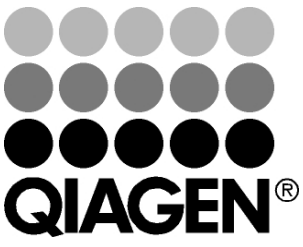
Third Edition **April 2010**

EZ1® Virus Mini Handbook

EZ1 Virus Mini Kit

For automated, simultaneous purification of viral nucleic acids from serum and plasma samples using EZ1 instruments



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

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Contents

Kit Contents

EZ1 Virus Mini Kit	**(48)**
Catalog no.	**955338**
Number of preps	**48**
Reagent Cartridges, Virus Mini*†	48
Disposable Tip Holders	50
Disposable Filter-Tips	50
Sample Tubes (2 ml)	50
Elution Tubes (1.5 ml)	150
QIAGEN® Protease (lyophilized)‡	1
Protease Resuspension Buffer†	6 ml
Carrier RNA	310 µg
Buffer AVE†	2 ml
Handbook	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for safety information.

† Contains sodium azide as a preservative.

‡ Resuspension volume 4.4 ml per vial.

Storage

The EZ1 Virus Mini Kit is shipped at ambient temperature. All buffers and reagents can be stored at room temperature (15–25°C). Do not freeze the reagent cartridges.

Lyophilized carrier RNA is stable for up to 1 year when stored at room temperature.

Lyophilized QIAGEN Protease can be stored at room temperature for up to 1 year. For longer storage, or if ambient temperatures exceed 25°C, lyophilized QIAGEN Protease should be stored dry at 2–8°C.

Product Use Limitations

The EZ1 Virus Mini 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.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the EZ1 Virus Mini Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of EZ1 Virus Mini Kits is tested against predetermined specifications to ensure consistent product quality.

Safety Information

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

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

Some buffers in the reagent cartridges contain guanidine hydrochloride, 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 EZ1 Advanced or the BioRobot® EZ1, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite, followed by water.

The following risk and safety phrases apply to the components of the EZ1 Virus Mini Kit:

Reagent Cartridge, Virus Mini

Contains ethanol, isopropanol, and guanidine hydrochloride: highly flammable, harmful, irritant. Risk and safety phrases:* R11-22-36/38-67, S13-26-36/37/39-46

QIAGEN Protease

Contains protease: subtilisin, sensitizer, irritant. Risk and safety phrases:* R37/38-41-42 S22-24-26-36/37/39-46

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R11: Highly flammable; R22: Harmful if swallowed; R36/38: Irritating to eyes and skin; R37/38: Irritating to respiratory system and skin; R41: Risk of serious damage to eyes; R42: May cause sensitization by inhalation; R67: Vapours may cause drowsiness and dizziness. S13: Keep away from food, drink and animal feedingstuffs; S22: Do not breathe dust; S24: Avoid contact with skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36/37/39: Wear suitable protective clothing, gloves and eye/face protection; S46: If swallowed, seek medical advice immediately and show the container or label.

Introduction

The EZ1 Virus Mini Kit provides a fully automated procedure for purification of viral nucleic acids* from serum and plasma using EZ1 instruments. The kit can be used to purify nucleic acids from a broad range of DNA and RNA viruses. However, kit performance is not guaranteed for each virus species and must be validated by the user. Magnetic-particle technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. The purified nucleic acids are ready to use for highly sensitive detection in downstream assays, such as amplification or other enzymatic reactions. EZ1 instruments perform all steps of the purification procedure.

Principle and procedure

Magnetic-particle technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles. The purification procedure is designed to ensure safe and reproducible handling of potentially infectious samples. The purification procedure comprises 4 steps: lyse, bind, wash, and elute (see below, and flowchart, page 10).

Lysis with QIAGEN Protease

Serum and plasma samples are proteolysed under highly denaturing conditions at elevated temperatures. Lysis is performed in the presence of QIAGEN Protease and Buffer AL, which together ensure digestion of viral coat proteins and inactivation of RNases.

Binding to magnetic particles

Isopropanol is added to the lysed samples to adjust binding conditions. Lysates are thoroughly mixed with magnetic particles to allow optimal adsorption of viral nucleic acids to the silica surface. Salt and pH conditions ensure that protein and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not bound to the magnetic particles.

* For purification of viral RNA, we recommend use of the EZ1 Virus Mini Kit v2.0 (cat. no. 955134).

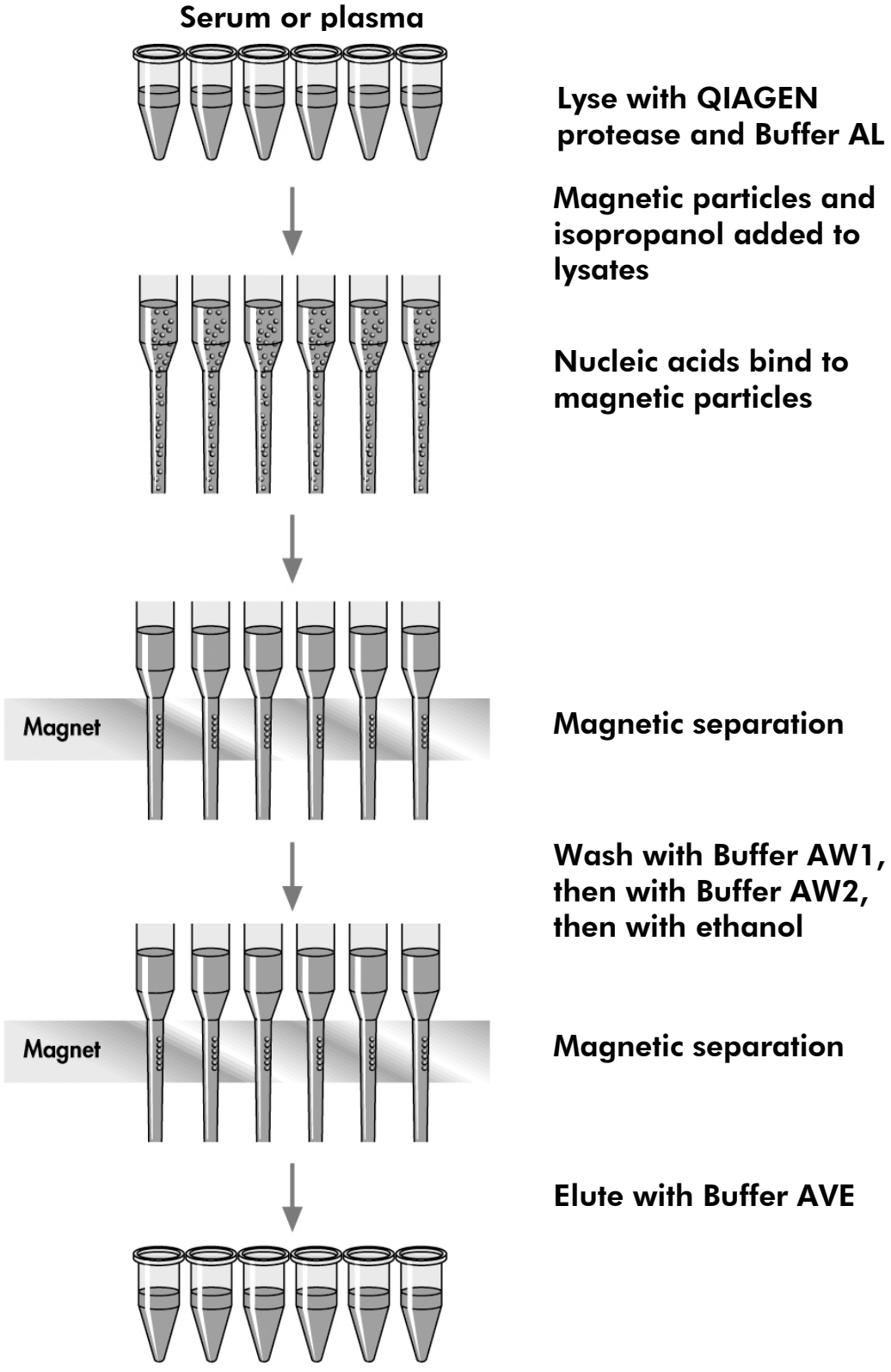
Washing of bound nucleic acids

While viral nucleic acids remain bound to the magnetic particles, contaminants are efficiently washed away during a sequence of wash steps using first Buffer AW1, then Buffer AW2, and then ethanol.

Elution of pure nucleic acids

In a single step, highly pure viral DNA and RNA are eluted in Buffer AVE. The purified nucleic acids can be either used immediately in downstream applications or stored for future use.

EZ1 Virus Mini Procedure



Equipment and Reagents to Be Supplied by User

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

All protocols

- Sterile, RNase-free pipet tips and disposable gloves
- Soft paper tissue
- Water

For EZ1 Advanced XL users

- EZ1 Advanced XL instrument (cat. no. 9001492)
- EZ1 Advanced XL Virus Card (cat. no. 9018707)

For EZ1 Advanced users

- EZ1 Advanced instrument (cat. no. 9001410)
- EZ1 Advanced Virus Card (cat. no. 9018304)

For EZ1 Advanced and EZ1 Advanced XL Users

For documentation purposes, one of the following is required:

- EZ1 Advanced Communicator Software (supplied with EZ1 Advanced and EZ1 Advanced XL instruments), PC (cat. no. 9016310; can be connected with up to 4 EZ1 Advanced and EZ1 Advanced XL instruments), and monitor (cat. no. 9016308)
- EZ1 Advanced Communicator Software (supplied with EZ1 Advanced and EZ1 Advanced XL instruments) and your own PC and monitor (connection with up to 4 EZ1 Advanced and EZ1 Advanced XL instruments not recommended)
- Printer (cat. no. 9018464) and accessory package for printer (cat. no. 9018465)

For BioRobot EZ1 users

- BioRobot EZ1 instrument (cat. no. 9000705)
- EZ1 Virus Card (cat. no. 9016386)

Important Notes

EZ1 Virus Mini v2.0 Kit

QIAGEN recently launched an improved version of the EZ1 Virus Mini Kit which is called the EZ1 Virus Mini Kit v2.0. The EZ1 Virus Mini Kit v2.0 is for purification of viral DNA and RNA from plasma, serum, CSF, urine, and respiratory samples. For higher sensitivity and improved results, it is recommended to validate your purification process on the EZ1 instruments with the EZ1 Virus Mini Kit v2.0 (cat. no. 955134).

Preparing samples

The purification procedure is optimized for use with 100 µl, 200 µl, or 400 µl plasma or serum samples. Blood samples treated with EDTA or citrate as an anticoagulant can be used for plasma preparation (blood samples treated with heparin should not be used, as heparin can interfere with downstream applications). Samples can be either fresh or frozen, provided that they have not been refrozen after thawing.

After collection and centrifugation, plasma or serum can be stored at 2–8°C for up to 6 hours. For longer storage, it is recommended to freeze aliquots at –20°C or –80°C. Thaw samples at room temperature (15–25°C), and process the samples immediately when they have equilibrated to room temperature. Do not refreeze the aliquots after thawing. Repeated freeze–thawing leads to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral nucleic acids. If cryoprecipitates are visible in the samples, centrifuge at 6800 x *g* for 3 minutes, transfer the supernatants to fresh tubes without disturbing the pellets, and start the purification procedure immediately.

Reconstituting QIAGEN Protease

Add 4.4 ml Protease Resuspension Buffer to a vial of lyophilized QIAGEN Protease, and mix carefully to avoid foaming. Make sure that the QIAGEN Protease is completely dissolved. Store the reconstituted QIAGEN Protease at 2–8°C. We recommend storing aliquots at –20°C.

Note: Protease Resuspension Buffer contains cofactors for QIAGEN Protease that are not compatible with samples or internal controls containing phosphate (e.g., viral transport medium, cell culture supernatants, whole blood, or phosphate-buffered saline). If the sample or internal control is incompatible with Protease Resuspension Buffer, dissolve the QIAGEN Protease in Protease Solvent or Buffer AVE (see page 36 for ordering information). Label the resuspended QIAGEN Protease to indicate which buffer was used for resuspension.

Note: If you want to use large volumes of internal control in the purification procedure (see Appendix A, page 28), use less Protease Resuspension Buffer or Protease Solvent to produce a more concentrated stock solution. Accordingly, reduce the amount of concentrated QIAGEN Protease added to each reaction.

Preparing carrier RNA

General remarks

Carrier RNA serves two purposes during the purification procedure. Firstly, it enhances binding of viral nucleic acids to the silica surface of the magnetic particles, especially if the sample contains very few target molecules. Secondly, the addition of large amounts of carrier RNA reduces the chances of viral RNA degradation in the rare event that RNases are not denatured by the chaotropic salts and detergent in Buffer AL. If carrier RNA is not added to the reaction, recovery of viral DNA or RNA may be reduced.

The lyophilized carrier RNA provided with the kit is sufficient for 48 sample preparations. The concentration of carrier RNA used in the purification procedure allows the EZ1 Virus Mini Kit to be compatible with many different amplification systems and suitable for purifying nucleic acids from a wide range of DNA and RNA viruses. However, amplification systems vary in efficiency depending on the total amount of nucleic acids present in the reaction. Eluates obtained using this kit contain viral nucleic acids and carrier RNA, and the amount of carrier RNA in each eluate greatly exceeds the amount of viral nucleic acids. The amount of eluate to add to downstream amplification reactions should therefore be based on the amount of carrier RNA in the eluate. To obtain the highest levels of sensitivity in amplification reactions, it may be necessary to adjust the amount of carrier RNA solution added.

Dissolving carrier RNA

Dissolve the lyophilized carrier RNA thoroughly in 300 µl Buffer AVE, divide it into conveniently sized aliquots, and store at –20°C. Thawed aliquots of this stock solution can be stored at 2–8°C for up to 6 weeks. Do not freeze–thaw the aliquots more than 3 times.

For each sample processed, add 3 µl carrier RNA stock solution to 7 µl Buffer AVE (containing internal control if used) and 75 µl reconstituted QIAGEN Protease. A 75 µl aliquot of this carrier RNA–internal control–protease solution is transferred to the lysis mix, corresponding to 2.7 µg carrier RNA.

Note: If you do not want to add an internal control, the carrier RNA can be dissolved in 1000 µl Buffer AVE. For each sample processed, add 10 µl carrier RNA solution to 75 µl reconstituted QIAGEN Protease.

Note: The purification procedure is optimized so that 2.7 µg carrier RNA is added to each sample. If a different amount of carrier RNA has been shown to be better for a specific amplification system, use a different concentration of stock solution. Use of different amounts of carrier RNA must be validated for each particular sample type and downstream assay. Carrier RNA is also available separately from QIAGEN if larger amounts are required (see ordering information, page 36).

Using an internal control

Use of the EZ1 Virus Mini Kit in combination with commercially available amplification systems may require introduction of internal controls into the purification procedure to monitor the efficiency of sample preparation and downstream assay. The internal control is added to the mixture of carrier RNA and QIAGEN Protease.

The amount of internal control added (IC_{SAM}) depends on the assay system and the elution volume chosen within the EZ1 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. Take into account that only 75 µl of the total 85 µl protease–carrier RNA–internal control mixture are used for the purification procedure. Refer to Appendix A (page 28) for further guidance.

The volume of the internal control to be added to the purification procedure (IC_{SAM}) should be between 1 µl and 7 µl. If IC_{SAM} is less than 7 µl, add Buffer AVE to bring the volume up to 7 µl. Internal control DNA or RNA should be combined with carrier RNA (3 µl) and reconstituted QIAGEN Protease (75 µl).

Note: If you want to add more than 7 µl (up to a maximum of 40 µl) internal control solution, refer to Appendix A (page 28).

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

Note: If the internal control is stable in plasma or serum (e.g., armored RNA), it can be added to the sample shortly before beginning the sample preparation procedure. Use a mixture of QIAGEN Protease and carrier RNA as described above.

If desired, carrier RNA–internal control mixture can be stored for long periods by dividing it into aliquots and storing at –20°C.

Working with EZ1 instruments

The main features of the EZ1 instruments include:

- Purification of high-quality nucleic acids
- Small footprint with no external computer to save laboratory space
- Preprogrammed EZ1 Cards containing ready-to-use protocols for nucleic acid purification
- Prefilled, sealed reagent cartridges for easy, safe, and fast setup of the EZ1 worktable
- Complete automation of nucleic acid purification from opening of reagent cartridges to elution of nucleic acids, with no manual centrifugation steps

Additional features of the EZ1 Advanced and EZ1 Advanced XL include:

- Bar code reading and sample tracking
- UV lamp to help eliminate sample carryover from run to run and to allow decontamination of the worktable surfaces

Note: UV decontamination helps to reduce possible pathogen contamination of the EZ1 Advanced and EZ1 Advanced XL worktable surfaces. The efficiency of inactivation must be determined for each specific organism and depends, for example, on layer thickness and sample type. QIAGEN cannot guarantee complete eradication of specific pathogens.

EZ1 Cards, EZ1 Advanced Cards, and EZ1 Advanced XL Cards

Protocols for nucleic acid purification are stored on preprogrammed EZ1 Cards (integrated circuit cards). The user simply inserts an EZ1 Advanced XL Card into the EZ1 Advanced XL, an EZ1 Advanced Card into the EZ1 Advanced, or an EZ1 Card into the BioRobot EZ1, and the instrument is then ready to run a protocol (Figure 1). The availability of various protocols increases the flexibility of the EZ1 instruments.

Figure 1. Ease of protocol setup using EZ1 Cards. Inserting an EZ1 Card, containing a protocol, into an EZ1 instrument. The instrument should only be switched on after an EZ1 Card is inserted. EZ1 Cards should not be exchanged while the instrument is switched on.

The EZ1 Virus Mini Kit requires use of the EZ1 Advanced XL Virus Card with the EZ1 Advanced XL, or use of the EZ1 Advanced Virus Card with the EZ1 Advanced, or use of the EZ1 Virus Card with the BioRobot EZ1. These EZ1 Cards contain protocols for purifying viral nucleic acids from serum and plasma.

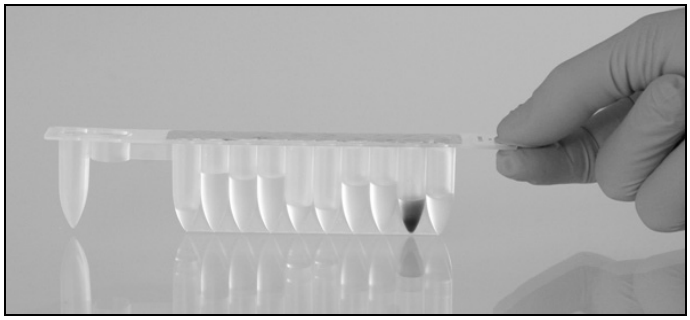
EZ1 instruments should only be switched on after an EZ1 Card is inserted. Make sure that the EZ1 Card is completely inserted (Figure 2), otherwise essential instrument data could be lost, leading to a memory error. EZ1 Cards should not be exchanged while the instrument is switched on.

Figure 2. Complete insertion of EZ1 Card. The EZ1 Card must be completely inserted before the EZ1 instrument is switched on.

Reagent cartridges

Reagents for the purification of nucleic acids from a single sample are contained in a single reagent cartridge (Figure 3). Each well of the cartridge contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or RNase-free elution buffer.

A



B

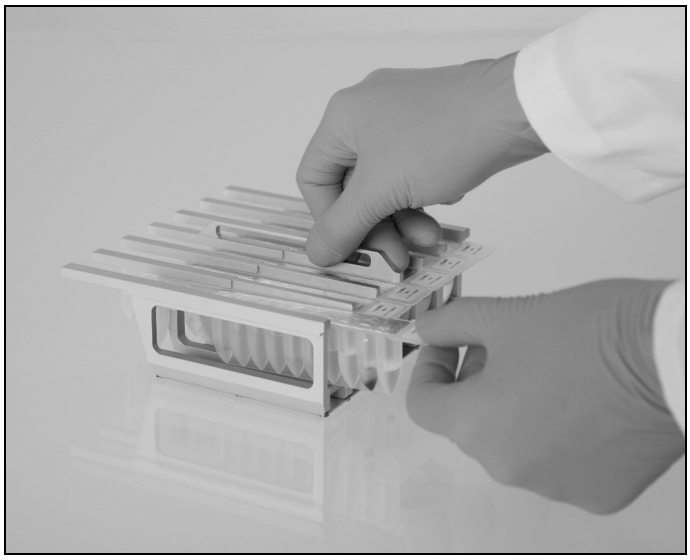


Figure 3. Ease of instrument setup using reagent cartridges. A A sealed, prefilled reagent cartridge. Fill levels vary, depending on the type of reagent cartridge. B Loading reagent cartridges into the cartridge rack. The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges must be loaded.

The reagent cartridges supplied with the EZ1 Virus Mini Kit are prefilled with all the necessary reagents for viral nucleic acid purification, except QIAGEN Protease, carrier RNA, and internal control (optional). These components must be added in a tube outside the reagent cartridge at the start of the purification procedure.

Worktable

The worktable of the EZ1 instrument is where the user loads samples and the components of the EZ1 Virus Mini Kit (Figure 4).

Details on worktable setup are provided in the protocol in this handbook, and are also displayed in the vacuum fluorescent display (VFD) of the EZ1 Advanced and EZ1 Advanced XL or the liquid crystal display (LCD) of the BioRobot EZ1 control panel when the user starts worktable setup.

The display also shows updates during the automated purification procedure.

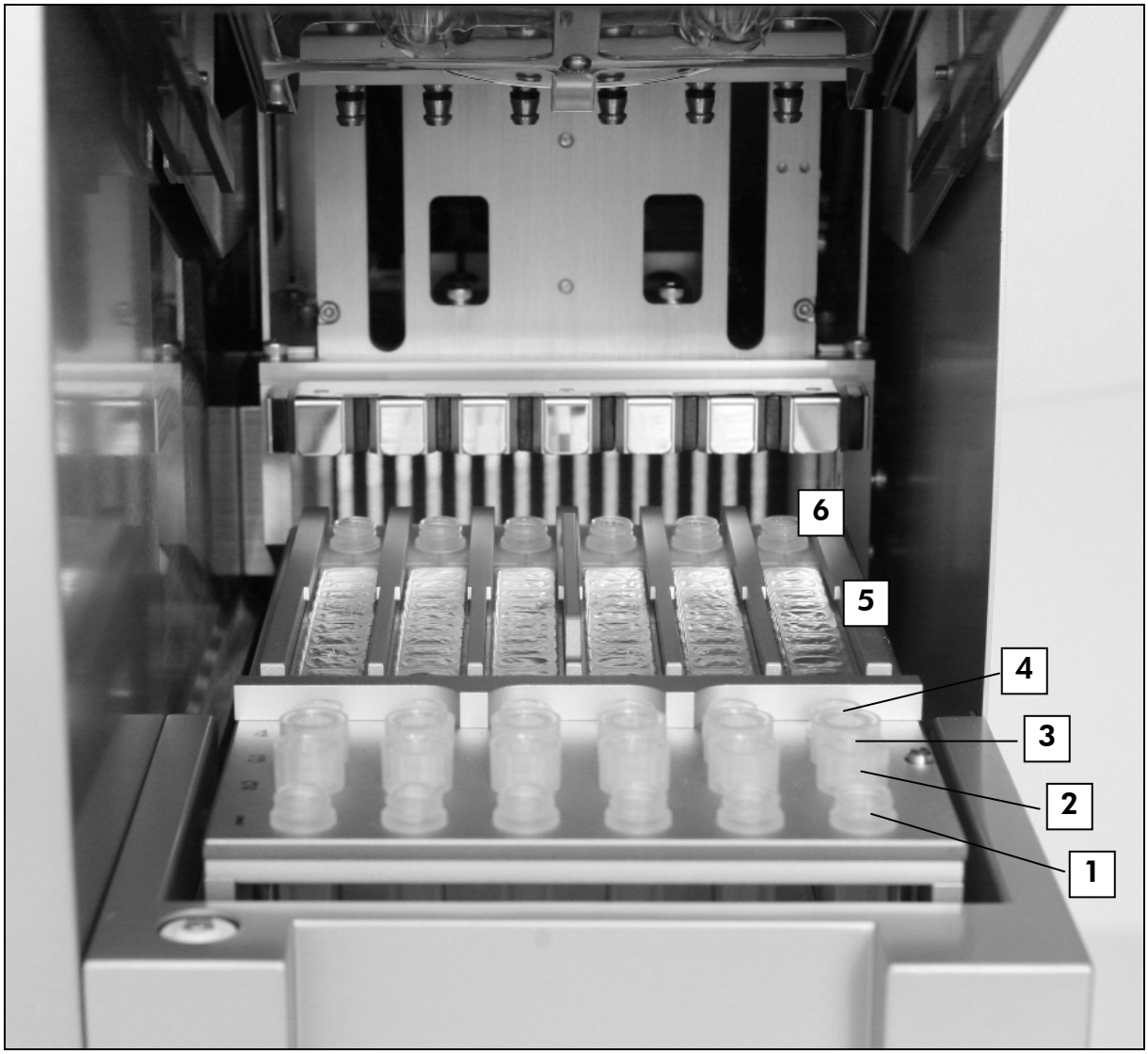


Figure 4. Typical EZ1 worktable.

1. Elution tubes (1.5 ml) loaded into the first row.
2. Tip holders containing filter-tips loaded into the second row.
3. Tubes (1.5 ml) containing QIAGEN Protease, carrier RNA, and internal control (if used), loaded into the third row.
4. Sample tubes (2 ml) loaded into the fourth row.
5. Reagent cartridges loaded into the cartridge rack.
6. Heating block with 1.5 ml tubes in the reagent cartridges.

Data tracking with the EZ1 Advanced and EZ1 Advanced XL

The EZ1 Advanced and EZ1 Advanced XL enable complete tracking of a variety of data for increased process control and reliability. A user ID can be entered manually using the keypad or by scanning bar codes using the handheld bar code reader. Cartridge lot numbers and sample and assay information can also be optionally entered at the start of the protocol. At the end of the protocol run, a report file is automatically generated. The EZ1 Advanced and EZ1 Advanced XL can store up to 10 report files, and the data can be transferred to a PC or directly printed on a printer (for ordering information, see "Equipment and Reagents to Be Supplied by User" on page 11).

To receive report files on a PC, the EZ1 Advanced Communicator software needs to be installed. The software receives the report file and stores it in a folder that you define. After the PC has received the report file, you can use and process the file with a LIMS (Laboratory Information Management System) or other programs. An example of a report file is shown in Appendix C (page 33). In report files, the 6 pipetting channels of the EZ1 Advanced are named, from left to right, channels A to F, or the 14 pipetting channels of the EZ1 Advanced XL are named, from left to right, channels 1–14.

When scanning a user ID bar code with the bar code reader, a beep confirms data input. After the information is displayed for 2 seconds, it is automatically stored, and the next display message is shown. When scanning sample ID, assay kit ID, or notes, a beep confirms data input, the information is displayed, and a message prompts you to enter the next item of information. After scanning sample ID, assay kit ID, and notes, press "ENT" once to confirm that the information entered is correct. If, for example, a wrong bar code was scanned for one of the samples, press "ESC" and then rescan all sample bar codes according to the onscreen instructions. For user ID and notes, you can enter the numbers using the keypad, or you can easily generate your own bar codes to encode these numbers.

For details about data tracking on the EZ1 Advanced and EZ1 Advanced XL and using EZ1 Advanced Communicator software, see the *EZ1 Advanced User Manual* or the *EZ1 Advanced XL User Manual*.

Workflow of EZ1 Virus operation

Insert EZ1 Card into the EZ1 Card slot

↓

Switch on the EZ1 instrument

↓

Follow onscreen messages for data tracking*

↓

Follow onscreen messages for worktable setup

↓

Start the protocol

↓

Collect purified nucleic acids

↓

UV decontamination*

Yields of viral nucleic acids

The yields of viral nucleic acids obtained in the purification procedure are normally below 1 µg and therefore difficult to quantify using a spectrophotometer. We recommend using quantitative amplification methods to determine yields. Remember that the purified nucleic acids contain much more carrier RNA than viral nucleic acids.

Storing viral nucleic acids

For short-term storage of up to 24 hours, it is recommended to store the purified viral DNA and RNA at 2–8°C. For long-term storage of over 24 hours, storage at –20°C is recommended.

* EZ1 Advanced and EZ1 Advanced XL only.

Protocol: Purification of Viral DNA and RNA from Plasma and Serum

Important points before starting

- If using the EZ1 Virus Mini Kit for the first time, read "Important Notes" (page 12).
- After receiving the kit, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Services or your local distributor. In the case of liquid spillage, refer to "Safety Information" (page 6). Do not use damaged kit components, since their use may lead to poor kit performance or contamination of the EZ1 instrument.
- The reagent cartridges contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for safety information.
- Perform all steps of the protocol at room temperature (15–25°C). During the setup procedure, work quickly.
- In some steps of the procedure, one of 2 choices can be made. Choose ▲ if using the EZ1 Advanced or the EZ1 Advanced XL; choose ● if using the BioRobot EZ1.

Things to do before starting

- Buffer AL in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature.
- Prepare serum and plasma samples as described in "Preparing samples", page 12. If cryoprecipitates are visible in the thawed samples, centrifuge at 6800 x *g* for 3 minutes, transfer the supernatants to fresh tubes without disturbing the pellets, and start the purification procedure immediately.
- Reconstitute QIAGEN Protease, as described in "Reconstituting QIAGEN Protease", page 12, before using it for the first time. Store unused, reconstituted QIAGEN Protease at 2–8°C or frozen in aliquots at –20°C.
- Prepare a carrier RNA stock solution (with optional internal control) before using it for the first time. Dissolve the lyophilized carrier RNA in 300 µl Buffer AVE (provided in the kit), and mix it with the internal control (optional) as described in "Preparing carrier RNA" and "Using an internal control", starting on page 13.

Procedure

1. **For each sample, prepare a solution containing 75 µl reconstituted QIAGEN Protease and 10 µl dissolved carrier RNA (with optional internal control and Buffer AVE) in a 1.5 ml tube (supplied). Mix gently by pipetting the solution 10 times. Do not vortex.**

   The 1.5 ml tube is loaded into the third row in step 8.

   Note: Make sure that the protease–carrier RNA solution is at the bottom of the 1.5 ml tube. This ensures that the appropriate amount can be transferred by the EZ1 instrument.

2. **Transfer 100 µl, 200 µl, or 400 µl serum or plasma into 2 ml sample tubes, and equilibrate to room temperature (15–25°C) before loading on the worktable. If using frozen samples, thaw and equilibrate at room temperature, and mix well by vortexing.**

   Note: For optimal performance it is essential to use the 2 ml tubes provided with the kit.

   Note: Do not refreeze thawed samples or store samples for over 6 hours at 2–8°C, as this leads to significantly reduced yields of viral nucleic acids.

   We recommend using 100 µl, 200 µl, or 400 µl of serum or plasma. If you want to use less sample, bring the volume up to 100 µl, 200 µl, or 400 µl with the appropriate amount of Buffer AVE (Buffer AVE is available separately; see page 36 for ordering information).

3. **Insert ▲ the EZ1 Advanced XL Virus Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or the EZ1 Advanced Virus Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or ● the EZ1 Virus Card completely into the EZ1 Card slot of the BioRobot EZ1.**
4. **Switch on the EZ1 instrument.**

   The power switch is located on the left rear of the instrument.

5. **Press "START" to start worktable setup of the EZ1 Virus Mini protocol.**
6. **Open the instrument door.**
7. **Invert reagent cartridges 3 times to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells.**

8. **Follow the onscreen instructions for worktable setup, protocol variable selection, and ▲ data tracking.**

   Note: After sliding a reagent cartridge into the cartridge rack, press down on the cartridge until it clicks into place.

   If there are fewer than 6 (BioRobot EZ1, EZ1 Advanced) or 14 (EZ1 Advanced XL) reagent cartridges, they can be loaded in any order on the rack. However, when loading the other labware, ensure that they also follow the same order.

   ▲ When using the data tracking option, ensure that the sample ID follows the same order as the samples on the worktable to avoid data mix up.

9. **Close the instrument door.**
10. **Press "START" to start the protocol.**
11. **When the protocol ends, the display shows "Protocol finished". ▲ Press "ENT" to generate the report file.**

    The EZ1 Advanced and EZ1 Advanced XL can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.

12. **Open the instrument door.**
13. **Remove the elution tubes containing the purified viral nucleic acids. Discard the sample-preparation waste.***
14. **▲ Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.**
15. **Carry out the regular maintenance procedure as described in the user manual supplied with your EZ1 instrument.**

    Regular maintenance must be carried out at the end of each protocol run. It consists of cleaning the piercing unit and the worktable surfaces.

    Note: The piercing unit is sharp! Use of double gloves is recommended.

16. **To run another protocol, press "START", carry out steps 1 and 2 of the protocol, and then follow the protocol from step 5. Otherwise press "STOP" twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.**

    Steps 3–4 are not necessary when running another protocol. Skip these steps.

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 6 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
General handling	
a) Error message in instrument display	Refer to the user manual supplied with your EZ1 instrument.
b) Report file not printed	Check whether the printer is connected to the EZ1 Advanced/EZ1 Advanced XL via the "PC/Printer" serial port. Check whether the serial port is set for use with a printer.
c) Report file not sent to the PC	Check whether the PC is connected to the EZ1 Advanced/EZ1 Advanced XL via the "PC/Printer" serial port. Check whether the serial port is set for use with a PC.
Low yield of viral DNA and RNA	
a) Magnetic particles not completely resuspended	Ensure that you invert the reagent cartridges twice to resuspend the magnetic particles.
b) Insufficient reagent aspirated	After inverting the reagent cartridges to resuspend the magnetic particles, ensure that you tap the cartridges to deposit the reagents at the bottom of the wells.
c) Reagents loaded onto worktable in wrong order	Ensure that all tubes and the tip holders with the tips are loaded onto the worktable in the correct order. Repeat the purification procedure with new samples.

	Comments and suggestions
d) Carrier RNA not added to QIAGEN Protease	Reconstitute carrier RNA in Buffer AVE and mix with QIAGEN Protease as described in the protocol. Repeat the purification procedure with new samples.
e) QIAGEN Protease–carrier RNA not sufficiently mixed	Mix reconstituted QIAGEN Protease and carrier RNA by pipetting at least 10 times.
f) RNA degraded	RNA may have been degraded by RNases in the original serum or plasma samples. Ensure that the samples are processed immediately after collection or removal from storage.
g) Varying pipetting volumes	To ensure pipetting accuracy, it is important that buffer volumes in the reagent cartridges are correct and that the filter tips fit optimally to the tip adapter. Ensure that samples are thoroughly mixed and that reagent cartridges have not passed their expiry date. Perform regular maintenance as described in the instrument User Manual. Check the fit of the filter tips regularly as described in the User Manual.

RNA or DNA does not perform well in downstream enzymatic reactions

a) Little or no nucleic acid in the eluate	See "Low yield of viral DNA and RNA", page 24 for possible reasons. Increase the amount of eluate added to the downstream enzymatic reaction, if possible.
b) Frozen serum or plasma samples not mixed properly after thawing	Thaw frozen samples at room temperature (15–25°C) and mix by pulse vortexing for 15 s.
c) Nucleic acids in samples already degraded prior to purification	This can occur if samples were refrozen after thawing once or stored at room temperature for too long. Always use fresh samples or samples thawed only once. Repeat the purification procedure with new samples.

	Comments and suggestions
d) Insufficient sample lysis	This can occur if reconstituted QIAGEN Protease was stored at elevated temperatures for too long. Repeat the purification procedure using new samples and fresh QIAGEN Protease.
e) Salt carryover during elution	For best results, ensure that the reagent cartridges are at 20–30°C.
f) 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 QIAGEN Protease accordingly.
g) Too much eluate in the amplification reaction	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce the volume of eluate added to the amplification reaction or increase the elution volume accordingly.
h) Varying performance of purified nucleic acids in downstream assays	The salt and ethanol components of Buffer AW2 may have separated due to long-term storage. Always invert the cartridges thoroughly and tap them before a purification procedure.
i) Lack of sensitivity because of phosphate in the sample	Either avoid phosphate in the sample, or reconstitute the QIAGEN Protease in Protease Solvent or Buffer AVE (see page 36 for ordering information) instead of Protease Resuspension Buffer.
j) Lack of sensitivity because of inhibitory substances	Clean the piercing unit after each run.
k) Lack of sensitivity because of inhibitory substances	Increase the elution volume.
l) New combination of reverse transcriptase and *Taq* DNA polymerase	If the enzymes are changed, it may be necessary to readjust the amount of carrier RNA added to Buffer AL and the amount of eluate used.

	Comments and suggestions
m) Carryover of magnetic particles	Carryover of magnetic particles in the eluates will not affect most downstream applications, including RT-PCR. If the risk of magnetic-particle carryover needs to be minimized (e.g., for applications such as real-time PCR), first place the tubes containing eluate in a suitable magnet (e.g., 12-Tube Magnet [cat. no. 36912]) for 1 min, and then transfer the eluates to clean tubes. If a suitable magnet is not available, centrifuge the tubes containing eluates in a microcentrifuge at full speed for 1 min to pellet any remaining magnetic particles, and transfer the supernatants to clean tubes.

Appendix A: Calculating the Amount of Internal Control

To monitor the efficiency of sample preparation and downstream assay, an internal control may need to be added to the sample preparation process.

If you want to introduce an internal control into the purification procedure of the EZ1 Virus Mini kit, it is added to the mixture of carrier RNA and QIAGEN Protease. The amount of internal control added (IC_{SAM}) depends on the assay system and the elution volume chosen within the EZ1 protocol. In addition, it must be taken into account that only an aliquot of the internal control–carrier RNA–protease mixture is used in the purification procedure (i.e., a 75 µl aliquot of the 85 µl mixture is used).

As an example, a user is working with an assay that is optimized for use with 1 µl internal control solution per reaction (IC_{RXN}) and 20 µl of eluate per reaction (EL_{RXN}). The user follows the EZ1 Virus Mini protocol, and a 75 µl elution volume (EL_{SAM}) has been selected. To determine the amount of internal control, the following formula is used to calculate IC_{SAM}:

$$IC_{SAM} = IC_{RXN} \times \frac{EL_{SAM}}{EL_{RXN}} \times \frac{85}{75}$$

where:

IC_{SAM} = volume of internal control added to a purification sample

IC_{RXN} = volume of internal control added to a downstream reaction

EL_{SAM} = elution volume

EL_{RXN} = volume of eluate per downstream reaction

In this example, IC_{SAM} is 4.25 µl. For each sample to be processed, the following mixture is manually pipetted into the 1.5 ml tube in position 3 of the EZ1 instrument worktable:

IC_{SAM}	= 4.25 µl
Buffer AVE	= 2.75 µl
Carrier RNA	= 3.00 µl
QIAGEN Protease (reconstituted in 4.4 ml Protease Resuspension Buffer)	= 75.0 µl

If you want to add more than 7 µl internal control, prepare a concentrated QIAGEN Protease solution and decrease the amount of protease accordingly, e.g., reconstitute one vial of QIAGEN Protease with 2.2 ml Protease

Resuspension Buffer, use 37.5 µl of this 2x QIAGEN Protease. Add Protease Resuspension Buffer to bring the total volume to 85 µl, as in the following examples:

Example:

IC_{SAM}	=	10.0 µl
Protease Resuspension Buffer	=	34.5 µl
Carrier RNA	=	3.00 µl
2x QIAGEN Protease (reconstituted in 2.2 ml Protease Resuspension Buffer)	=	37.5 µl

Example:

IC_{SAM}	=	15.0 µl
Protease Resuspension Buffer	=	29.5 µl
Carrier RNA	=	3.00 µl
2x QIAGEN Protease (2x) (reconstituted in 2.2 ml Protease Resuspension Buffer)	=	37.5 µl

Note: If you do not have any information from the manufacturer of the downstream assay, you may calculate the amount of internal control required in the EZ1 Virus Mini protocol from another known purification procedure.

This calculation can be performed as in the following description: a user prepares a mixture of internal control (IC_{PRE}), lysis buffer (LB_{PRE}), and carrier RNA solution ($cRNA_{PRE}$). An aliquot (LB_{SAM}) of this buffer–internal control–carrier RNA mixture is added to each purification sample. After the sample is eluted in the specified elution volume (EL_{SAM}), an aliquot of the eluate (EL_{RXN}) is taken for the downstream analysis. The volume of internal control that is present in the downstream assay, can be determined using the following formula:

$$IC_{RXN} = IC_{PRE} \times \frac{LB_{SAM}}{(LB_{PRE} + cRNA_{PRE} + IC_{PRE})} \times \frac{EL_{RXN}}{EL_{SAM}}$$

where:

IC_{RXN}	=	volume of internal control added to the downstream reaction
IC_{PRE}	=	volume of internal control in the buffer–internal control–carrier RNA mixture
LB_{SAM}	=	volume of buffer–internal control–carrier RNA mixture added to each sample

LB_{PRE} = volume of lysis buffer in the buffer–internal control–carrier RNA mixture

$cRNA_{PRE}$ = volume of carrier RNA solution in the buffer–internal control–carrier RNA mixture

EL_{RXN} = volume of eluate added to downstream reaction

EL_{SAM} = elution volume

For example, a user has added 300 µl of internal control solution to 22.1 ml lysis buffer and 152 µl carrier RNA. During the sample preparation, 435 µl of this buffer–internal control–carrier RNA mixture is added per sample. An elution volume of 75 µl has been selected by the user, and 50 µl of the eluate will be added to the downstream reaction. The volume of internal control solution in each downstream reaction is as follows:

$$IC_{RXN} = 300\ \mu l \times \frac{435\ \mu l}{22{,}100\ \mu l + 152\ \mu l + 300\ \mu l} \times \frac{50\ \mu l}{75\ \mu l} = 3.86\ \mu l$$

The final downstream reactions contain 3.86 µl of internal control solution.

Appendix B: General Remarks on Handling RNA

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 even 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 isolation procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep isolated RNA on ice when aliquots are pipetted for downstream applications.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA* followed by RNase-free water (see "Solutions", page 32). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

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

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for four or more hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with a detergent solution (e.g., 0.5% SDS),* thoroughly rinsed with RNase-free water, and then rinsed with ethanol*† and allowed to dry.

Solutions

Solutions (water and other solutions)* should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Note: The buffers of the EZ1 Virus Mini Kit are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

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

† Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

Appendix C: Example of an EZ1 Advanced Report File

This appendix shows a typical report file generated on the EZ1 Advanced. The EZ1 Advanced XL generates a similar report file containing instrument and protocol information relevant to the EZ1 Advanced XL and information for channels 1–14. The values for each parameter will differ from the report file generated on your EZ1 Advanced. Please note that "User ID" is allowed a maximum of 9 characters, and that "Assay kit ID" and "Note" are allowed a maximum of 14 characters.

REPORT - FILE EZ1 Advanced:

Serial no. EZ1 Advanced: ..... SN 0001
User ID: 9876543210
Firmware version: ..... V 1.0.0
Installation date of instr.: ..... Jan 05, 2008
Weekly maintenance done on: ..... Jun 13, 2008
Yearly maintenance done on: ..... Jan 10, 2008
Date of last UV-run: ..... Jun 13, 2008
Start of last UV-run: 16:06
End of last UV-run: 16:26
Status UV-run: ..... o.k.

Protocol name: ..... Virus 1.10
.....
Date of run: ..... _ Jun 19, 2008
Start of run: 12:57
End of run: 13:50
Status run: ..... o.k.
Error code: .....
Sample input volume [ul]: ..... _ 400
Elution volume [ul]: 150

Channel A:
Sample ID: 123456789
Reagent Kit number: ..... _ 955338
Reagent Lot number: 127135225
Reagent Expiry date: .....
Assay Kit ID: 848373922
Note: .....

Channel B:
Sample ID: 234567891
Reagent Kit number: ..... _ 955338
Reagent Lot number: 127135225
Reagent Expiry date: .....
Assay Kit ID: 848373922
Note: .....

Channel C:
Sample ID: 345678912

Reagent Kit number: ..... _ 955338
Reagent Lot number: 127135225
Reagent Expiry date: .....
Assay Kit ID: 848373922
Note: .....

Channel D:
Sample ID: 456789123
Reagent Kit number: ..... _ 955338
Reagent Lot number: 127135225
Reagent Expiry date: .....
Assay Kit ID: 848373922
Note: .....

Channel E:
Sample ID: 567891234
Reagent Kit number: ..... _ 955338
Reagent Lot number: 127135225
Reagent Expiry date: .....
Assay Kit ID: 848373922
Note: .....

Channel F:
Sample ID: 678912345
Reagent Kit number: ..... _ 955338
Reagent Lot number: 127135225
Reagent Expiry date: .....
Assay Kit ID: 848373922
Note: .....

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.
EZ1 Virus Mini Kit (48)	For 48 virus nucleic acid preps: Reagent Cartridges, Disposable Tips, Disposable Tip-Holders, Sample Tubes, Elution Tubes, Buffers	955338
EZ1 Virus Card	Preprogrammed card for EZ1 Virus purification protocols on the BioRobot EZ1	9016386
EZ1 Advanced Virus Card	Preprogrammed card for EZ1 Virus purification protocols on the EZ1 Advanced	9018304
EZ1 Advanced XL Virus Card	Preprogrammed card for EZ1 Virus purification protocols on the EZ1 Advanced XL	9018707
EZ1 Virus Mini Kit v2.0 (48)	For 48 virus nucleic acid preps: Reagent Cartridges, Disposable Tips, Disposable Tip-Holders, Sample Tubes, Elution Tubes, Buffers	955134
EZ1 Advanced XL Virus Card v2.0	Preprogrammed card for purification of viral DNA and RNA on an EZ1 Advanced XL instrument using the EZ1 Virus Mini Kit v2.0	9018708
EZ1 Advanced Virus Card v2.0	Preprogrammed card for purification of viral DNA and RNA on an EZ1 Advanced instrument using the EZ1 Virus Mini Kit v2.0	9018303
EZ1 Virus Card v2.0	Preprogrammed card for purification of viral DNA and RNA on a BioRobot EZ1 instrument using the EZ1 Virus Mini Kit v2.0	9017330

Product	Contents	Cat. no.
Accessories		
EZ1 Advanced XL	Robotic instrument for automated purification of nucleic acids from up to 14 samples using EZ1 Kits, 1-year warranty on parts and labor*	9001492
EZ1 Advanced	Robotic instrument for automated purification of nucleic acids using EZ1 Kits, 1-year warranty on parts and labor*	9001410
Filter-Tips and Holders, EZ1 (50)	50 Disposable Filter-Tips, 50 Disposable Tip Holders; additional tips and holders for use with EZ1 Kits	994900
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912
Buffer AVE (108 x 2 ml)	108 tubes containing 2 ml Buffer AVE	1020953
Carrier RNA (12 x 1350 µg)	12 tubes containing 1350 µg carrier RNA	1017647
Protease Solvent	1 tube containing 6 ml of Protease Solvent	1021055
PC	PC capable of connection with up to 4 EZ1 Advanced instruments	9016310
TFT Monitor, 17"	Monitor for use with PC	9016308
Printer	Printer for connection with EZ1 Advanced instrument	9018464
Printer Accessory Package	Accessories for printer connected to EZ1 Advanced instrument	9018465

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* Warranty PLUS 2 (cat. no. 9237720) recommended: 3-year warranty, 1 preventive maintenance visit per year, 48-hour priority response, all labor, travel, and repair parts.

Notes

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the EZ1 Virus Mini Kit to the following terms:

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