretreatment is for extraction of viral RNA and DNA from fecal samples.

Important point before starting

- This pretreatment is not suitable for isolation of bacterial DNA.

Procedure

1. Add up to 100 mg fecal material to a 2 ml microcentrifuge tube.
2. Add 1 ml water and suspend fecal material by vigorous vortexing.
3. Centrifuge for 1 min at maximum speed (>14,000 x g).
4. Use up to 200 µl supernatant as starting material for the protocol, "Purification of Pathogen Nucleic Acids from Fluid Samples'' (page 23).

   Ensure that no solid particles are transferred to the purification protocol.

   If using a lower volume, adjust to 200 µl with water.

Pretreatment F2 — for Isolation of Bacterial and Viral DNA from Feces

Note: Due to the high diversity of fecal samples, QIAGEN has developed a variety of pretreatments for QIAamp® *cador* Pathogen Kits. If any issues regarding inhibition or sensitivity (e.g. difficult-to-lyse bacteria) are encountered, contact QIAGEN Technical Services for advice.

Procedure

1. Add 100 – 220 mg fecal material to a 2 ml microcentrifuge tube and place the tube on ice.
2. Add 1 ml Buffer ASL and vortex continuously for 1 min or until the sample is thoroughly homogenized.
3. Incubate suspension for 5 minutes at 70°C.

   Note: The lysis temperature can be increased to 95°C for cells that are difficult to lyse (such as Gram-positive bacteria).

   An additional incubation on ice for 5 min may facilitate precipitation of additional inhibitors.
4. Centrifuge at 14,000 x g for 1 min.
5. Use up to 200 µl supernatant as starting material for the protocol, "Purification of Pathogen Nucleic Acids from Fluid Samples'' (page 23).

   Ensure that no solid particles are transferred to the purification protocol.

   If using a lower volume, adjust to 200 µl with PBS or water.

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/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Low yield of DNA and RNA	
a) MagAttract Suspension G not completely resuspended	Ensure that the MagAttract Suspension G is fully resuspended before adding to the Buffer VXL mixture. Vortex for at least 3 min before the first use, and for 1 min before subsequent uses.
b) Buffer VXL mixture prepared incorrectly	Ensure that Buffer VXL 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 24 and 25). Repeat the DNA purification procedure with new samples.
c) Buffer AW1 or Buffer AW2 prepared incorrectly	Check that Buffer AW or Buffer AW2 concentrate was diluted with the correct volume of 96–100% ethanol, as indicated on the bottle. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification protocol 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 purification protocol with new samples.
e) Insufficient sample lysis	Proteinase K was stored at elevated temperatures for too long. Repeat the purification procedure using new samples and fresh proteinase K (see storage recommendations on 5). For some DNA viruses and bacteria, heated lysis may improve lysis efficiency. For this purpose, an off-board-lysis protocol is available. Please contact QIAGEN Technical Services.
f) Carrier RNA not added to Buffer VXL or degraded carrier RNA	Please refer to the recommendations for preparation, storage, and addition of carrier RNA.

		Comments and suggestions
g)	Buffer VXL– Proteinase K-carrier RNA mixture mixed insufficiently	Mix well by pipetting with a large pipette.
h)	RNase contamination in Buffer AVE	Take care not to introduce RNases, which can degrade viral RNA. This may occur if tubes containing Buffer AVE are opened repeatedly. In case of RNase contamination, replace the open vial of Buffer AVE with a new vial. Repeat the purification procedure with new samples.
i)	Nucleic acids in samples already degraded prior to purification	Samples were freeze-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.
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" (above) 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 39) 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)	Excessive 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.
d)	Degraded RNA	RNA may have been degraded by RNases in the original samples. Ensure that the samples are processed immediately after collection or recovery from storage. Repeat the purification protocol with new samples.
e)	Nucleic acids in samples already degraded prior to purification	Samples were freeze-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 VXL mixture	Reconstitute carrier RNA in Buffer AVE and mix with an appropriate volume of Buffer AVE, as described in "Using carrier RNA and internal controls" (page 19). Repeat the purification protocol with new samples.

		Comments and suggestions
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 VXL mixture, accordingly.
h)	Degraded carrier RNA	Carrier RNA reconstituted in Buffer AVE was not stored at –30 to –15°C or underwent multiple freeze–thaw cycles. 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 and feces) may contain high amounts of PCR inhibiting substances. Removal of inhibitors may 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*.
Precipitate in buffers		
a)	Precipitate in Buffer VXL or Buffer ACB	Precipitate may form after storage at low temperature or prolonged storage. To dissolve precipitate, incubate Buffer VXL or ACB for 30 min at 37°C, with occasional shaking.
b)	Precipitate in sample-Buffer VXL mixture	If using sample fluid containing Buffer ATL, e.g., after enzymatic digestion of tissue, precipitate may form after addition of Buffer VXL to the sample (step 3 of the protocol "Purification of Pathogen Nucleic Acids from Fluid Samples", page 23). The precipitate does not influence subsequent protocol steps and can be dissolved by brief incubation at 56°C.

Ordering Information

Product	Contents	Cat. no.
MagAttract 96 *cador* Pathogen Kit (384)	For 384 preps: Large 96-Rod Covers, S-Blocks, MagAttract Suspension G, Buffers and Reagents	947457
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 ASL	4 x 140 ml Stool Lysis Buffer	19082
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 workstation	1031668
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
96-Well Microplates MP (20)	96-well microplates for use with the BioSprint 96, 20 per case	1031656
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

Product	Contents	Cat. no.
96-Well Microplates FB (24)	96-well microplates with flat-bottom wells, 24 per case, for use with the 96-Well Magnet	36985
TissueLyser		
TissueLyser II	Bead mill, 100-120/220-240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96*	85300
TissueLyser Adapter Set 2 x 24	2 sets of adapter plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II	69982
TissueLyser Adapter Set 2 x 96	2 sets of adapter plates for use with Collection Microtubes (racked) on the TissueLyser II	69984
TissueLyser LT	Compact bead mill, 100-240 V AC, 50–60 Hz; requires the TissueLyser LT Adapter, 12-Tube†	85600
TissueLyser LT Adapter, 12-Tube	Adapter for disruption of up to 12 samples in 2 ml microcentrifuge tubes on the TissueLyser LT	69980
Pathogen Lysis Tubes L	50 Pathogen Lysis Tubes and 1 vial of Reagent DX	19092
Pathogen Lysis Tubes S	50 Pathogen Lysis Tubes and 1 vial of Reagent DX	19091
Stainless Steel Beads, 5 mm (200)	200 stainless steel beads (5 mm diameter), suitable for use with TissueLyser systems	69989

* The TissueLyser II must be used in combination with the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96.

† The TissueLyser LT must be used in combination with the TissueLyser LT Adapter, 12-Tube.

QIAGEN 96-Well Centrifugation System

Centrifuge 4-16S	Universal laboratory centrifuge with brushless motor	81500* 81510† 81525‡ 81520§
Centrifuge 4-16KS	Refrigerated universal laboratory centrifuge with brushless motor	81600* 81610† 81625‡ 81620§
Plate Rotor 2 x 96	Rotor for 2 QIAGEN 96-well plates, for use with QIAGEN Centrifuges	81031

Related Products

QuantiFast Pathogen PCR +IC Kit (100)**	For 100 x 25 µl reactions: Master Mix, lyophilized Internal Control Assay, lyophilized Internal Control DNA, ROX Dye Solution, High-ROX Dye Solution, RNase-Free Water, Nucleic Acid Dilution Buffer, Buffer TE	211352

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.

* Japan (Centrifuge 4-16S: 100 V, 50/60 Hz; Centrifuge 4-16KS: 200 V, 50/60 Hz)
† North America (Centrifuge 4-16S: 120 V, 60 Hz; Centrifuge 4-16KS: 220–240 V, 50/60 Hz)
‡ UK (220 V, 50 Hz)
§ Rest of world (220 V, 50 Hz)
** Other kit sizes are available; see www.qiagen.com

Revision History

Document revision history	
R2 02/2018	Added notes about not adding proteinase K directly to Buffer VXL mixture, page 25

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