March 2024

EZ1&2™ RNA Tissue Mini Handbook

For purification of total RNA from tissue and cells using EZ1® instruments

Sample to Insight

Contents

Kit Contents

EZ1&2 RNA Tissue Mini Kit Catalog no. Number of preps	 959034 48
Reagent Cartridge, Tissue RNA*	48
Disposable Tip Holders	100
Disposable Filter-Tips	100
Sample Tubes (2 mL)	50
Elution Tubes (1.5 mL)	50
Buffer RLT*	45 mL
RNase-Free DNase I (lyophilized)	750 Kunitz units†
RNase-Free Water	1.5 mL
Quick-Start Protocol	1

* Contains guanidine salt. Not compatible with disinfectants containing bleach. See page 7 for safety information.

† Kunitz units are the commonly used units for measuring DNase I, defined as the amount of DNase I that causes an increase in A_{260} of 0.001 per minute per milliliter at 25°C, pH 5.0, with highly polymerized DNA as the substrate (Kunitz, M. [1950] J. Gen. Physiol. 33, 349 and 363).

Additional filter-tips, tip holders, Buffer RLT, and QIAzol® Lysis Reagent are available separately. The RNase-Free DNase Set is available separately for DNase digestion during the EZ1 RNA Universal Tissue procedure. See Ordering Information starting on page 75.

Shipping and Storage

The EZ1&2 RNA Tissue Mini Kit is shipped at ambient temperature. The box containing RNase-free DNase I and RNase-free water should be stored immediately upon receipt at 2–8°C. The remaining components of the kit should be stored dry at room temperature (15–25°C). All kit components are stable for at least 9 months under these conditions.

Intended Use

The EZ1&2 Tissue Mini Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

The EZ1&2 RNA Tissue Mini Kit is intended to be used with EZ1 or EZ2® Connect instruments from QIAGEN®.

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 RLT, QIAzol Lysis Reagent, and some buffers in the reagent cartridges contain guanidine thiocyanate or 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 XL, EZ1 Advanced, or BioRobot® EZ1, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite, followed by water.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the EZ1&2 RNA Tissue Mini Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The EZ1&2 RNA Tissue Mini Kit is for purification of total RNA from human and animal tissues (e.g., liver, spleen, and kidney) and cells.

This user manual describes processing of this kit with EZ1 instruments. For usage of EZ1&2 RNA Tissue Mini Kit with EZ2 instruments, please refer to quick-start protocol (**www.qiagen.com/HB-2975**).

Magnetic-particle technology provides high-quality RNA which is suitable for direct use in downstream applications such as amplification or other enzymatic reactions. EZ1 instruments perform all steps of the sample preparation procedure. Up to 6 samples are processed in a single run using the BioRobot EZ1 or the EZ1 Advanced. Up to 14 samples are processed in a single run using the EZ1 Advanced XL.

Principle and Procedure

Magnetic-particle technology combines the speed and efficiency of silica-based RNA purification with the convenient handling of magnetic particles (see flowchart, page 11). Nucleic acids are purified from lysates in one step through their binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet, and the DNA is removed by treatment with RNase-free DNase I. Then, the magnetic particles are efficiently washed, and the RNA is eluted in elution buffer.

Difficult samples are homogenized in QIAzol Lysis Reagent.* After addition of chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. RNA partitions to the upper, aqueous phase, while DNA partitions to the interphase and proteins to the lower,

* To be ordered separately.

organic phase or the interphase. The upper, aqueous phase is extracted, and nucleic acids are purified in one step following the automated EZ1 RNA procedure.

When purifying total nucleic acids, instead of total RNA from cells and easy-to-lyse tissues, the DNase digestion step is omitted. For details about total nucleic acid purification, see “Supplementary Protocols” on page 28.

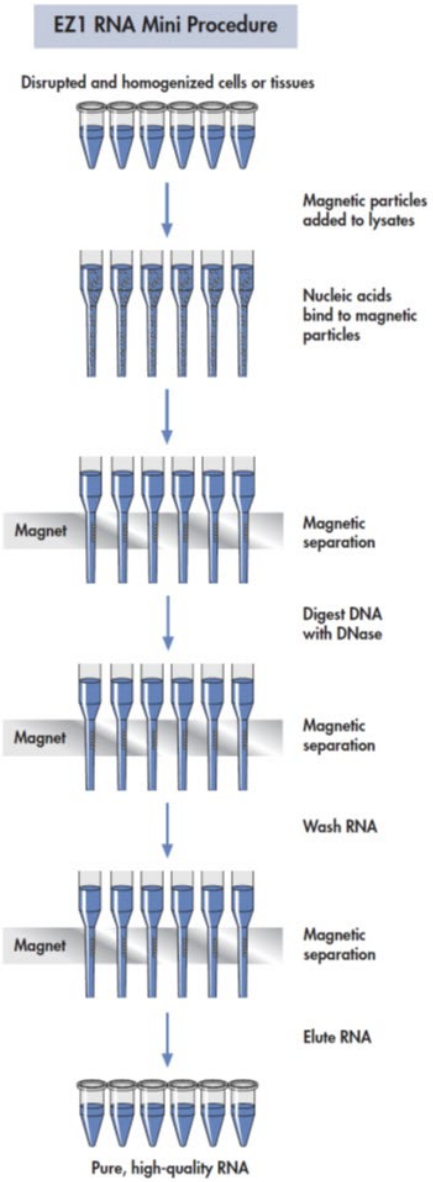

Figure 1. EZ1 RNA Mini Procedure

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 safety data sheets (SDSs), available from the product supplier.

For all protocols

- Pipettes and sterile, RNase-free pipette tips
- Reagent for RNA stabilization (for details, see page 17):
    - For cells: RNAprotect® Cell Reagent (cat. no. 76526), or liquid nitrogen, and dry ice
    - For tissues: RNAprotect® Tissue Reagent* (stabilizes RNA only)
- Allprotect® Tissue Reagent* (stabilizes DNA, RNA, and protein), or liquid nitrogen and dry ice
- Equipment for sample disruption and homogenization (for details, see pages 18–21) Depending on the method chosen, one or more of the following are required:
    - Trypsin and PBS
    - QIAshredder*
    - Blunt needle and syringe
    - Mortar and pestle
    - TissueRuptor® and TissueRuptor Disposable Probes*
    - TissueLyser LT, TissueLyser II, and TissueLyser accessories (TissueLyser LT Adapter, 12-Tube, or TissueLyser Adapter Set 2 x 24; Stainless Steel Beads, 5 mm; and optionally, TissueLyser Single-Bead Dispenser 5 mm)*
- 2 mL microcentrifuge tubes or other suitably sized vessel for homogenization

* For ordering information, see page 75.

- Soft paper tissue
- Water
- 70% ethanol

For EZ1 Advanced XL users

- EZ1 Advanced XL instrument (cat. no. 9001492)
- EZ1 Advanced XL RNA Card (cat. no. 9018705)

For EZ1 Advanced users

- EZ1 Advanced instrument* (discontinued)
- EZ1 Advanced RNA Card (cat. no. 9018297)

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. 9016319; 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)

* Ensure that the instruments have been checked, maintained, and calibrated regularly according to the manufacturer's recommendations.

For BioRobot EZ1 users

- BioRobot EZ1 DSP instrument* (discontinued)
- EZ1 RNA Card (cat. no. 9015590)

For Total RNA Purification from Cells and Easy-to-Lyse Tissues

- **Optional**: 14.3 M β-mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M)
- **Optional**: 2 M dithiothreitol (DTT)

For Total RNA Purification from Any Type of Tissues Using QIAzol Lysis Reagent

- QIAzol Lysis Reagent (cat. no. 79306)
- Chloroform (without added isoamyl alcohol)
- Microcentrifuge at 4°C capable of centrifuging 2 mL microcentrifuge tubes at 12,000 x *g*

* Ensure that the instruments have been checked, maintained, and calibrated regularly according to the manufacturer's recommendations.

Important Notes

Determining the amount of starting material

The EZ1&2 RNA Tissue Mini Kit is optimized for RNA purification cells and tissues as listed in Table 1. If you use more than these amounts, you may not achieve further increases in RNA yields.

Table 1. Amounts of starting material and elution volumes used in EZ1 RNA procedures*

Sample	EZ1 RNA Card protocol	Amount of starting material	Elution volume (µL)
Cultured cells	EZ1 RNA Cell	10 – 1 x 10^6 cells†	50–200
Cultured cells	EZ1 RNA Universal Tissue	≤1 x 10^7 cells‡	50–200
White blood cells	EZ1 RNA Cell	10 – 2 x 10^6 cells†	50–200
Easy-to-lyse tissue§	EZ1 RNA Tissue	≤10 mg tissue†	50–200
Standard tissue, flash-frozen§	EZ1 RNA Universal Tissue	≤50 mg tissue‡	50–200
Adipose tissue	EZ1 RNA Universal Tissue	≤100 mg tissue‡	50–200
Liver, thymus, or spleen, flash-frozen tissue	EZ1 RNA Universal	≤25 mg tissue‡	50–200
RNAprotect/Allprotect stabilized tissue	EZ1 RNA Universal Tissue	≤25 mg tissue‡	50–200

* Supplementary protocols (e.g., for purification of total nucleic acids) are available at **www.qiagen.com/literature**
† Sample volume 300 µL.
‡ Sample volume 300–400 µL.
§ For example, kidney, liver, and spleen.

Direct counting is the most accurate way to quantify the number of cells. However, as a guide, the number of HeLa cells obtained in various culture dishes after confluent growth is given in Table 2. When using the EZ1&2 RNA Tissue Mini Kit to purify total RNA from 1 × 10^6 HeLa cells and 1 × 10^6 HL-60 cells, the average yields are 15 µg and 5 µg, respectively. RNA yield can vary due to factors such as cell type, developmental stage, and growth conditions. Typical RNA yields are given in Table 2.

Table 2. Growth area and number of HeLa cells in various culture dishes

Cell culture vessel	Growth area (cm^2)*	Number of cells†
Multiwell plates		
96-well	0.32–0.6	$4–5 \times 10^4$
48-well	1	1×10^5
24-well	2	2.5×10^5
12-well	4	5×10^5
6-well	9.5	1×10^6
Dishes		
35 mm	8	1×10^6

* Per well, if multiwell plates are used; varies slightly depending on the supplier.

† Cell numbers are given for HeLa cells (approximate length = 15 µm), assuming confluent growth. Numbers will vary for different kinds of animal cells, which can vary in length from 10 to 30 µm.

Weighing is the most accurate way to quantify the amount of tissue. As a guide, a 1.5 mm cube (volume, approximately 3.4 mm^3) of most animal tissues weighs 3.5–4.5 mg. The average yield of total RNA varies depending on the type of tissue sample being processed. In addition, RNA yield can vary due to factors such as species, developmental stage, and growth conditions.

Handling and storing starting material

Cells

After harvesting, cells should be immediately lysed in Buffer RLT or QIAzol Lysis Reagent to prevent unwanted changes in the gene expression profile. The highly denaturing lysis conditions inactivate RNases and other proteins to prevent RNA degradation as well as downregulation or upregulation of transcripts.

If the cells are to be shipped to another lab for RNA purification, they should be pelleted, frozen in liquid nitrogen, and transported on dry ice. Alternatively, the cells can be mixed with RNAprotect Cell Reagent at room temperature (15–25°C) and then shipped at ambient temperature.

Tissues

The RNA in harvested tissue is not protected until the sample is treated with RNAprotect Tissue Reagent, flash-frozen, or disrupted and homogenized in the presence of RNase-inhibiting or denaturing reagents. Otherwise, unwanted changes in the gene expression profile will occur. It is therefore important that tissue samples are immediately frozen in liquid nitrogen and stored at –90°C to –65°C, or immediately immersed in RNAprotect Tissue Reagent. An alternative to RNAprotect Tissue Reagent is Allprotect Tissue Reagent, which provides immediate stabilization of DNA, RNA, and protein in tissue samples at room temperature (15–25°C).

The procedures for tissue harvesting and RNA protection should be carried out as quickly as possible. Frozen tissue samples should not be allowed to thaw during handling or weighing. After disruption and homogenization in Buffer RLT (lysis buffer) or QIAzol Lysis Reagent, samples can be stored at –90°C to –65°C for months.

Disrupting and homogenizing starting material

Efficient disruption and homogenization of the starting material is an absolute requirement at the start of the EZ1 RNA Tissue Mini procedure. Disruption and homogenization are 2 distinct steps:

- Disruption: Complete disruption of plasma membranes of cells and organelles releases all the RNA contained in the sample. Incomplete disruption results in significantly reduced yields.
- Homogenization: Homogenization reduces the viscosity of the cell lysates produced by disruption. Homogenization shears high-molecular-weight genomic DNA and other high-molecular-weight cellular components to create a homogeneous lysate. Incomplete homogenization results in inefficient binding of RNA to the magnetic particles and therefore significantly reduced RNA yields.

Some disruption methods simultaneously homogenize the sample, while others require an additional homogenization step.

Disruption of cells is achieved by vortexing or mixing in Buffer RLT. The method of homogenization depends on the cell count of the sample. If the cell count is 1 x 10^5 cells or fewer, efficient homogenization is achieved by vortexing the sample. If the cell count is higher (1 × 10^5 to 1 × 10^6), homogenization must be performed using one of the 4 types of homogenizers:

- QIAshredder homogenizer
- TissueRuptor or other rotor–stator homogenizer
- Syringe and needle
- TissueLyser LT, TissueLyser II, or other bead mill

Disruption and homogenization of tissue can be performed using one of the 3 methods:

- Disruption and homogenization using the TissueRuptor or other rotor–stator homogenizer
- Disruption using a mortar and pestle, and homogenization using a QIAshredder homogenizer or syringe and needle
- Disruption and homogenization using the TissueLyser LT, TissueLyser II, or other bead mill

Note: After storage in RNAprotect Tissue Reagent or Allprotect Tissue Reagent, tissues become slightly harder than fresh or thawed tissues. Disruption and homogenization of this tissue, however, is usually not a problem.

The different disruption and homogenization methods are described in more detail below.

Disruption and homogenization using the TissueRuptor

The TissueRuptor is a rotor–stator homogenizer that thoroughly disrupts and simultaneously homogenizes single tissue samples in the presence of lysis buffer in 15–90 seconds, depending on the toughness and size of the sample. The TissueRuptor can also be used to homogenize cell lysates. The blade of the TissueRuptor disposable probe rotates at a very high speed, causing the sample to be disrupted and homogenized by a combination of turbulence and mechanical shearing. For guidelines on using the TissueRuptor, refer to the *TissueRuptor Handbook*. For other rotor–stator homogenizers, refer to suppliers' guidelines.

Disruption and homogenization using the TissueLyser LT or TissueLyser II

In bead-milling, tissues can be disrupted by rapid agitation in the presence of beads and lysis buffer. Disruption and simultaneous homogenization occur by the shearing and crushing action of the beads as they collide with the cells. The TissueLyser II disrupts and homogenizes up to 48 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 × 24, which holds 48 × 2 mL microcentrifuge tubes containing stainless steel beads of 5 mm mean diameter. The TissueLyser II can also disrupt and homogenize up to 192 tissue samples

simultaneously when used in combination with the TissueLyser Adapter Set 2 × 96, which holds 192 × 1.2 mL microtubes containing stainless steel beads of 5 mm mean diameter. The TissueLyser II can also be used to homogenize cell lysates. The optimal beads for use with human or animal cells are 3–7 mm diameter steel beads. For guidelines on using the TissueLyser II, refer to the *TissueLyser Handbook*. For other bead mills, refer to suppliers' guidelines.

Note: Tungsten carbide beads react with Buffer RLT and QIAzol Lysis Reagent, and must not be used to disrupt and homogenize tissues.

The TissueLyser LT is a small bead mill which provides fast, effective disruption of up to 12 samples at the same time. This throughput matches that of the QIAcube®, which automates sample preparation using trusted QIAGEN spin-column kits. Simultaneous disruption and homogenization are achieved through high-speed shaking of samples in 2 mL microcentrifuge tubes with stainless steel or glass beads. The TissueLyser LT must be used in combination with the coolable TissueLyser LT Adapter, which holds tubes during the disruption process. Additional accessories include beads and bead dispensers.

Disruption using a mortar and pestle

For disruption using a mortar and pestle, freeze the tissue sample immediately in liquid nitrogen and grind to a fine powder under liquid nitrogen. Transfer the suspension (tissue powder and liquid nitrogen) into a liquid-nitrogen-cooled, appropriately sized tube and allow the liquid nitrogen to evaporate without allowing the sample to thaw. Add lysis buffer and continue as quickly as possible with the homogenization according to one of the 2 methods below:

Note: Grinding the sample using a mortar and pestle will disrupt the sample but will not homogenize it. Homogenization must be performed separately before proceeding with EZ1 RNA procedures. Homogenization must be done by using QIAshredder homogenizers.

Homogenization using QIAshredder

Using QIAshredder homogenizers is a fast and efficient way to homogenize cell and tissue lysates without cross-contamination of samples. Up to 700 µL of lysate is loaded onto a QIAshredder spin column placed in a 2 mL collection tube and spun for 2 minutes at maximum speed in a microcentrifuge. The lysate is homogenized as it passes through the spin column.

Homogenization using a syringe and needle

Cell lysates can be homogenized by using a syringe and needle. A high-molecular-weight DNA can be sheared by passing the lysate through a 20-gauge (0.9 mm) needle attached to a sterile plastic syringe at least 5 times or until a homogeneous lysate is achieved. Increasing the volume of lysis buffer may facilitate handling and minimize loss.

Working with EZ1 Instruments

The main features of the EZ1 instruments include:

- Purification of high-quality nucleic acids from 1–6 or 1–14 samples per run
- Small footprint 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 EZ1 instruments
- 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 has to 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 2). The availability of various protocols increases the flexibility of EZ1 instruments.

Figure 2. 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&2 RNA Tissue Mini Kit requires the use of the EZ1 Advanced XL RNA Card with the EZ1 Advanced XL, or use of the EZ1 Advanced RNA Card with the EZ1 Advanced, or use of the EZ1 RNA Card with the BioRobot EZ1. These EZ1 Cards contain protocols for purifying total RNA or total nucleic acids (for details about total nucleic acid purification, see “Supplementary Protocols” on page 28).

EZ1 instruments should only be switched on after an EZ1 Card is inserted. Make sure that the EZ1 Card is completely inserted (Figure 3), 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 3. 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 4). Each well of the cartridge contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or RNase-free water. Since each well contains only the required amount of reagent, generation of additional waste due to leftover reagent at the end of the purification procedure is avoided.

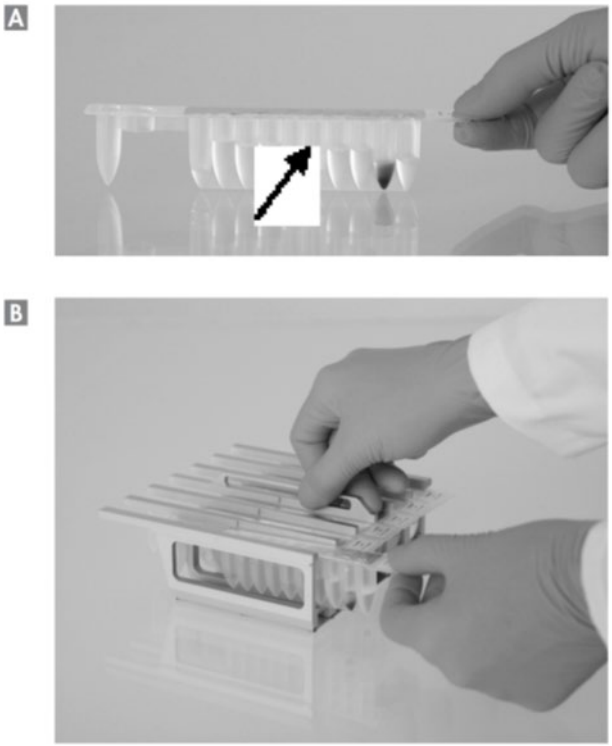

Figure 4. Ease of setup using reagent cartridges. (A) A sealed, prefilled reagent cartridge. Fill levels vary, depending on the type of reagent cartridge. Well 5, where DNase I is added by the user, is indicated with an arrow. **(B)** Loading reagent cartridges into the cartridge rack. The cartridge 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&2 RNA Tissue Mini Kit are prefilled with all the necessary reagents for RNA purification, except DNase I. Since DNase I has different storage requirements, the user needs to add DNase I to each reagent cartridge at the start of the purification procedure.

Addition of DNase I is optional when using the EZ1 RNA Universal Tissue protocols. The DNase I is not used in EZ1 RNA protocols for purification of total nucleic acids.

Worktable

The worktable of EZ1 instruments is where the user loads samples and the components of the EZ1&2 RNA Tissue Mini Kit (Figure 5).

Details on worktable setup are provided in the protocols 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 protocol status during the automated purification procedure.

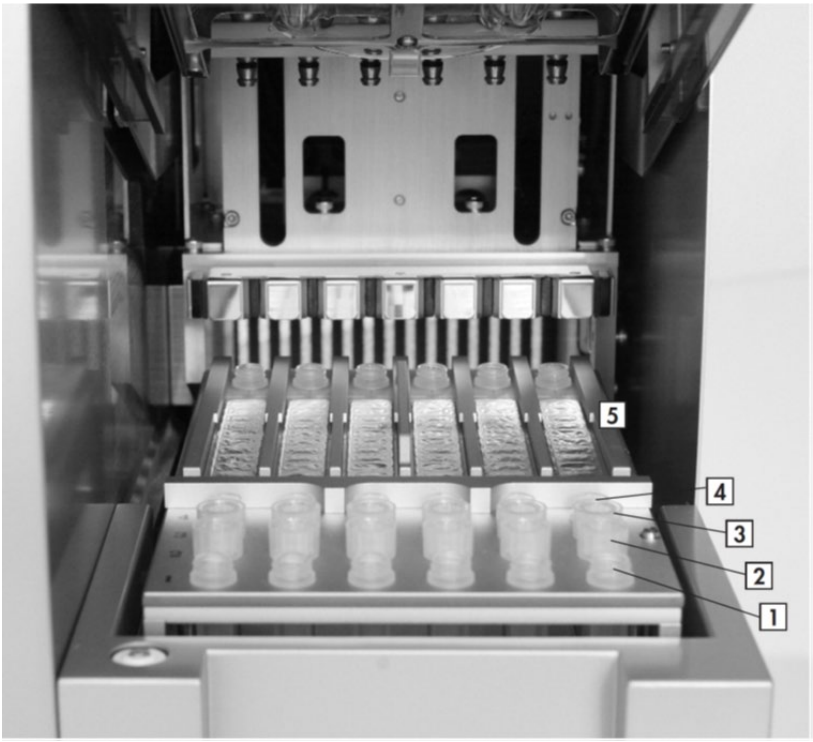

Figure 5. Typical EZ1 worktable. **1.) First row**: In EZ1 RNA protocols, elution tubes (1.5 mL) are loaded here. **2.) Second row**: In EZ1 RNA protocols, tip holders containing filter-tips loaded are loaded here. **3.) Third row**: In EZ1 RNA protocols, tip holders containing filter-tips loaded are loaded here. **4.) Fourth row**: In EZ1 RNA protocols, sample tubes (2 mL) are loaded here. **5.)** Reagent cartridges loaded into the cartridge rack.

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 and the reagent cartridge lot number can be entered manually using the keypad or by scanning bar codes using the handheld bar code reader. 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 12).

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 Laboratory Information Management System (LIMS) or other programs. An example of a report file is shown in Appendix F (page 72). 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 to 14. When scanning a user ID or reagent cartridge lot number with the bar code reader, a beep confirms the 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 and using EZ1 Advanced Communicator software, see the *EZ1 Advanced User Manual* or the *EZ1 Advanced XL User Manual*.

Workflow of EZ1 RNA 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*

Supplementary Protocols

The EZ1&2 RNA Tissue Mini Kit can also be used to purify total nucleic acids from tissues. Additional supplementary protocols are continuously being developed for new applications. Visit **www.qiagen.com/literature** to download the supplementary protocols for EZ1 RNA Kits.

Quantification of RNA

Carryover of magnetic particles may affect the absorbance reading at 260 nm (A_{260}) of the purified RNA but should not affect downstream applications. The measured absorbance at 320 nm (A_{320}) should be subtracted from all absorbance readings. See Appendix B, page 59, for more information.

* EZ1 Advanced and EZ1 Advanced XL only.

Protocol: Purification of Total RNA from Cells

This protocol is designed for purification of total RNA from human and animal cells.

Important points before starting

- If using the EZ1&2 RNA Tissue Mini Kit for the first time, read "Important Notes" (page 15).
- If working with RNA for the first time, read Appendix A: General Remarks on Handling RNA (page 56).
- If working with blood cells, read Appendix D: Preparing Human Blood Cells for Purification of Total RNA (page 66).
- Cell pellets can be stored at –90°C to –65°C for later use or used directly in the procedure. Determine the number of cells before freezing. Frozen cell pellets should be thawed slightly so that they can be dislodged by flicking the tube in step 2. Homogenized cell lysates from step 3 can be stored at –90°C to –65°C for several months. To thaw frozen lysates, incubate at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity. If any insoluble material is visible, centrifuge for 5 min at 3000–5000 x *g*. Transfer the supernatant to a new RNase-free glass or polypropylene tube and continue with step 4.
- Cells stored in RNAprotect Cell Reagent can also be used in the procedure. Transfer the entire sample, including any material deposited at the bottom of the storage vessel, to a centrifuge tube. Pellet the cells by centrifuging for 5 min at 5000 x *g,* and remove the supernatant by pipetting (if necessary, thaw the sample before centrifuging). Proceed immediately to step 2.
- Buffer RLT and the reagent cartridges contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 7 for safety information.
- Perform all steps of the procedure at room temperature (15–25°C).

- In some steps of the procedure, one of the 2 choices can be made. Choose ■ (blue) if using the EZ1 Advanced or the EZ1 Advanced XL; choose ▲ (red) if using the BioRobot EZ1.

Things to do before starting

- Buffer RLT may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).
- **Optional**: For cell lines with high RNase activity, such as white blood cells, we recommend adding either β-mercaptoethanol (β-ME) or dithiothreitol (DTT) to Buffer RLT before use. Add 10 µL β-ME per 1 mL Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature for up to 1 month. Alternatively, add 20 µL of 2 M DTT per 1 mL Buffer RLT. The stock 2 M DTT solution should be prepared in water and used immediately or frozen in single-use aliquots. Buffer RLT containing DTT can be stored at room temperature for up to 1 month.
- Prepare DNase I stock solution before using the RNase-free DNase I for the first time. Dissolve the lyophilized DNase I (750 Kunitz units) in 550 µL of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. **Do not vortex**.
- For long term storage of reconstituted DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –30°C to –15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

1. Harvest cells according to the type of growth:

   Cells grown in suspension (do not use more than 1 x 10^6 cells)

   1a. Determine the number of cells.
   1b. Pellet the appropriate number of cells by centrifuging for 5 min at 300 x *g* in a centrifuge tube (not supplied).
   1c. Carefully remove all supernatant by aspiration.
   1d. Proceed to step 2.
   1e. **Note**: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate which may reduce the RNA yield by affecting the conditions for binding of RNA to the magnetic particles.

   Cells grown in a monolayer (do not use more than 1 x 10^6 cells)

   Cells grown in a monolayer in cell-culture vessels can be either lysed directly in the vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet before lysis. Cells grown in a monolayer in cell-culture flasks should always be trypsinized.

   To **lyse** cells directly:

   1a. Determine the number of cells.
   1b. Completely aspirate the cell-culture medium.
   1c. Proceed immediately to step 2.

   Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, which may reduce RNA yield by affecting the conditions for binding of RNA to the magnetic particles.

To **trypsinize** and collect cells:

1a. Determine the number of cells.
1b. Aspirate the medium and wash the cells with PBS.
1c. Aspirate the PBS and add 0.10–0.25% trypsin in PBS.
1d. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 x *g* for 5 min.
1e. Completely aspirate the supernatant and proceed to step 2.

Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, which may reduce RNA yield by affecting the conditions for binding of RNA to the magnetic particles.

2. Disrupt the cells by adding Buffer RLT.

For pelleted cells:

2a. Loosen the cell pellet thoroughly by flicking the tube.
2b. Add 300 µL Buffer RLT.
2c. Vortex or pipette to mix.
2d. Proceed to step 3.

Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields.

For direct lysis of cells grown in a monolayer:

2a. Add 300 µL Buffer RLT to the cell culture dish.
2b. Collect the lysate with a rubber cell scraper.
2c. Pipette the lysate into a microcentrifuge tube (not supplied).
2d. Vortex or pipette to mix and ensure that no cell clumps are visible before proceeding to step 3.

3. Homogenize the lysate according to the method used.

   See "Disrupting and Homogenizing Starting Material" on page 18 for more details on disruption and homogenization. If processing ≤1 × 10^5 cells, they can be homogenized by vortexing for 1 min. After homogenization, proceed to step 4.

   Note: Incomplete homogenization can affect binding of nucleic acids to the magnetic particles and lead to significantly reduced RNA yields. Homogenization with the TissueLyser LT, TissueLyser II, or QIAshredder homogenizers generally results in higher RNA yields than with a syringe and needle.

   3a. Pipette the lysate directly into a QIAshredder spin column (not supplied) placed in a 2 mL collection tube, and centrifuge for 2 min at full speed. Proceed to step 4.

   3b. Place the tip of the TissueRuptor disposable probe into the lysate and operate the TissueRuptor at full speed until the lysate is homogenous (usually 30 s). Proceed to step 4.

   Note: To avoid damage to the TissueRuptor and disposable probe during operation, make sure the tip of the probe remains submerged in the buffer.

   3c. Pass the lysate at least 5 times through a blunt 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe. Proceed to step 4.

   3d. Transfer the lysate to a 2 mL microcentrifuge tube (not supplied) and add 1 stainless steel bead (5 mm diameter). Attach the caps to the tubes and place the tubes in the TissueLyser LT Adapter, 12-Tube, or the TissueLyser Adapter 2 × 24. Homogenize the lysate using the TissueLyser LT or TissueLyser II for 1 min at 20 Hz. Rotate the rack of tubes so that the tubes nearest to the TissueLyser LT or TissueLyser II are now outermost and homogenize for another 1 min at 20 Hz. Centrifuge the lysate for 3 min at full speed. Proceed to step 4.

   Note: The instructions in step 3d are only guidelines. They may need to be changed depending on the cell sample being processed and on the bead mill being used.

4. Transfer the homogenized lysates to the 2 mL sample tubes supplied with the kit.

5. Insert ■ the EZ1 Advanced RNA Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL RNA Card completely into the EZ1

Advanced XL Card slot of the EZ1 Advanced XL or ▲ the EZ1 RNA Card completely into the EZ1 Card slot of the BioRobot EZ1.

6. Switch on the EZ1 instrument.

   The power switch is located at the rear of the instrument.

7. Press **START** to start protocol setup. ■ Follow the onscreen instructions for data tracking.
8. Press **1** to select purification of total RNA, and then press **1** to select the EZ1 RNA Mini protocols.
9. Press **1** to start worktable setup for the protocol for purification from cells.
10. Press **1** to select an elution volume of 50 µL, **2** to select an elution volume of 100 µL, or **3** to select an elution volume of 200 µL.
11. Open the instrument door.
12. Invert the reagent cartridges 3 times to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells.
13. Follow the onscreen instructions for worktable setup. Before loading reagent cartridges into the cartridge rack, use a pipette tip to puncture the foil covering well 5 of each cartridge (see Figure 4A, page 24). Then pipette 10 µL reconstituted DNase into the liquid in well 5 of each cartridge.

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

    If there are fewer reagent cartridges than slots in the cartridge rack, load them onto the cartridge rack starting from the left slot and do not leave any empty slots between the cartridges. Be sure to load the other labware (i.e., tubes and tips) in the same order.

    Note: When using the data tracking option, ensure that the sample ID follows the same order as the samples on the worktable to avoid data mixup.

14. Close the instrument door.
15. Press **START** to start the protocol.
16. 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.

17. Open the instrument door.
18. Remove the elution tubes containing the purified RNA from the first row. Discard the sample-preparation waste.*

    Total RNA is eluted in RNase-free water. During elution, the eluate is heated, causing the RNA to denature. Denaturation of RNA is essential for some downstream applications. It is not necessary to denature the sample a second time. The RNA remains denatured after freezing, storage, and thawing.
19. ■ **Optional**: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.

    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.
20. To run another protocol, press **START**, carry out steps 1–4 of the protocol, and then follow the protocol from step 7. Otherwise press **STOP** twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.

    Steps 5–6 are not necessary when running another protocol. Skip these steps.

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 7 for safety information.

Protocol: Purification of Total RNA from Easy-to-Lyse Tissues

This protocol is designed for purification of total RNA from easy-to-lyse human and animal tissues (e.g., liver, spleen, and kidney) using the EZ1&2 RNA Tissue Mini Kit. For purification of total RNA from any type of human or animal tissue using the EZ1 RNA Universal Tissue protocols, see page 44.

Determining the correct amount of starting material

It is essential to use the correct amount of starting material to obtain optimal RNA yield and purity. Generally, this protocol can be used with a maximum of:

- 10 mg flash-frozen tissue
- 5 mg flash-frozen high-cell density tissue, such as spleen
- 4–6 mg RNAprotect or Allprotect stabilized tissue*
- 2–3 mg RNAprotect or Allprotect stabilized high-cell density tissue, such as spleen*

Using fresh tissue is not recommended unless it is homogenized in Buffer RLT immediately, since RNA in not-stabilized fresh tissue is not protected from degradation.

Important points before starting

- If using the EZ1&2 RNA Tissue Mini Kit for the first time, read "Important Notes" (page 15).
- If working with RNA for the first time, read Appendix A: General Remarks on Handling RNA (page 56).

* Since RNAprotect or Allprotect stabilized tissues are partially dehydrated, a lower amount is used as starting material.

- If using the TissueRuptor, ensure that you are familiar with operating it by referring to the *TissueRuptor User Manual and TissueRuptor Handbook*.
- If using the TissueLyser LT or TissueLyser II, ensure that you are familiar with operating it by referring to the operating instructions and the *TissueLyser LT Handbook or the TissueLyser Handbook*.
- For optimal results, stabilize harvested tissues immediately in RNAprotect Tissue Reagent (see the *RNAprotect Handbook*) or Allprotect Tissue Reagent (see the *Allprotect Tissue Reagent Handbook*). Tissues can be stored in the reagent at 37°C for up to 1 day, at 15–25°C for up to 7 days, or at 2–8°C for up to 4 weeks (RNAprotect) or 6 months (Allprotect). Alternatively, tissues can be archived at –30°C to –15°C or –90°C to –65°C.
- Fresh, frozen, or RNAprotect/Allprotect stabilized tissues can be used. To freeze tissues for storage at –90°C to –65°C for several months, flash-freeze in liquid nitrogen and transfer immediately to –90°C to –65°C. Do not allow the tissues to thaw during weighing or handling before disruption in Buffer RLT. Homogenized tissue lysates from step 2 can also be stored at –90°C to –65°C for several months. To thaw frozen lysates, incubate at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity. Continue with step 3.
- Buffer RLT and the reagent cartridges contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 7 for safety information.
- Perform all steps of the procedure at room temperature (15–25°C).
  In some steps of the procedure, one of 2 choices can be made. Choose ■ (blue) if using the EZ1 Advanced or the EZ1 Advanced XL; choose ▲ (red) if using the BioRobot EZ1.

Things to do before starting

- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming at 37°C and then place at room temperature (15–25°C).
- **Optional**: For tissues with high RNase activity, we recommend adding either β-mercaptoethanol (β-ME) or dithiothreitol (DDT) to Buffer RLT before use. Add 10 µL β-ME

per 1 mL Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature for up to 1 month. Alternatively, add 20 µL of 2 M DTT per 1 mL Buffer RLT. The stock 2 M DTT solution should be prepared in water and used immediately, or frozen in single-use aliquots. Buffer RLT containing DTT can be stored at room temperature for up to 1 month.

- Prepare DNase I stock solution before using the RNase-free DNase I for the first time. Dissolve the lyophilized DNase I (750 Kunitz units) in 550 µL of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. **Do not vortex**.
- For long term storage of reconstituted DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –30°C to –15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

1. Excise the tissue sample from the animal or remove it from storage. Determine the amount of tissue. Do not use more than amounts described in “Determining the amount of starting material”, page 36. Proceed immediately to step 2.

   Weighing tissue is the most accurate way to determine the amount. If necessary, cut the tissue on a clean surface and weigh the piece to be used.

   For RNAprotect or Allprotect stabilized tissues:
   Remove the tissue from the stabilization reagent using forceps and be sure to remove any crystals that may have formed. RNA in RNAprotect or Allprotect stabilized tissues is protected during cutting and weighing of tissues at ambient temperature (15–25°C). It is not necessary to cut the tissues on ice or dry ice or in a refrigerated room. Remaining tissues can be stored in RNAprotect or Allprotect Reagent. Previously stabilized tissues can be stored at –90°C to –65°C without the reagent.

For not-stabilized fresh or frozen tissues:
RNA in harvested tissues is not protected until the tissues are treated with RNAprotect or Allprotect Reagent, flash-frozen, or disrupted and homogenized in step 2. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible. Remaining fresh tissues can be placed into RNAprotect Reagent to stabilize RNA or in Allprotect Tissue Reagent to stabilize DNA, RNA, and protein. However, previously frozen tissues thaw too slowly in the reagent, preventing the reagent from diffusing into the tissues quickly enough to prevent RNA degradation.

2. Disrupt the tissue and homogenize the lysate in Buffer RLT according to the method used.

   See “Disrupting and homogenizing starting material”, page 18, for more details on disruption and homogenization.

   Note: After addition of Buffer RLT, homogenization must be started as soon as possible.

   After storage in RNAprotect or Allprotect Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization using standard methods is usually not a problem.

   Note: Incomplete homogenization leads to significantly reduced RNA yields. Homogenization with the TissueRuptor, TissueLyser LT, or TissueLyser II generally results in higher RNA yields than with other methods.

   Disruption and homogenization using the TissueRuptor:

   2a. Place the tissue in a suitably sized vessel. Add 300 µL Buffer RLT.
   Note: Use a suitably sized vessel with sufficient extra headspace to accommodate foaming, which may occur during homogenization.

   Generally, round-bottomed tubes allow more efficient disruption and homogenization than conical-bottomed tubes.

   2b. Place the tip of the disposable probe into the vessel and operate the TissueRuptor at full speed until the lysate is homogeneous (usually 30 s).

2c. Proceed to step 3.

Note: To avoid damage to the TissueRuptor and disposable probe during operation, make sure the tip of the probe remains submerged in the buffer.

Foaming may occur during homogenization. If this happens, let the homogenate stand at room temperature (15–25°C) for 2–3 min until the foam subsides before continuing with the procedure.

Disruption using a mortar and pestle followed by homogenization using a QIAshredder homogenizer or a needle and syringe:

2a. Immediately place the weighed tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle.
2b. Decant the tissue powder and liquid nitrogen into an RNase-free, liquid nitrogen–cooled, 2 mL microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate but do not allow the tissue to thaw.
2c. Add 300 µL Buffer RLT.
2d. Pipette the lysate directly into a QIAshredder spin column placed in a 2 mL collection tube and centrifuge for 2 min at full speed. Alternatively, pass the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNase-free syringe.
2e. Proceed to step 3.

Disruption and homogenization using the TissueLyser LT or the TissueLyser II:

2a. Place the tissues in 2 mL microcentrifuge tubes containing 1 stainless steel bead (5 mm mean diameter).
If handling frozen tissue samples, keep the tubes on dry ice.
2b. Place the tubes at room temperature. Immediately add 300 µL Buffer RLT per tube.
2c. Attach the caps to the tubes, and place the tubes in the TissueLyser LT Adapter, 12-Tube, or TissueLyser Adapter Set 2 × 24.
2d. Operate the TissueLyser LT or the TissueLyser II for 2–5 min at 25 Hz.

The time depends on the tissue being processed and can be extended until the tissue is completely homogenized.

2e. Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser LT or the TissueLyser II are now outermost, and reassemble the adapter set. Operate the TissueLyser LT or the TissueLyser II for another 2–5 min at 25 Hz. Rearranging the tubes allows even homogenization.

2f. Proceed to step 3.

Note: Do not reuse the stainless steel beads.

3. Centrifuge the lysates for 3 min at full speed. Carefully pipette the supernatants into the 2 mL sample tubes supplied with the kit.

   In some preparations, a very small amount of insoluble material will be present after the 3-min centrifugation, making the pellet invisible.

4. Insert ■ the EZ1 Advanced RNA Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL RNA Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ▲ the EZ1 RNA Card completely into the EZ1 Card slot of the BioRobot EZ1.

5. Switch on the EZ1 instrument.

   The power switch is located at the rear of the instrument.

6. Press **START** to start protocol setup. ■ Follow the onscreen instructions for data tracking.

7. Press **1** to select purification of total RNA, and then press **1** to select the EZ1 RNA Mini protocols.

8. Press **2** to start worktable setup for the protocol for purification from tissues.

9. Press **1** to select an elution volume of 50 µL, **2** to select an elution volume of 100 µL, or **3** to select an elution volume of 200 µL.

10. Open the instrument door.

11. Invert reagent cartridges 3 times to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells.

12. Follow the onscreen instructions for worktable setup. Before loading reagent cartridges into the cartridge rack, use a pipette tip to puncture the foil covering well 5 of each cartridge (see Figure 4A, page 24). Then pipette 10 µL reconstituted DNase into the liquid in well 5 of each cartridge.

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

    If there are fewer reagent cartridges than slots in the cartridge rack, load them onto the cartridge rack starting from the left slot, and do not leave any empty slots between the cartridges. Be sure to load the other labware (i.e., tubes and tips) in the same order.

    Note: 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.

13. Close the instrument door.
14. Press **START** to start the protocol.
15. 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.

16. Open the instrument door.
17. Remove the elution tubes containing the purified RNA from the first row. Discard the sample-preparation waste.*

    Total RNA is eluted in RNase-free water. During elution, the eluate is heated, causing the RNA to denature. Denaturation of RNA is essential for some downstream applications. It is not necessary to denature the sample a second time. The RNA remains denatured after freezing, storage, and thawing.

18. ■ **Optional**: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 6 for safety information.

19. 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.

20. To run another protocol, press **START**, carry out steps 1–3 of the protocol, and then follow the protocol from step 6. Otherwise, press **STOP** twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.

    Steps 4–5 are not necessary when running another protocol. Skip these steps.

Protocol: Purification of Total RNA from Any Type of Tissue

This protocol is designed for purification of total RNA from any type of human or animal tissue. For purification of total RNA from human and animal cells, see Appendix E: Purification of Total RNA from Cells, page 71.

Determining the correct amount of starting material

It is essential to use the correct amount of starting material to obtain optimal RNA yield and purity. Generally, this protocol can be used with a maximum of:

- 50 mg flash-frozen tissue
- 100 mg flash-frozen adipose tissue
- 25 mg flash-frozen liver, thymus, or spleen tissue
- 25 mg RNAprotect or Allprotect stabilized tissue*

Using fresh tissue is not recommended unless it is homogenized in QIAzol Lysis Reagent immediately, since RNA in not-stabilized fresh tissue is not protected from degradation.

Important points before starting

- If working with RNA for the first time, read Appendix A (page 56).
- If using the TissueRuptor, ensure that you are familiar with operating it by referring to the *TissueRuptor User Manual and TissueRuptor Handbook*.
- For optimal results, stabilize harvested tissues immediately in RNAprotect Tissue Reagent (see the *RNAprotect Handbook*) or Allprotect Tissue Reagent (see the *Allprotect Tissue*

* Since RNAprotect or Allprotect stabilized tissues are partially dehydrated, a lower amount is used as starting material.

Reagent Handbook). Tissues can be stored in the reagent at 37°C for up to 1 day, at 15–25°C for up to 7 days, or at 2–8°C for up to 4 weeks (RNAprotect) or 6 months (Allprotect). Alternatively, tissues can be archived at –30°C to –15°C or –90°C to –65°C.

- Fresh, frozen, or RNAprotect/Allprotect stabilized tissues can be used. To freeze tissues for storage at –90°C to –65°C for several months, flash-freeze in liquid nitrogen and transfer immediately to –90°C to –65°C. Do not allow the tissues to thaw during weighing or handling before disruption in QIAzol Lysis Reagent. Homogenized tissue lysates from step 2 can also be stored at –90°C to –65°C for several months. To thaw frozen lysates, incubate at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity. Continue with step 3.
- Generally, DNase digestion is not required since integrated QIAzol and EZ1 technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be desirable for certain RNA applications that are sensitive to very small amounts of DNA. In these cases, small residual amounts of DNA remaining can be removed by using the automated protocol with an optional, integrated DNase digestion step, or by a DNase digestion after RNA purification (please contact QIAGEN Technical Services for a protocol).
- QIAzol Lysis Reagent and the reagent cartridges contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for safety information.
- Perform the centrifugation step to separate the aqueous from the organic phase (step 6) at 4°C. Perform all other steps of the procedure at room temperature (15–25°C).
- In some steps of the procedure, one of 2 choices can be made. Choose ■ (blue) if using the EZ1 Advanced or the EZ1 Advanced XL; choose ▲ (red) if using the BioRobot EZ1.

Things to do before starting

- **Optional**: If using the protocol with integrated DNase digestion, prepare the DNase I stock solution before using the RNase-Free DNase Set (cat. no. 79254) for the first time. Dissolve the lyophilized DNase I (1500 Kunitz units) in 1100 µL of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. **Do not vortex**.
- For long-term storage of reconstituted DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –30°C to –15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

1. Excise the tissue sample from the animal or remove it from storage. Determine the amount of tissue. Do not use more than amounts described in “Determining the correct amount of starting material”, page 44. Proceed immediately to step 2.

   Weighing tissue is the most accurate way to determine the amount. If necessary, cut the tissue on a clean surface and weigh the piece to be used.

   For RNAprotect or Allprotect stabilized tissues:

   Remove the tissue from the stabilization reagent using forceps and be sure to remove any crystals that may have formed. RNA in RNAprotect or Allprotect stabilized tissues is protected during cutting and weighing of tissues at ambient temperature (15–25°C). It is not necessary to cut the tissues on ice or dry ice or in a refrigerated room. Remaining tissues can be stored in RNAprotect or Allprotect Reagent. Previously stabilized tissues can be stored at –90°C to –65°C without the reagent.

For unstabilized fresh or frozen tissues:

RNA in harvested tissues is not protected until the tissues are treated with RNAprotect or Allprotect Reagent, flash-frozen, or disrupted and homogenized in step 2. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible. Remaining fresh tissues can be placed into RNAprotect Reagent to stabilize RNA or in Allprotect Tissue Reagent to stabilize DNA, RNA, and protein.

However, previously frozen tissues thaw too slowly in the reagent, preventing the reagent from diffusing into the tissues quickly enough to prevent RNA degradation.

2. Disrupt the tissue and homogenize the lysate in QIAzol Lysis Reagent according to the method used.

   See “Disrupting and homogenizing starting material”, page 18, for more details on disruption and homogenization.

   After storage in RNAprotect or Allprotect Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization using standard methods is usually not a problem.

   Note: Incomplete homogenization leads to significantly reduced RNA yields. Homogenization with the TissueRuptor, TissueLyser LT, or TissueLyser II generally results in higher RNA yields than with other methods.

   Disruption and homogenization using the TissueRuptor:

   2a. Place the tissue in a suitably sized vessel. Add 750 µL QIAzol Lysis Reagent.
   Note: Use a suitably sized vessel with sufficient extra headspace to accommodate foaming, which may occur during homogenization.

   2b. Generally, round-bottomed tubes allow more efficient disruption and homogenization than conical-bottomed tubes.

   2c. Place the tip of the disposable probe into the vessel and operate the TissueRuptor at full speed until the lysate is homogeneous (usually 30 s).

   2d. Proceed to step 3.

Note: To avoid damage to the TissueRuptor and disposable probe during operation, make sure the tip of the probe remains submerged in the buffer.
Foaming may occur during homogenization. If this happens, let the homogenate stand at room temperature (15–25°C) for 2–3 min until the foam subsides before continuing with the procedure.
Some exceptionally tough tissues (e.g., human skin) may not be completely homogenized. This does not affect the protocol since undisrupted pieces of tissue are removed after phase separation. However, total RNA yields will be reduced.

Disruption and homogenization using the TissueLyser LT or TissueLyser II:

2a. Place the tissues in 2 mL microcentrifuge tubes containing 1 stainless steel bead (5 mm mean diameter).
If handling frozen tissue samples, keep the tubes on dry ice.

2b. Place the tubes at room temperature. Immediately add 750 µL QIAzol Lysis Reagent per tube.

2c. Attach the caps to the tubes, and place the tubes in the TissueLyser LT Adapter, 12-Tube, or TissueLyser Adapter Set 2 × 24.

2d. Operate the TissueLyser LT or TissueLyser II for 2–5 min at 25 Hz.
The time depends on the tissue being processed and can be extended until the tissue is completely homogenized.

2e. Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser LT or TissueLyser II are now outermost, and reassemble the adapter set. Operate the TissueLyser LT or TissueLyser II for another 2–5 min at 25 Hz.
Rearranging the tubes allows even homogenization.

2f. Proceed to step 3.

Note: Do not reuse the stainless steel beads.
Some exceptionally tough tissues (e.g., pig skin) may not be completely homogenized. This does not affect the protocol, however, since undisrupted pieces of tissue are removed after phase separation.

3. Place the tubes containing the homogenates on the benchtop at room temperature for 5 min.
4. Add 150 µL chloroform to each tube. Securely cap the tubes and shake them vigorously for 15 s.

   Thorough mixing is important for subsequent phase separation.
5. Place the tubes on the benchtop at room temperature for 2–3 min.
6. Centrifuge at 12,000 x *g* for 15 min at 4°C.

   Centrifugation at 4°C is important for optimal phase separation and removal of genomic DNA.

   After centrifugation, the sample separates into 3 phases:

   - An upper, colorless, aqueous phase containing RNA
   - A white interphase
   - A lower, red, organic phase

     For tissues with an especially high fat content, an additional, clear phase may be visible below the red, organic phase. The volume of the aqueous phase should be approximately 350 µL.

7. Transfer the upper, aqueous phases (300–400 µL each) to the 2 mL sample tubes supplied with the kit.
8. Insert ■ the EZ1 Advanced RNA Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL RNA Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ▲ the EZ1 RNA Card completely into the EZ1 Card slot of the BioRobot EZ1.
9. Switch on the EZ1 instrument.

   The power switch is located at the rear of the instrument.
10. Press **START** to start protocol setup. ■ Follow the onscreen instructions for data tracking.
11. Press **1** to select purification of total RNA and then press **2** to select the EZ1 RNA Universal Tissue protocol.

12. Press **1** to start worktable setup for the standard EZ1 RNA Universal Tissue protocol without integrated DNase digestion or press **2** to start worktable setup for the EZ1 RNA Universal Tissue protocol with integrated DNase digestion.
13. Press **1** to select an elution volume of 50 µL, **2** to select an elution volume of 100 µL, or **3** to select an elution volume of 200 µL.
14. Open the instrument door.
15. Invert reagent cartridges 3 times to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells.
16. Follow the onscreen instructions for worktable setup. For the protocol with integrated DNase digestion: Before loading reagent cartridges into the cartridge rack, use a pipette tip to puncture the foil covering well 5 of each cartridge (see Figure 4A, page 24). Then 10 µL reconstituted DNase into the liquid in well 5 of each cartridge.

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

    If there are fewer reagent cartridges than slots in the cartridge rack, load them onto the cartridge rack starting from the left slot. Do not leave any empty slots between the cartridges. Be sure to load the other labware (i.e., tubes and tips) in the same order.

    Note: When using the data tracking option, ensure that the sample ID follows the same order as the samples on the worktable to avoid data mixup.
17. Close the instrument door.
18. Press **START** to start the protocol.
19. When the protocol ends, the display shows “Protocol finished”. ■ Press **ENT** to generate the report file.

    The EZ1 Advanced and the 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.
20. Open the instrument door.

21. Remove the elution tubes containing the purified RNA from the first row. Discard the sample-preparation waste.*

    Total RNA is eluted in RNase-free water. During elution, the eluate is heated, causing the RNA to denature. Denaturation of RNA is essential for some downstream applications. It is not necessary to denature the sample a second time.

    The RNA remains denatured after freezing, storage, and thawing.

22. ■ **Optional**: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.

23. 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.

24. To run another protocol, press **START**, carry out steps 1–7 of the protocol, and then follow the protocol from step 10. Otherwise press **STOP** twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.

    Steps 8–9 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 or protocols in this handbook (for contact information, 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 or 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 or EZ1 Advanced XL via the "PC/Printer" serial port. Check whether the serial port is set for use with a PC.
EZ1 RNA Universal Tissue protocol: phases do not separate completely		
a)	No chloroform added or chloroform not pure	Make sure to add chloroform that does not contain isoamyl alcohol or other additives.
b)	Homogenate not sufficiently mixed before centrifugation	After addition of chloroform (step 4), the homogenate must be vigorously shaken. If the phases are not well separated, shake the rack vigorously while inverting it for at least 15 s, and repeat the incubation and centrifugation (steps 5 and 6).
c)	Organic solvents in samples used for purification	Make sure that the starting sample does not contain organic solvents (e.g., ethanol, DMSO), strong buffers, or alkaline reagents. These can interfere with the phase separation
Low RNA yield		
a)	Incomplete sample lysis	Before use, check that the Buffer RLT does not contain a precipitate by shaking the bottle. Check again before pipetting Buffer RLT. If necessary, incubate for 30 min at 37°C with occasional shaking to dissolve precipitate.

		Comments and suggestions
b)	Magnetic particles not completely resuspended	Ensure that you invert the reagent cartridges several times to resuspend the magnetic particles.
c)	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.
d)	DNase I added to the wrong well of the reagent cartridge	Ensure that DNase I is added to well 5 of the reagent cartridges (see Figure 4A, page 24). Repeat the purification procedure with new samples.
e)	Insufficient disruption and homogenization	See "Disrupting and homogenizing starting material" (pages 18–21) for a detailed description of homogenization methods. In subsequent preparations, reduce the amount of starting material (see page 15) and/or increase the homogenization time.
f)	Too much starting material	In subsequent preparations, reduce amounts of starting material. It is essential to use the correct amount of starting material (see page 15).
g)	Incomplete removal of cell-culture medium	When processing cultured cells, ensure that the cell-culture medium is completely removed after harvesting cells.
h)	Buffer temperatures too low	All buffers must be at room temperature (15–25°C) throughout the procedure.

RNA concentration too low

a)	Elution volume too high	Reduce the elution volume (down to 50 µL). Although eluting with less buffer results in increased RNA concentrations, total yields might be reduced, especially for tissues with low amounts of RNA, such as adipose tissue or skin.

RNA does not perform well in downstream applications

a)	Insufficient RNA used in downstream application	Quantify the purified RNA by spectrophotometric measurement of the absorbance at 260 nm (see "Appendix B: Storage, Quantification, and Determination of Quality of RNA, page 59).
b)	Excess RNA used in downstream application	Excess RNA can inhibit some enzymatic reactions. Quantify the purified RNA by spectrophotometric measurement of the absorbance at 260 nm (see "Appendix B: Storage, Quantification, and Determination of Quality of RNA, page 59).
c)	Degraded RNA obtained from tissue samples	Too much starting material may have been used. For most tissue types, 10 mg per 300 µL Buffer RLT is sufficient when using the EZ1&2 RNA Tissue Mini Kit. Larger amounts of tissue can be used with the EZ1 RNA Universal Tissue protocols, as indicated in Table 1 on page 15.

		Comments and suggestions
d)	Salt carryover during elution	Ensure that the reagent cartridges are at 20–30°C.
e)	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.
A_{260}/A_{280} ratio for RNA is low		
a)	Absorbance reading at 320 nm was not subtracted from the absorbance readings at 260 nm and 280 nm	To correct for the presence of magnetic particles in the eluate, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm (see "Quantification of RNA", Appendix B, page 59).
b)	Wrong buffer used for RNA dilution	Use 10 mM Tris·Cl, pH 7.5*, not RNase-free water, to dilute the sample before measuring purity (see "Appendix B: Storage, Quantification, and Determination of Quality of RNA, page 59).
c)	**EZ1 RNA Universal Tissue protocol**: Not enough QIAzol Lysis Reagent used for homogenization	In subsequent preparations, reduce the amount of starting material and/or increase the volume of QIAzol Lysis Reagent and the homogenization time.
d)	**EZ1 RNA Universal Tissue protocol**: Sample not incubated for 5 min after homogenization	Place the sample at room temperature (15–25°C) for 5 min after homogenization, as indicated in the protocol.
RNA degraded		
a)	Tissue sample not immediately stabilized	Submerge the sample in the appropriate volume of RNAprotect Tissue Reagent or Allprotect Tissue Reagent immediately after harvesting the material.
b)	Too much tissue sample for proper stabilization	Reduce the amount of starting material or increase the amount of RNAprotect Tissue Reagent (see *RNAprotect Handbook*) or Allprotect Tissue Reagent (see *Allprotect Tissue Reagent Handbook*) used for stabilization.
c)	Tissue sample too thick for stabilization	Cut large samples into slices less than 0.5 cm thick for stabilization in RNAprotect Tissue Reagent or Allprotect Tissue Reagent.

* 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.

		Comments and suggestions
d)	Frozen tissue samples used for stabilization	Use only fresh, unfrozen material for stabilization.
e)	Storage duration exceeded	Storage of RNAprotect /Allprotect stabilized material is possible for up to 1 day at 37°C, up to 7 days at 15–25°C, and up to 4 weeks (RNAprotect) or 6 months (Allprotect) at 2–8°C. Store at –30°C to –15°C or –90°C to –65°C for archival storage.
f)	Sample inappropriately handled	For frozen cell pellets, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –90°C to –65°C. Perform the protocol quickly, especially the first few steps. See Appendix A (page 56), and the cell protocol (page 29).
g)	RNase contamination	Although all buffers have been tested and guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the procedure or later handling. See Appendix A (page 56). Do not put RNA samples into a vacuum dryer that has been used in DNA preparation where RNases may have been used.
h)	Tissue incubated in lysis buffer for too long before homogenization was started	After adding Buffer RLT or QIAzol Lysis Reagent to tissue, proceed with homogenization immediately.
EZ1 RNA Universal Tissue protocol: DNA contamination in downstream experiments		
a)	Phase separation performed at too high a temperature	The phase separation should be performed at 4°C to allow optimal phase separation and removal of genomic DNA from the aqueous phase. Make sure that the centrifuge does not heat above 10°C during the centrifugation.
b)	Interphase contamination of aqueous phase	Contamination of the aqueous phase with the interphase results in an increased DNA content in the eluate. Make sure to transfer the aqueous phase without interphase contamination.
c)	No DNase treatment	Use the protocol with integrated DNase digestion using the RNase-Free DNase Set. Alternatively, after the EZ1 RNA Universal Tissue procedure, DNase digest the eluate containing the RNA. After inactivating DNase by heat treatment, the RNA can be either used directly in the subsequent application without further treatment or repurified using an RNA cleanup protocol.
Low reproducibility between samples		
a)	Incomplete homogenization	Some types of tissues are more difficult to homogenize, resulting in greater variability from sample to sample.
b)	Variability between tissue samples	RNA yields from tissue samples can vary more than, for example, cultured cells due to the heterogeneous nature of most tissues and donor-to-donor variability.

Appendix A: 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 plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. To create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non-disposable 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 purified RNA on ice* when aliquots are pipetted for downstream applications. To remove RNase contamination from bench surfaces, nondisposable plasticware, and laboratory equipment (e.g., pipettes and electrophoresis tanks), use of RNaseKiller (cat. no. 2500080) from VWR (**www.vwr.com**) is recommended. RNase contamination can alternatively be removed using general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 57), or rinse with chloroform* if the plasticware is chloroform-resistant. To decontaminate electrophoresis tanks, clean with

* 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.

detergent (e.g., 0.5% SDS),* rinse with RNase-free water, rinse with ethanol (if the tanks are ethanol-resistant), and allow to dry.

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.

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 at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with diethyl pyrocarbonate (DEPC)* as described in "Solutions" below.

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 min 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.

* 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.

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

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 min.

Note: The buffers of the EZ1&2 RNA Tissue Mini Kit are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

Appendix B: Storage, Quantification, and Determination of Quality of RNA

Storage of RNA

Purified RNA may be stored at –30°C to –15°C or –90°C to –65°C.

Quantification of RNA

The concentration of RNA can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer (see "Spectrophotometric quantification of RNA" below). For small amounts of RNA, however, it may not be possible to accurately determine amounts photometrically. Small amounts of RNA can be quantified using the QIAxcel® system or Agilent® 2100 Bioanalyzer®, fluorometric quantification, or quantitative, real-time RT-PCR.

Spectrophotometric quantification of RNA

To ensure significance, A_{260} readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per mL (A_{260}=1 → 44 µg/mL). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH. As discussed below (see "Purity of RNA", page 60), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH, 1 mM EDTA*, followed by washing with RNase-free water (see

* 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.

"Solutions", page 57). Use the buffer in which the RNA is diluted to zero the spectrophotometer.

Carryover of magnetic particles in the eluate may affect the A_{260} reading, but should not affect the performance of the RNA in downstream applications. If it is necessary to minimize magnetic-particle carryover, the tube containing the eluate should first be placed in a suitable magnet (e.g., QIAGEN 12-Tube Magnet, cat. no. 36912 for 1 min) and the eluate transferred to a clean tube. If a suitable magnet is not available, centrifuge the tube containing the RNA for 1 min at full speed in a microcentrifuge to pellet any remaining magnetic particles.

When quantifying RNA samples, be sure also to measure the absorbance at 320 nm. Subtract the absorbance reading obtained at 320 nm from the reading obtained at 260 nm to correct for the presence of magnetic particles.

Concentration of RNA sample = 44 µg/mL × (A_{260} – A_{320}) × dilution factor

Total amount of RNA purified = concentration × volume of sample in milliliters

Purity of RNA

The ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV spectrum, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an A_{260} – A_{280} ratio of 1.9–2.1† in

* Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques 22, 474.

† Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris·Cl, pH 7.5) with some spectrophotometers.

10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution used for dilution.

For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration (A_{260} reading of 1 = 44 µg/mL RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "Spectrophotometric quantification of RNA", page 60).

When determining the purity of RNA samples, be sure also to measure the absorbance at 320 nm. Subtract the absorbance reading obtained at 320 nm from the readings obtained at 260 nm and 280 nm to correct for the presence of magnetic particles.

Purity of RNA sample = $(A_{260} - A_{320}) / (A_{280} - A_{320})$

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. In the EZ1 RNA Universal Tissue procedure, the vast majority of cellular DNA will be removed in the organic extraction step. In the EZ1 RNA Tissue procedures, the integrated DNase digestion step will very efficiently remove genomic DNA, so that no additional DNA removal should be required, even for the most sensitive applications.

For analysis of very low abundance targets, any interference by residual DNA contamination can be detected by performing real-time RT-PCR control experiments in which no reverse transcriptase is added prior to the PCR step.

To prevent any interference by DNA in real-time RT-PCR applications, such as with Applied Biosystems® and Rotor-Gene® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiTect® Primer Assays from QIAGEN (www.qiagen.com/GeneGlobe) are designed for SYBR® Green-based real-

time RT-PCR analysis of RNA sequences (without detection of genomic DNA) where possible. For real-time RT-PCR assays where amplification of genomic DNA cannot be avoided, the QuantiTect Reverse Transcription Kit provides fast cDNA synthesis with integrated removal of genomic DNA contamination (see Ordering Information, page 75). Alternatively, gene expression analysis can be performed using QuantiFast® Probe Assays and the QuantiFast Probe RT-PCR Plus Kit, which includes an integrated genomic DNA removal step.

Integrity of RNA

The integrity and size distribution of total RNA purified with EZ1 RNA Kits can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining* or by using the QIAxcel system or Agilent 2100 Bioanalyzer. The respective ribosomal RNAs should appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S rRNA should be approximately 2:1. If the ribosomal bands or peaks of a specific sample are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the sample suffered major degradation either before or during RNA purification.

The Agilent 2100 bioanalyzer also provides an RNA Integrity Number (RIN) as a useful measure of RNA integrity. Ideally, the RIN should be close to 10, but in many cases (particularly with tissue samples), how well the original sample is preserved greatly influences RNA quality.

* 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.

Appendix C: Protocol for Formaldehyde Agarose Gel Electrophoresis

The following protocol for formaldehyde agarose (FA) gel electrophoresis is routinely used at QIAGEN and gives enhanced sensitivity for gel and subsequent analysis (e.g., northern blotting). A key feature is the concentrated RNA loading buffer that allows a larger volume of RNA sample to be loaded onto the gel than conventional protocols (e.g., Sambrook, J. and Russell, D.W. [2001] *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

1.2% FA Gel Preparation

To prepare FA gel (1.2% agarose)* of size 10 x 14 x 0.7 cm, mix:

- 1.2 g agarose
- 10 mL 10x FA gel buffer (see composition below)
- Add RNase-free water to 100 mL

If smaller or larger gels are needed, adjust the quantities of components proportionately.

Heat the mixture to melt agarose. Cool to 65°C in a water bath. Add 1.8 mL of 37% (12.3 M) formaldehyde* and 1 µL of a 10 mg/mL ethidium bromide* stock solution. Mix thoroughly and pour onto gel support. Before running the gel, equilibrate in 1x FA gel running buffer (see composition below) for at least 30 minutes.

* 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.

RNA sample preparation for FA Gel Electrophoresis

Add 1 volume of 5x RNA loading buffer (see composition below) per 4 volumes of RNA sample (for example, 10 µL of loading buffer and 40 µL of RNA) and mix. Incubate for 3–5 minutes at 65°C, chill on ice, and load onto the equilibrated FA gel.

Gel running conditions

Run gel at 5–7 V/cm in 1x FA gel running buffer.

Composition of FA gel buffers

10x FA gel buffer

- 200 mM 3-[N-morpholino] propanesulfonic acid (MOPS) (free acid)*
- 50 mM sodium acetate*
- 10 mM EDTA*
- pH to 7.0 with NaOH*

1x FA gel running buffer

- 100 mL 10x FA gel buffer
- 20 mL 37% (12.3 M) formaldehyde
- 880 mL RNase-free water

* 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.

5x RNA loading buffer

- 16 µL saturated aqueous bromophenol blue solution*†
- 80 µL 500 mM EDTA, pH 8.0
- 720 µL 37% (12.3 M) formaldehyde
- 2 mL 100% glycerol*
- 3084 µL formamide*
- 4 mL 10x FA gel buffer
- RNase-free water to 10 mL
- Stability: Approximately 3 months at 4°C

* 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.

† To make a saturated solution, add solid bromophenol blue to distilled water. Mix and continue to add more bromophenol blue until no more will dissolve. Centrifuge to pellet the undissolved powder, and carefully pipette the saturated supernatant.

Appendix D: Preparing Human Blood Cells for Purification of Total RNA

The protocol for purification of total RNA from cells (page 29) can be used to purify total RNA from human blood cells. This appendix contains information on how to prepare blood cells for this protocol. Please read this information before starting the protocol.

Collecting, storing, and handling samples

The EZ1&2 RNA Tissue Mini Kit is suitable for purification of total cellular RNA from fresh, whole human blood. Whole blood should be collected in the presence of an anticoagulant, preferably EDTA, although other anticoagulants such as citrate, heparin, or acid citrate dextrose (ACD) can also be used.

Since mRNA levels can change after blood collection, blood samples should be processed within a few hours of collection. mRNAs from blood cells have different stabilities. mRNAs of regulatory genes have shorter half-lives than mRNAs of housekeeping genes. To ensure that the purified RNA contains a representative distribution of mRNAs, blood samples should not be stored for long periods before purifying RNA.

Note: The EZ1&2 RNA Tissue Mini Kit cannot be used for frozen blood samples.

Starting amounts of samples

Blood

Maximum RNA yields using the EZ1&2 RNA Tissue Mini Kit are generally determined by 2 criteria: lysis volume and binding capacity of the magnetic particles. Using the maximum

amount of leukocytes that can be processed in the procedure (2 x 10^6), however, the binding capacity of the magnetic particles is not usually attained due to the low RNA content of leukocytes.

Lysis and Homogenization

Blood cells are lysed in 2 separate steps: erythrocyte lysis and leukocyte lysis. Erythrocytes (red blood cells) of human blood do not contain nuclei and are therefore not important for RNA purification since they neither synthesize nor contain RNA. In contrast, RNA can be purified from leukocytes (white blood cells), which are nucleated and therefore do contain RNA. Leukocytes consist of 3 main cell types: lymphocytes, monocytes, and granulocytes.

Erythrocyte lysis

Since healthy blood contains approximately 1000 times more erythrocytes than leukocytes, removing the erythrocytes simplifies RNA purification. The procedure described in this appendix (see below) uses selective lysis of erythrocytes to achieve this. Erythrocytes are more susceptible than leukocytes to hypotonic shock and burst rapidly in the presence of a hypotonic buffer.

Intact leukocytes are then recovered by centrifugation. The conditions for selective lysis of erythrocytes in the procedure below have been optimized to allow fast removal of erythrocytes without affecting the stability of the leukocytes. The erythrocyte-lysis step can be scaled up for volumes of whole blood >500 µL.

A common alternative to erythrocyte lysis is Ficoll® density-gradient centrifugation. In contrast to erythrocyte lysis, Ficoll density-gradient centrifugation only recovers mononuclear cells (lymphocytes and monocytes) and removes granulocytes. Mononuclear cells isolated by Ficoll density-gradient centrifugation can be processed using the EZ1&2 RNA Tissue Mini Kit.

Both erythrocyte lysis and Ficoll density-centrifugation rely upon intact blood cells, so fresh blood must be used.

Leukocyte lysis

During the EZ1&2 RNA Tissue Mini procedure, leukocytes are efficiently lysed under highly denaturing conditions that immediately inactivate RNases, allowing purification of intact RNA.

Additional 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.

- Buffer EL (cat. no. 79217)
- Ice
- **Recommended**: 14.3 M β-mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M) or 2 M dithiothreitol (DTT)
- Microcentrifuge at 4°C capable of centrifuging 2 mL microcentrifuge tubes at
- 4000 x *g*

Things to do before starting

- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).
- **Recommended**: Add β-mercaptoethanol (β-ME) to Buffer RLT before beginning the protocol. Add 10 µl β-ME per 1 mL Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature for up to 1 month. Alternatively, add 20 µL of 2 M DTT per 1 mL Buffer RLT. The stock 2 M DTT solution should be prepared in water and used immediately, or frozen in single-use aliquots. Buffer RLT containing DTT can be stored at room temperature for up to 1 month.

Procedure

1. Mix 1 volume of whole human blood with 5 volumes of Buffer EL (cat. no. 79217) in an appropriately sized tube (not provided).

   For optimal results, the volume of the mixture (blood and Buffer EL) should not exceed 3/4 of the volume of the tube to allow efficient mixing. For example, add 1000 µL of Buffer EL to 200 µL of whole blood, and mix in a tube which has a total volume of ≥1600 µL.

   Note: Use an appropriate amount of whole blood. Up to 500 µL of healthy blood (typically 4000–7000 leukocytes per microliter) can be processed. Reduce the amount appropriately if blood with elevated numbers of leukocytes is used.

2. Incubate for 10–15 min on ice. Mix by vortexing briefly 2 times during incubation. The cloudy suspension becomes translucent during incubation, indicating lysis of erythrocytes. If necessary, incubation time can be extended to 20 min.

3. Centrifuge at 400 x *g* for 10 min at 4°C and completely remove and discard the supernatant.

   Leukocytes will form a white pellet after centrifugation. Ensure the supernatant is completely removed. Trace amounts of erythrocytes, which give the pellet a red tint, will be eliminated in step 4.

   However, if erythrocyte lysis is incomplete, the white pellet may not be visible and large amounts of erythrocytes will form a red pellet. If this happens, incubate for an additional 5–10 min on ice after addition of Buffer EL at step 4.

4. Add Buffer EL to the cell pellet (use 2 volumes of Buffer EL per volume of whole blood used in step 1). Resuspend the cells by vortexing briefly.

   For example, add 400 µL of Buffer EL per 200 µL of whole blood used in step 1.

5. Count the cells and transfer a volume of cell suspension that corresponds to 2 x 10^6 cells to a microcentrifuge tube (not supplied). Centrifuge at 400 x *g* for 10 min at 4°C and completely remove and discard the supernatant.

Note: Incomplete removal of the supernatant will interfere with lysis and subsequent binding of RNA to the magnetic particles, resulting in lower RNA yield.

6. Continue with step 2 of the Protocol: Purification of Total RNA from Cells (page 29).

Appendix E: Purification of Total RNA from Cells

The EZ1&2 RNA Tissue Mini Kit can be used for purification of total RNA from any type of human and animal cells or tissues. The kit is optimized for use with up to 1×10^7 human or animal cells. If you use more than this amount, you may not achieve further increases in RNA yields.

For purification of total RNA from human or animal cells, proceed as follows:

- Harvest cells as described in step 1 of the protocol for purification of total RNA from cells (page 29).
- Immediately pipette 750 µL QIAzol Lysis Reagent into each tube containing cells. For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Vortex or pipette to mix.
- Proceed with step 2 of the EZ1 RNA Universal Tissue protocol (page 46). Step 2 describes the addition of 750 µL QIAzol Lysis Reagent to the sample but this is not necessary as the cells are already mixed with the reagent.

Appendix F: Example of an EZ1 Advanced Report File

This appendix shows a typical report file generated on the EZ1 Advanced. 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.

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.

REPORT - FILE EZ1 Advanced:

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

Serial no. EZ1 Advanced: _ _ _ _ _ _ _ _ _ 0301F0172
User ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 987654321
Firmware version: _ _ _ _ _ _ _ _ _ _ _ _ _ _ V 1.0.0
Installation date of instr.: _ _ _ _ _ _ _ _ _ Jan 05, 2008
Weekly maintenance done on: _ _ _ _ _ Jun 15, 2008
Yearly maintenance done on: _ _ _ _ _ _ Jan 07, 2008
Date of last UV-run: _ _ _ _ _ _ _ _ _ _ _ Feb 17, 2008
Start of last UV-run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 16:06
End of last UV-run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 16:26
Status UV-run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ o.k.

Protocol name: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ RNA
_ Cell

Date of run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ Feb 17, 2008
Start of run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 12:57

End of run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 13:51
Status run: _ o.k.
Error code: _
Sample input volume [uL]: _ _ _ _ _ _ _ _ _ _ _ _ _ 300
Elution volume [uL]: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 100

Channel A:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 123456789
Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 959034
Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 848373922
Note: _ 2000

Channel B:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 234567890
Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 959034
Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 836266738
Note: _

Channel C:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 345678901
Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 959034
Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 883727832

Notes: _ 1000

Channel D:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 456789012
Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 959034
Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 763684837
Note: _

Channel E:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 567890123
Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 959034
Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 4387728002
Note: _

Channel F:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 678901234
Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 959034
Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 509389403
Note: _ 50

Ordering Information

Product	Contents	Cat. no.
EZ1&2 RNA Tissue Mini Kit (48)	For 48 preps: Reagent Cartridges (Tissue RNA), Disposable Filter-Tips, Disposable Tip-Holders, Sample Tubes (2 mL), Elution Tubes (1.5 mL), Buffer RLT, RNase-Free DNase I, RNase-free water	959034
EZ1 Advanced XL RNA Card	Preprogrammed card for purification of RNA using the EZ1 Advanced XL	9018705
EZ1 Advanced RNA Card	Preprogrammed card purification of RNA using the EZ1 Advanced	9018297
EZ1 RNA Card	Pre-programmed card for EZ1 RNA purification protocols	9015590
Accessories		
Filter-Tips and Holders, EZ1 (50)	50 Disposable Filter-Tips, 50 Disposable TipHolders; additional tips and holders for use with EZ1 Kits	994900
12-Tube Magnet	Magnet for separating magnetic beads in 12 x 1.5 mL or 2 mL tubes	36912
Buffer EL (1000 mL)	1000 mL Erythrocyte Lysis Buffer	79217
Printer Accessory Package	Accessories for printer connected to EZ1 Advanced or EZ1 Advanced XL instruments	9018465

QIAshredder (50)*	50 disposable cell-lysate homogenizers for use in nucleic acid minipreps, caps	79654
TissueRuptor	Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes	Varies†
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor II (and the old TissueRuptor)	990890
TissueLyser LT	Compact bead mill, 100-240 V AC, 50-60 Hz; requires the TissueLyser LT Adapter, 12-Tube (available separately)	85600
TissueLyser LT Adapter, 12-Tube	Adapter for disruption of up to 12 samples in 2 mL microcentrifuge tubes on the TissueLyser LT	69980
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 (available separately)	85300
TissueLyser Adapter Set 2 x 24	Two sets of adapter plates and 2 racks for use with 2 mL microcentrifuge tubes on the TissueLyser II	69982
Stainless Steel Beads, 5 mm (200)	200 stainless steel beads (5 mm diameter), suitable for use with the TissueLyser systems	69989
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
Buffer RLT (220 mL)	220 mL RNeasy Lysis Buffer	79216

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

† For ordering information, visit **www.qiagen.com/TissueRuptor**.

QIAzol Lysis Reagent (200 mL)	200 mL QIAzol Lysis Reagent	79306
RNase-Free DNase Set (50)	1500 Kunitz units RNase-free DNase I, RNase-free Buffer RDD, and RNase-free water for 50 RNA minipreps	79254
RNAprotect Cell Reagent (250 mL)	250 mL RNAprotect Cell Reagent	76526
RNAprotect Tissue Reagent (50 mL)*	50 mL RNAprotect Tissue Reagent for stabilization of RNA in 25 x 200 mg tissue samples	76104
RNAprotect Tissue Tubes (50 x 1.5 mL)	For stabilization of RNA in 50 x 150 mg tissue samples: 50 screw-top tubes containing 1.5 mL RNAprotect Tissue Reagent each	76154
RNAprotect Tissue Tubes (20 x 5 mL)	For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 mL RNAprotect Tissue Reagent each	76163
Allprotect Tissue Reagent (100 mL)	100 mL Allprotect Tissue Reagent, Allprotect Reagent Pump	76405
QuantiTect Reverse Transcription Kit (50)	For 50 x 20 µL reactions: 100 µL 7x gDNA Wipeout Buffer, 50 µL Quantiscript Reverse Transcriptase, 200 µL 5x Quantiscript RT Buffer, 50 µL RT Primer Mix,1.9 mL RNase-Free Water	205311
RNase-Free DNase Set (50)	1500 Kunitz units RNase-free DNase I, RNase-free Buffer RDD, and RNase-free water for 50 RNA minipreps	79254

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

RNAprotect Cell Reagent (250 mL)	250 mL RNAprotect Cell Reagent	76526
RNAprotect Tissue Reagent (50 mL)	50 mL RNAprotect Tissue Reagent for stabilization of RNA in 25 x 200 mg tissue samples	76104
RNAprotect Tissue Tubes (50 × 1.5 mL)	For stabilization of RNA in 50 x 150 mg tissue samples: 50 screw-top tubes containing 1.5 mL RNAprotect Tissue Reagent each	76154
RNAprotect Tissue Tubes (20 × 5 mL)	For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 mL RNAprotect Tissue Reagent each	76163
Allprotect Tissue Reagent (100 mL)	100 mL Allprotect Tissue Reagent, Allprotect Reagent Pump	76405

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Document Revision History

Revision	Description
02/2022	Rebranded the name of the kit from “EZ1” to “EZ1&2”. Added the EZ2 Connect Instrument to the “Ordering Information” section. Changed occurrences of “RNAlater” to “RNAprotect”. Deleted Table 3 (Typical total RNA yields using the EZ1 RNA Universal Tissue Kit). Updated the Kit Contents section: removed the EZ1 RNA Cell Mini (cat. no. 958034) and EZ1 RNA Universal Tissue (cat. no. 956034) kits. Editorial and layout changes.
03/2024	Deleted reference to Table 3 from pages 14 and 43. Updated “Ordering Information” section to match the information on the website.

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