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TissueLyser LT Handbook

For disruption of up to 12 biological samples



Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

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- microRNA research and RNAi
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Product Contents

TissueLyser LT	
Catalog no.	85600
TissueLyser LT (100–240 V, 50/60 Hz)	1
Power Supply (24 V)	1
Cable Set (for different countries)	1
Allen Key	1
User Manual	1
Handbook	1

Important: The TissueLyser LT must be used in combination with the **TissueLyser LT Adapter, 12-Tube** (cat. no. 69980), which is available separately.

Storage

The TissueLyser LT should be stored upright in a dry environment at 5–40°C.

Product Use Limitations

The TissueLyser LT is intended for molecular biology applications. This product is neither intended for the diagnosis, prevention, or treatment of a disease, nor has it been validated for such use either alone or in combination with other products. Therefore, the performance characteristics of the product for clinical use (i.e., diagnostic, prognostic, therapeutic, or blood banking) are unknown.

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

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not

meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

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

Technical Assistance

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

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

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

Safety Information

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

24-hour emergency information

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

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Introduction

Principle

The TissueLyser LT provides rapid and efficient disruption of up to 12 biological samples, including animal and human tissues, plant tissues, bacteria, and yeast. Disruption and homogenization are achieved through the beating and grinding effect of beads on the sample material as they are shaken together in 2 ml sample tubes.

Disruption is critically important in order to release the nucleic acids from the sample material. Homogenization of the material acts to shear carbohydrates that may otherwise reduce binding of DNA and RNA to silica membranes or magnetic particles. Sample disruption using, for example, a mortar and pestle does not result in efficient homogenization. The TissueLyser LT both disrupts and homogenizes sample material in one simple and reliable step.

The TissueLyser LT is easily programmed to provide variable speeds from 15 to 50 Hz (900–3000 oscillations/minute) and run times from 1 second to 1 hour 59 minutes.

Applications

The ability to process up to 12 samples per run makes the TissueLyser LT the ideal front-end solution to access biological information for genomics, transcriptomics, and proteomics applications.

The TissueLyser LT enables fast and uniform disruption of animal and human tissues, plant tissues, bacteria, and yeast in the TissueLyser LT Adapter, which holds up to twelve 2 ml sample tubes. QIAGEN offers 2 ml microcentrifuge tubes as well as stainless steel beads for use with the TissueLyser LT Adapter. For more details about these and other accessories for the TissueLyser LT, see Appendix A (page 29) and the ordering information (page 32).

The TissueLyser LT provides efficient disruption of biological material in each sample tube for reproducible, high-quality results in downstream applications such as the purification of total DNA, RNA, or protein from a variety of human, animal, and plant tissues. A wide range of QIAGEN sample purification kits are compatible with the TissueLyser LT (see Tables 1–6, pages 7–10). Sample purification can be performed manually or can be automated using the QIAcube[®], QIASymphony[®] SP, QIAxtractor[™], EZ1[®] Advanced, EZ1 Advanced XL, or BioRobot[®] or BioSprint[®] workstations. For more information about automated solutions from QIAGEN, see Appendix B (page 30) and visit www.qiagen.com/automation.

This handbook provides guidelines on disrupting and homogenizing various sample materials for subsequent purification of DNA, RNA, or protein. Specific details on disruption and homogenization and nucleic acid or protein

purification, such as the amount of starting material and lysis buffer to use, can be found in the handbook supplied with each QIAGEN sample purification kit.

Table 1. Kits for RNA purification from animal or human tissues using spin columns

Sample type	Kit	Kit format	Page
Easy-to-lyse tissues (e.g., kidney, liver, and lung)	RNeasy [®] Micro Kit	Up to 5 mg tissue; automatable on QIAcube	17
	RNeasy Mini Kit	Up to 30 mg tissue; automatable on QIAcube	17
	RNeasy Protect Mini Kit	Up to 20 mg RNAlater [®] stabilized tissue; automatable on QIAcube	17
	RNeasy Plus Micro Kit	Up to 5 mg tissue; includes gDNA Eliminator spin columns	17
	RNeasy Plus Mini Kit	Up to 30 mg tissue; includes gDNA Eliminator spin columns; automatable on QIAcube	17
Fiber-rich tissues (e.g., heart and muscle)	RNeasy Fibrous Tissue Mini Kit	Up to 30 mg tissue; automatable on QIAcube	17
	RNeasy Fibrous Tissue Midi Kit	Up to 250 mg tissue	17
Any type of tissue, including fatty tissues (e.g., adipose tissue and brain)	RNeasy Lipid Tissue Mini Kit	Up to 100 mg tissue; automatable on QIAcube	17
	miRNeasy Mini Kit	Up to 100 mg tissue; automatable on QIAcube	17

Table 2. Kits for RNA purification from animal or human tissues using magnetic particles or 96-well plates

Sample type	Kit	Kit format	Page
Easy-to-lyse tissues (e.g., kidney, liver, and lung)	EZ1 RNA Tissue Mini Kit	Magnetic particles; up to 10 mg tissue; automated on EZ1 Advanced (1–6 samples per run) or EZ1 Advanced XL (1–14 samples per run)*	17
	MagAttract® RNA Tissue Mini M48 Kit	Magnetic particles; up to 10 mg tissue; automated on BioRobot M48 (6–48 samples per run)	17
	QIAsymphony RNA Kit	Magnetic particles; up to 50 mg tissue; automated on QIAsymphony SP (1–96 samples per run)	17
Any type of tissue	EZ1 RNA Universal Tissue Kit	Magnetic particles; up to 50 mg tissue; automated on EZ1 Advanced (1–6 samples per run) or EZ1 Advanced XL (1–14 samples per run)*	17
	MagAttract RNA Universal Tissue M48 Kit	Magnetic particles; up to 50 mg tissue; automated on BioRobot M48 (6–48 samples per run)	17
	RNeasy 96 Universal Tissue Kits	96-well plate; up to 100 mg tissue; automatable on BioRobot Universal System (up to 80 mg tissue)†	17
	miRNeasy 96 Kit	96-well plate; up to 100 mg tissue	17

* Also automatable on BioRobot EZ1 (no longer available).

† Also automatable on BioRobot Gene Expression — Real-Time RT-PCR and BioRobot 8000 (no longer available).

Table 3. Kits for RNA purification from plant tissues, bacteria, and yeast

Sample type	Kit	Kit format	Page
Plant tissue (e.g., leaf)	RNeasy Plant Mini Kit	Spin column; up to 100 mg tissue; automatable on QIAcube	19
	RNeasy 96 Kit	96-well plate; up to 25 mg tissue	19
Bacteria (Gram-positive and -negative)	RNeasy Protect Bacteria Mini Kit	Spin column; up to 2.5×10^8 cells; automatable on QIAcube	21
	RNeasy Protect Bacteria Midi Kit	Spin column; up to 1.5×10^9 cells	21
Yeast	RNeasy Mini Kit	Spin column; up to 5×10^7 cells	22

Table 4. Kits for DNA purification from animal or human tissues

Kit	Kit format	Page
DNeasy [®] Blood & Tissue Kit	Spin column; up to 25 mg tissue; automatable on QIAcube	23
DNeasy 96 Blood & Tissue Kit	96-well plate; up to 20 mg tissue	23
QIAamp [®] DNA Mini Kit	Spin column; up to 25 mg tissue; automatable on QIAcube	23
EZ1 DNA Tissue Kit	Magnetic particles; up to 40 mg tissue; automated on EZ1 Advanced (1–6 samples per run) or EZ1 Advanced XL (1–14 samples per run)*	23
MagAttract DNA Mini M48 Kit	Magnetic particles; up to 40 mg tissue; automated on BioRobot M48 (6–48 samples per run)	23
QIAsymphony DNA Mini Kit	Magnetic particles; up to 50 mg tissue; automated on QIAsymphony SP (1–96 samples per run)	23
Reagent Pack, DX, and Plasticware Pack, DX [†]	96-well plate; automated on QIAextractor	23

* Also automatable on BioRobot EZ1 (no longer available).

† These 2 products will soon be available together as the QIAextractor DX Kit.

Table 5. Kits for DNA purification from plant tissues

Kit	Kit format	Page
DNeasy Plant Mini Kit	Spin column; up to 100 mg tissue; automatable on QIAcube	25
DNeasy 96 Plant Kit	96-well plate; up to 50 mg tissue	25
MagAttract 96 DNA Plant Core Kit	Magnetic particles; up to 100 mg tissue; automatable on BioRobot Plant Science System — Genotyping*	25
BioSprint 15 DNA Plant Kit	Magnetic particles; up to 50 mg tissue; automated on BioSprint 15* (up to 15 samples per run)	25
BioSprint 96 DNA Plant Kit	Magnetic particles; up to 50 mg tissue; automated on BioSprint 96 (up to 96 samples per run)	25

* No longer available.

Table 6. Kits for simultaneous purification of multiple analytes from animal or human tissues

Analytes purified	Kit	Kit format	Page
DNA, RNA, and protein	AllPrep [®] DNA/RNA/Protein Mini Kit	Spin column; up to 30 mg tissue	17
DNA and RNA	AllPrep DNA/RNA Micro Kit	Spin column; up to 5 mg tissue	17
	AllPrep DNA/RNA Mini Kit	Spin column; up to 30 mg tissue; automatable on QIAcube	17
	AllPrep DNA/RNA 96 Kit	96-well plate; up to 10 mg tissue	17

QIAGEN Supplementary Protocols

Many of the protocols listed in this handbook are supplementary to the protocols found in the handbook of the specific kit being used. QIAGEN is constantly developing new protocols for existing products. These supplementary protocols can be obtained by contacting one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com) or visiting our Technical Support Center at www.qiagen.com/Support.

Supplementary protocols can be identified by their reference number, which is made up of 2 letters followed by 2 numbers (e.g., RY23 — *Isolation of total RNA from plants using the RNeasy 96 Kit*).

Equipment and Reagents to Be Supplied by User

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

- TissueLyser LT Adapter, 12-Tube (cat. no. 69980)
- Kit for purification of DNA, RNA, and/or protein (see ordering information on pages 32–37 or visit www.qiagen.com)
- 2 ml microcentrifuge tubes, such as Sample Tubes RB (2 ml) (cat. no. 990381)
- Stainless steel or acid-washed glass beads, such as:
 - Stainless Steel Beads, 5 mm (200) (cat. no. 69989)
 - Stainless Steel Beads, 7 mm (200) (cat. no. 69990)
 - Glass beads, acid-washed, 150–600 μm (e.g., cat. no. G 8772, Sigma-Aldrich [www.sigmaaldrich.com])
- Optional: Reagent DX (see page 16 for details)
- Optional: Dry ice (see the individual protocols)
- Optional: TissueLyser Single-Bead Dispenser, 5 mm (cat. no. 69965)
- Optional: TissueLyser Single-Bead Dispenser, 7 mm (cat. no. 69967)

Important Notes

General remarks on disruption and homogenization

Efficient disruption and homogenization of the starting material is an absolute requirement for all nucleic acid purification procedures. Disruption and homogenization are 2 distinct steps.

- **Disruption:** Complete disruption of cell walls and plasma membranes of cells and organelles is absolutely required to release all the nucleic acids contained in the sample. Different samples require different methods to achieve complete disruption. Incomplete disruption results in significantly reduced DNA and RNA yields.
- **Homogenization:** Homogenization is necessary to reduce the viscosity of the cell lysates produced by disruption. Homogenization shears carbohydrates to create a homogeneous lysate. Incomplete homogenization results in inefficient binding of nucleic acids to QIAGEN silica membranes and magnetic particles and therefore significantly reduced DNA and RNA yields.

Cellular disruption is one of the most critical steps in nucleic acid purification. Disruption in lysis buffer alone, without physical shearing, may result in nucleic acid degradation by endogenous DNases and RNases. Incomplete disruption prevents the lysis buffer, which inactivates nucleases, from contacting nucleic acids within the intact cells. Furthermore, cellular debris that is not disrupted can result in decreased yield and increases the risk of clogging the purification column. After sample disruption, there should be no visible particulates (except when disrupting materials containing hard, noncellular components, such as connective tissue, bone, or woody plant tissue). QIAGEN kits and protocols contain recommendations for the most appropriate method of sample disruption and homogenization to maximize the yield and quality of your DNA, RNA, and protein preparation.

Disruption and homogenization using the TissueLyser LT

In bead-milling, cells and tissues can be disrupted by rapid agitation in the presence of beads. Disruption and simultaneous homogenization occur by the shearing and crushing action of the beads as they collide with the sample. Disruption efficiency is influenced by:

- Size and composition of beads
- Ratio of buffer to samples (if buffer is used)
- Amount of starting material
- Configuration of TissueLyser LT (i.e., speed and duration)

- Consistency of sample
- Type of disruption vessel
- Temperature of the samples

Disruption and homogenization methods

When using the TissueLyser LT in combination with QIAGEN sample purification kits, one of 2 methods for disruption and homogenization is carried out:

- Samples are disrupted and homogenized in lysis buffer. For animal and human tissues that are either freshly isolated or frozen in liquid nitrogen, the sample tube and tissue sample need to be precooled on dry ice. For animal and human tissues that are stabilized in Allprotect Tissue Reagent or RNAlater RNA Stabilization Reagent, no precooling is required.
- Samples are precooled on dry ice for at least 30 minutes and then disrupted and homogenized without lysis buffer. Lysis buffer is added after disruption and homogenization. When precooling samples, the insert of the TissueLyser LT Adapter as well as the sample tubes, each containing the correct amount of sample and a grinding bead, are placed on dry ice.

Important: When using the TissueLyser LT Adapter, do not freeze the adapter and sample tubes in liquid nitrogen, as this may result in breakage of the tubes.

Note: After disrupting and homogenizing tissues using the TissueLyser LT, some debris may stick to the lids of the sample tubes. We therefore recommend a brief centrifugation before opening the sample tubes.

Bead selection

For human, animal, and plant tissues, the optimal beads to use are 5–7 mm (mean diameter) stainless steel beads. When disrupting tough tissue samples, we recommend using 7 mm beads instead of 5 mm beads to improve disruption efficiency. For disruption of cells, the optimal beads to use are 0.1–0.6 mm (mean diameter) glass beads for bacteria, and 0.5 mm glass beads for yeast and unicellular animal cells. It is essential that glass beads are pretreated before use by washing in concentrated nitric acid.* Pretreated (acid-washed) beads can be purchased from many vendors of biological supplies (e.g., Sigma, cat. nos. G1145, G1277, and G8772[†]). Disruption parameters for samples not addressed in this handbook must be determined empirically.

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

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

Operating the TissueLyser LT

The TissueLyser LT Adapter should be securely fixed onto the piston of the TissueLyser LT. For details, refer to the *TissueLyser LT User Manual*.

Disruption is carried out in high-speed (up to 50 Hz) shaking steps. Depending on the starting material and on the analytes to be purified, disruption for 40 seconds to 5 minutes at 30–50 Hz is usually sufficient to release DNA, RNA, or protein.

If fewer than 12 samples are to be processed, be sure to balance the TissueLyser LT Adapter (see Figure 1), otherwise the lid of the adapter may become twisted. If processing either 1 sample or 11 samples, load an additional, empty sample tube to balance the TissueLyser LT Adapter.

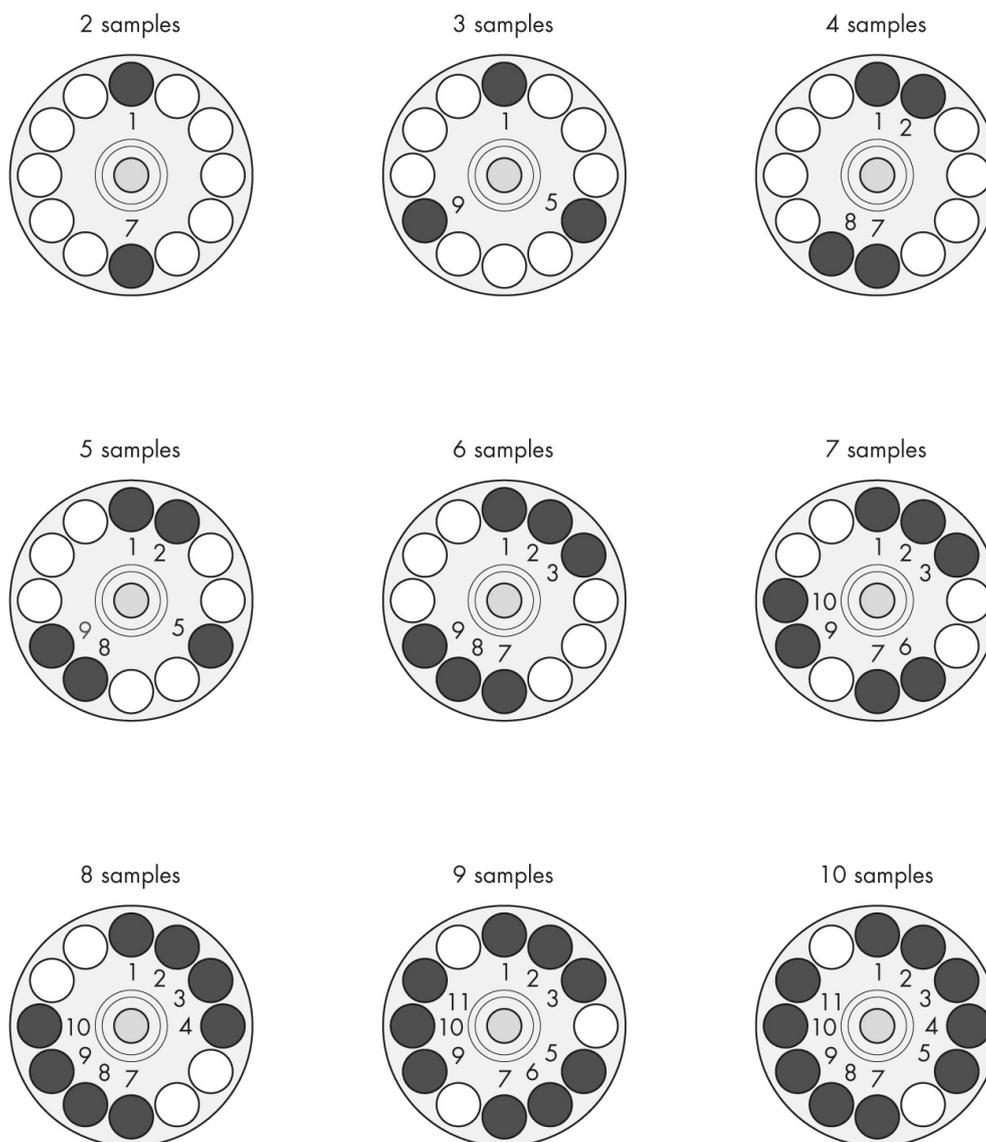


Figure 1. Loading the TissueLyser LT Adapter. Ensure that the TissueLyser LT Adapter is balanced by loading samples tubes as shown above.

Disruption and homogenization in Buffer RLT Plus

RNeasy Plus Kits and certain AllPrep Kits are supplied with Buffer RLT Plus, a lysis buffer that provides optimal sample lysis as well as appropriate conditions for DNA binding to gDNA Eliminator columns or AllPrep DNA columns. When disrupting and homogenizing tissues in Buffer RLT Plus, excessive foaming may occur. This foaming is substantially reduced by adding Reagent DX to Buffer RLT Plus at a final concentration of 0.5% (v/v) before starting disruption and homogenization. Reagent DX has been carefully tested with RNeasy Plus Kits and AllPrep Kits, and has no effect on RNA purity or on downstream applications such as real-time RT-PCR. Buffer RLT Plus containing Reagent DX can be stored at room temperature (15–25°C) for at least 9 months. Reagent DX is supplied separately; for ordering information, see page 33.

Protocol: Purification of RNA or Multiple Analytes from Animal and Human Tissues

This protocol provides guidelines on disrupting animal and human tissues for purification of RNA or for simultaneous purification of DNA and RNA or DNA, RNA, and protein. If using a QIAGEN sample purification kit (see Tables 1, 2, and 6 on pages 7, 8, and 10), refer to the supplied handbook, which contains a complete protocol for sample disruption and purification.

Important points before starting

- Before beginning the procedure, read “Important Notes” (page 13).
- Ensure that you are familiar with operating the TissueLyser LT by referring to the *TissueLyser LT User Manual*.
- If using a QIAGEN sample purification kit, read the supplied handbook carefully before starting.
- After storage in RNA/ater RNA Stabilization Reagent or Allprotect Tissue Reagent, tissues become slightly hard. If disrupting in Buffer RLT, we recommend increasing the volume of this buffer according to the protocols in the *RNeasy Mini Handbook*.
- When disrupting tough or very tough samples, we recommend using one or two 7 mm stainless steel beads, respectively, instead of one 5 mm stainless steel bead to guarantee optimal disruption.

Procedure

- 1. Place 2 ml microcentrifuge tubes containing 1 stainless steel bead (5 mm mean diameter) on dry ice for at least 15 min. Keep the insert of the TissueLyser LT Adapter at room temperature (15–25°C).**
- 2. Transfer up to 30 mg fresh or frozen tissue to the precooled tubes and incubate for another 15 min on dry ice.**

If handling tissue samples stabilized with RNA/ater RNA Stabilization Reagent or Allprotect Tissue Reagent, cooling on dry ice is not necessary.

- 3. Place the tubes into the insert of the TissueLyser LT Adapter, and incubate at room temperature for 2 min to avoid freezing of lysis buffer in step 4.**

Do not incubate for longer than 2 min, otherwise the tissue will thaw, resulting in potential RNA degradation.

- 4. Immediately add the appropriate volume of lysis buffer (e.g., Buffer RLT, Buffer RLT Plus, or QIAzol Lysis Reagent) to each tube.**

Note: If using Buffer RLT Plus, we recommend adding Reagent DX to prevent excessive foaming. For details, see “Disruption and homogenization in Buffer RLT Plus” (page 16).

- 5. Place the insert with sample tubes into the base of the TissueLyser LT Adapter, which is attached to the TissueLyser LT. Place the lid of the TissueLyser LT Adapter over the insert, and screw the knob until the lid is securely fastened.**

- 6. Operate the TissueLyser LT for 2–5 min at 50 Hz.**

The duration of disruption and homogenization depends on the tissue being processed and can be extended until no tissue debris is visible.

If processing fiber-rich tissues, complete disruption and homogenization may sometimes not be possible. However, small amounts of debris have no effect on subsequent RNA purification with QIAGEN kits and are usually digested in the proteinase K step.

- 7. Proceed with RNA, DNA/RNA, or DNA/RNA/protein purification.**

Do not reuse the stainless steel beads.

Protocol: Purification of RNA from Plant Tissues

This protocol provides guidelines on disrupting plant tissues for subsequent RNA purification. If using a QIAGEN kit for RNA purification (see Table 3, page 9), refer to the supplied handbook, which contains a complete protocol for sample disruption and RNA purification.

Important points before starting

- Before beginning the procedure, read “Important Notes” (page 13).
- Ensure that you are familiar with operating the TissueLyser LT by referring to the *TissueLyser LT User Manual*.
- If using a QIAGEN kit for RNA purification, read the supplied handbook carefully before starting.
- Soft, fresh tissues from plants such as *Nicotiana* and *Arabidopsis* can often also be disrupted and homogenized in lysis buffer. Hard tissues (e.g., woody plant materials) may require freezing and disruption under frozen conditions.
- When disrupting tough or very tough samples, we recommend using one or two 7 mm stainless steel beads, respectively, instead of one 5 mm stainless steel bead to guarantee optimal disruption.

Procedure

1. **Place 2 ml microcentrifuge tubes containing 1 stainless steel bead (5 mm mean diameter) into the insert of the TissueLyser LT Adapter. Incubate on dry ice for at least 30 min.**
2. **Determine the amount of fresh plant material. Do not use more than 100 mg per sample.**

Weighing tissue is the most accurate way to determine the amount.

3. **Transfer the weighed tissue to the precooled tubes and incubate for another 30 min on dry ice.**

Alternatively, plant tissues can be flash-frozen in liquid nitrogen prior to transfer to the precooled tubes. In this case, the additional 30 min incubation on dry ice is not necessary.

Note: Do not freeze the adapter and tubes in liquid nitrogen, as this may lead to breakage of the tubes.

4. **Place the precooled insert with sample tubes into the base of the TissueLyser LT Adapter, which is attached to the TissueLyser LT. Place the lid of the TissueLyser LT Adapter over the insert, and screw the knob until the lid is securely fastened.**

5. Immediately operate the TissueLyser LT for 2–5 min at 50 Hz.

Note: The duration of disruption and homogenization depends on the tissue being processed and can be extended until no tissue debris is visible. If operating the TissueLyser LT for longer than 2 min, stop the instrument every 2 min and place the insert of the TissueLyser LT Adapter and the sample tubes on dry ice for several minutes. This prevents thawing of the samples, which would result in reduced RNA yield and quality.

6. Remove the sample tubes from the insert of the TissueLyser LT Adapter, and incubate at room temperature (15–25°C) for 1 min to avoid freezing lysis buffer in step 7.

Do not incubate for longer than 1 min, otherwise the tissue will thaw, resulting in potential RNA degradation.

7. Add the appropriate volume of lysis buffer (e.g., Buffer RLT or Buffer RLC) to each tube, and proceed with RNA purification.

Do not reuse the stainless steel beads.

Protocol: Purification of RNA from Bacteria

This protocol provides guidelines on disrupting bacteria for subsequent RNA purification. If using an RNeasy Protect Bacteria Kit for RNA purification (see Table 3, page 9), refer to the supplied *RNAProtect® Bacteria Reagent Handbook*, which contains complete protocols for sample disruption and RNA purification.

Important points before starting

- Before beginning the procedure, read “Important Notes” (page 13).
- Ensure that you are familiar with operating the TissueLyser LT by referring to the *TissueLyser LT User Manual*.
- If using an RNeasy Protect Bacteria Kit for RNA purification, read the supplied handbook carefully before starting.
- Bead milling will disrupt most Gram-positive and Gram-negative bacteria, including mycobacteria. Gram-positive bacteria usually require more rigorous digestion (e.g., increased enzyme digestion time and temperature) and mechanical treatment than Gram-negative bacteria. For details, see the *RNAProtect Bacteria Reagent Handbook*.

Procedure

1. **Pellet the bacterial cells by centrifugation. Immediately add the appropriate volume of lysis buffer (e.g., Buffer RLT) to each sample and vortex vigorously.**
2. **Transfer each sample to 2 ml microcentrifuge tubes containing 25–50 mg acid-washed glass beads (150–600 μm mean diameter).**
3. **Place the tubes into the insert of the TissueLyser LT Adapter.**
4. **Place the insert with sample tubes into the base of the TissueLyser LT Adapter, which is attached to the TissueLyser LT. Place the lid of the TissueLyser LT Adapter over the insert, and screw the knob until the lid is securely fastened.**
5. **Operate the TissueLyser LT for 5 min at 50 Hz.**
The duration of disruption and homogenization depends on the sample being processed and can be extended until no debris is visible.
6. **Proceed with RNA purification.**

Protocol: Purification of RNA from Yeast

This protocol provides guidelines on disrupting yeast cells for subsequent RNA purification. If using the RNeasy Mini Kit for RNA purification (see Table 3, page 9), refer to the supplied *RNeasy Mini Handbook*, which contains a complete protocol for sample disruption and RNA purification.

Important points before starting

- Before beginning the procedure, read “Important Notes” (page 13).
- Ensure that you are familiar with operating the TissueLyser LT by referring to the *TissueLyser LT User Manual*.
- If using the RNeasy Mini Kit for RNA purification, read the supplied handbook carefully before starting.

Procedure

1. **Pellet the yeast cells by centrifugation. Immediately add the appropriate volume of lysis buffer (e.g., Buffer RLT) to each sample and vortex vigorously.**
2. **Transfer each sample to 2 ml microcentrifuge tubes containing approximately 600 μ l acid-washed glass beads (450–550 μ m mean diameter).**
3. **Place the tubes into the insert of the TissueLyser LT Adapter.**
4. **Place the insert with sample tubes into the base of the TissueLyser LT Adapter, which is attached to the TissueLyser LT. Place the lid of the TissueLyser LT Adapter over the insert, and screw the knob until the lid is securely fastened.**
5. **Operate the TissueLyser LT for 5 min at 50 Hz.**
The duration of disruption and homogenization depends on the sample being processed and can be extended until no debris is visible.
6. **Proceed with RNA purification.**

Protocol: Purification of DNA from Animal and Human Tissues

This protocol provides guidelines on disrupting animal and human tissues for subsequent DNA purification.

Important points before starting

- Before beginning the procedure, read “Important Notes” (page 13).
- Ensure that you are familiar with operating the TissueLyser LT by referring to the *TissueLyser LT User Manual*.
- If using a QIAGEN kit for DNA purification, read the supplied handbook and appropriate supplementary protocol carefully before starting.
- When disrupting tough or very tough samples, we recommend using one or two 7 mm stainless steel beads, respectively, instead of one 5 mm stainless steel bead to guarantee optimal disruption.

Procedure

1. **Place 2 ml microcentrifuge tubes containing 1 stainless steel bead (5 mm mean diameter) on dry ice for at least 15 min. Keep the insert of the TissueLyser LT Adapter at room temperature (15–25°C).**
2. **Transfer up to 25 mg fresh or frozen tissue to the precooled tubes and incubate for another 15 min on dry ice.**
3. **Place the tubes into the insert of the TissueLyser LT Adapter, and incubate at room temperature for 2 min to avoid freezing of lysis buffer in step 4.**

Do not incubate for longer than 2 min, otherwise the tissue will thaw, resulting in potential DNA degradation.

4. **Immediately add the appropriate volume of lysis buffer (e.g., Buffer ATL) to each tube.**
5. **Place the insert with sample tubes into the base of the TissueLyser LT Adapter, which is attached to the TissueLyser LT. Place the lid of the TissueLyser LT Adapter over the insert, and screw the knob until the lid is securely fastened.**
6. **Operate the TissueLyser LT for 40 s at 30 Hz.**

Note: Depending on the type of tissue, exceeding this homogenization time and intensity may lead to significant fragmentation of genomic DNA. However, for tough samples, it may be necessary to exceed this homogenization time and/or intensity to improve disruption efficiency.

If working with fibrous tissues, cutting the tissue into smaller pieces before starting disruption will improve disruption efficiency.

7. Proceed with DNA purification.

Do not reuse the stainless steel beads.

Protocol: Purification of DNA from Plant Tissues

This protocol provides guidelines on using the TissueLyser LT to disrupt plant tissues for subsequent DNA purification. If using a QIAGEN kit for DNA purification (see Table 5, page 10), refer to the supplied handbook, which contains a complete protocol for sample disruption and DNA purification.

Important points before starting

- Before beginning the procedure, read “Important Notes” (page 13).
- Ensure that you are familiar with operating the TissueLyser LT by referring to the *TissueLyser LT User Manual*.
- If using a QIAGEN kit for DNA purification, read the supplied handbook carefully before starting.
- When disrupting tough or very tough samples, we recommend using one or two 7 mm stainless steel beads, respectively, instead of one 5 mm stainless steel bead to guarantee optimal disruption.

Procedure

- 1. Place 2 ml microcentrifuge tubes containing 1 stainless steel bead (5 mm mean diameter) into the insert of the TissueLyser LT Adapter. Incubate on dry ice for at least 30 min.**
- 2. Determine the amount of fresh plant material. Do not use more than 100 mg per sample.**

Weighing tissue is the most accurate way to determine the amount.

- 3. Transfer the weighed tissue to the precooled tubes and incubate for another 30 min on dry ice.**

Alternatively, plant tissues can be flash-frozen in liquid nitrogen prior to transfer to the precooled tubes. In this case, the additional 30 min incubation on dry ice is not necessary.

Note: Do not freeze the adapter and tubes in liquid nitrogen, as this may lead to breakage of the tubes.

- 4. Place the precooled insert with sample tubes into the base of the TissueLyser LT Adapter, which is attached to the TissueLyser LT. Place the lid of the TissueLyser LT Adapter over the insert, and screw the knob until the lid is securely fastened.**

5. Immediately operate the TissueLyser LT for 2 min at 50 Hz.

Note: Depending on the type of tissue, exceeding this homogenization time and intensity may lead to significant fragmentation of genomic DNA. However, for tough samples, it may be necessary to exceed this homogenization time and/or intensity to improve disruption efficiency. If operating the TissueLyser LT for longer than 2 min, stop the instrument every 2 min and place the insert of the TissueLyser LT Adapter and the sample tubes on dry ice for several minutes. This prevents thawing of the samples, which would result in reduced DNA yield and quality.

6. Remove the sample tubes from the insert of the TissueLyser LT Adapter, and incubate at room temperature (15–25°C) for 1 min to avoid freezing lysis buffer in step 7.

Do not incubate for longer than 1 min, otherwise the tissue will thaw, resulting in potential DNA degradation.

7. Add the appropriate volume of lysis buffer (e.g., Buffer AP1) to each tube, and proceed with DNA purification.

Do not reuse the stainless steel beads.

Protocol: Purification of Protein from Animal and Human Tissues

This protocol provides guidelines on disrupting animal and human tissues for purification of protein.

Important points before starting

- Before beginning the procedure, read “Important Notes” (page 13).
- Ensure that you are familiar with operating the TissueLyser LT by referring to the *TissueLyser LT User Manual*.
- If using a QIAGEN sample purification kit, read the supplied handbook carefully before starting.
- After storage in Allprotect Tissue Reagent, tissues become slightly hard. The disruption time may need to be extended.
- When disrupting tough or very tough samples, we recommend using one or two 7 mm stainless steel beads, respectively, instead of one 5 mm stainless steel bead to guarantee optimal disruption.

Procedure

- 1. Place 2 ml microcentrifuge tubes containing 1 stainless steel bead (5 mm mean diameter) on dry ice for at least 15 min. Keep the insert of the TissueLyser LT Adapter at room temperature (15–25°C).**
- 2. Transfer up to 30 mg fresh or frozen tissue to the precooled tubes and incubate for another 15 min on dry ice.**

If handling tissue samples stabilized with Allprotect Tissue Reagent, cooling on dry ice is not necessary.

- 3. Place the tubes into the insert of the TissueLyser LT Adapter, and incubate at room temperature for 2 min to avoid freezing of lysis buffer in step 4.**

Do not incubate for longer than 2 min, otherwise the tissue will thaw, resulting in potential protein degradation.

- 4. Immediately add the appropriate volume of lysis buffer (e.g., Mammalian Cell Lysis Buffer) to each tube.**
- 5. Place the insert with sample tubes into the base of the TissueLyser LT Adapter, which is attached to the TissueLyser LT. Place the lid of the TissueLyser LT Adapter over the insert, and screw the knob until the lid is securely fastened.**
- 6. Operate the TissueLyser LT for 2–5 min at 50 Hz.**

The duration of disruption and homogenization depends on the tissue being processed and can be extended until no tissue debris is visible.

- 7. Proceed with protein purification.**
Do not reuse the stainless steel beads.

Appendix A: TissueLyser LT Accessories

TissueLyser LT Adapter

This adapter allows disruption of 12 samples in parallel using standard 2 ml microcentrifuge tubes, such as Sample Tubes RB (2 ml) (cat. no. 990381) from QIAGEN. Sample disruption can be carried out at room temperature or after storing the adapter on dry ice for at least 30 minutes. The adapter set can be cleaned with detergent, microbicides, or up to 96% ethanol. For more information, see the *TissueLyser LT User Manual*.

TissueLyser Single-Bead Dispenser, 5 mm

This bead dispenser dispenses individual beads (5 mm diameter) into any sample container. The reservoir holds approximately 150 beads. The TissueLyser Single-Bead Dispenser can be cleaned with water or detergent. For more information, see the product sheet supplied with the TissueLyser Single-Bead Dispenser.

TissueLyser Single-Bead Dispenser, 7 mm

This bead dispenser dispenses individual beads (7 mm diameter) into any sample container. The reservoir holds approximately 45 beads. The TissueLyser Single-Bead Dispenser can be cleaned with water or detergent. For more information, see the product sheet supplied with the TissueLyser Single-Bead Dispenser.

Appendix B: Automated Solutions

Automated purification using QIAGEN spin-column kits

Purification of genomic DNA or total RNA from tissues can be fully automated on the QIAcube. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using DNeasy Kits, QIAamp Kits, RNeasy Kits, AllPrep DNA/RNA Kits, and the miRNeasy Mini Kit for purification of high-quality DNA or RNA. For more information about the automated procedure, see the relevant protocol sheet available at www.qiagen.com/MyQIAcube.



The QIAcube.

The QIAcube is preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at www.qiagen.com/MyQIAcube.

Automated purification using magnetic particles and 96-well plates

Complete automated solutions from QIAGEN allow purification of genomic DNA or total RNA from human, animal, or plant tissues at a range of different throughputs using magnetic particles or 96-well plates (see Table 7, page 31). For more information about QIAGEN automation, visit www.qiagen.com/automation.

Instrument service and support

QIAGEN Instrument Service provides comprehensive support services to ensure the continued success of your automated applications. For more information about QIAGEN Instrument Services, visit www.qiagen.com/service.

Table 7. Automated purification of genomic DNA and total RNA from tissues

Workstation	Capability
EZ1 Advanced	Purification of genomic DNA or total RNA from 1–6 human samples per run
EZ1 Advanced XL	Purification of genomic DNA or total RNA from 1–14 human samples per run
QIAasymphony SP	Purification of genomic DNA or total RNA from 1–96 animal or human samples per run
BioRobot Universal System	Purification of genomic DNA or total RNA in 96-well format from animal or human samples, plus downstream reaction setup
BioSprint 96	Purification of genomic DNA from up to 96 animal or plant samples per run

Low-throughput sample disruption

The TissueRuptor® is a handheld rotor–stator homogenizer that provides rapid and efficient disruption of individual samples for a wide range of downstream applications. The TissueRuptor uses transparent disposable probes, which helps to minimize the risk of cross-contamination and enables visual control of the sample disruption process. For more information about the TissueRuptor, visit www.qiagen.com/TissueRuptor.

Medium- to high-throughput sample disruption

The TissueLyser II is a bead mill that rapidly and effectively disrupts up to 48 or 192 samples at the same time. A wide variety of samples, including human, animal, and plant tissues, bacteria, and yeast, can be disrupted to release high-quality DNA, RNA, and protein for subsequent purification and analysis. For more information about the TissueLyser II, visit www.qiagen.com/TissueLyserII.

Automated multicapillary gel electrophoresis

The revolutionary QIAxcel® System enables fully automated and sensitive, high-resolution capillary electrophoresis for up to 96 samples per run. Ready-to-go gel cartridges reduce manual handling errors and eliminate the need for tedious gel preparation. With the QIAxcel System, analysis of DNA fragments, singleplex or multiplex PCR products, and qualitative and quantitative RNA analysis is now easier and faster than ever. To find out more, visit www.qiagen.com/QIAxcel.

Ordering Information

Product	Contents	Cat. no.
TissueLyser LT	Bead mill for low- to medium-throughput sample disruption	85600
TissueLyser LT Adapter, 12-Tube	Adapter for disruption of up to 12 samples in 2 ml microcentrifuge tubes on the TissueLyser LT	69980
Sample Tubes RB (2 ml)	1000 safe-lock microcentrifuge tubes (2 ml) for use with the TissueLyser LT	990381
Stainless Steel Beads, 5 mm (200)	5mm Stainless Steel Beads, suitable for use with the TissueLyser LT system	69989
Stainless Steel Beads, 7 mm (200)	7 mm Stainless Steel Beads, suitable for use with the TissueLyser LT system	69990
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
TissueLyser Single-Bead Dispenser, 7 mm	For dispensing individual beads (7 mm diameter)	69967
Related products		
RNeasy Kits — for purification of total RNA from cells, tissues, and yeast		
RNeasy Micro Kit (50)	For 50 preps: RNeasy MinElute® Spin Columns, Collection Tubes, DNase I, Carrier RNA, Buffers	74004
RNeasy Mini Kit (50)	For 50 preps: RNeasy Spin Columns, Collection Tubes, Buffers	74104
RNeasy Protect Kits — for stabilization and purification of total RNA from tissues		
RNeasy Protect Mini Kit (50)	For 50 preps: RNA _{later} RNA Stabilization Reagent, RNeasy Spin Columns, Collection Tubes, Buffers	74124

Product	Contents	Cat. no.
RNeasy Plus Kits — for purification of total RNA from cells and tissues using gDNA Eliminator spin columns		
RNeasy Plus Micro Kit (50)	For 50 preps: RNeasy MinElute Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, Carrier RNA, Buffers	74034
RNeasy Plus Mini Kit (50)	For 50 preps: RNeasy Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, Buffers	74134
Reagent DX	1 ml Reagent DX in a screw-cap tube	19088
RNeasy Fibrous Tissue Kits — for purification of total RNA from fiber-rich tissues		
RNeasy Fibrous Tissue Mini Kit (50)	For 50 preps: RNeasy Spin Columns, Collection Tubes, Proteinase K, DNase I, Buffers	74704
RNeasy Fibrous Tissue Midi Kit (10)	For 10 preps: RNeasy Spin Columns, Collection Tubes, Proteinase K, DNase I, Buffers	75742
RNeasy Lipid Tissue Kits — for purification of total RNA from all types of tissue, including fatty tissues		
RNeasy Lipid Tissue Mini Kit (50)	For 50 preps: RNeasy Spin Columns, Collection Tubes, QIAzol Lysis Reagent, Buffers	74804
RNeasy 96 Universal Tissue Kits — for purification of total RNA from all types of tissue in 96-well format		
RNeasy 96 Universal Tissue Kit (4)	For 4 x 96 preps: RNeasy 96 Plates, Elution Microtubes CL, Caps, S-Blocks, Airpore Tape Sheets, QIAzol Lysis Reagent, Buffers	74881
RNeasy 96 Universal Tissue 8000 Kit (12)	For 12 x 96 preps on the BioRobot Universal System: RNeasy 96 Plates, Collection Microtubes, Elution Microtubes CL, Caps, S-Blocks, QIAzol Lysis Reagent, Buffers	967852

Product	Contents	Cat. no.
EZ1 RNA Tissue Mini Kit — for purification of total RNA from easy-to-lyse human tissues on EZ1 workstations		
EZ1 RNA Tissue Mini Kit (48)	For 48 preps: Reagent Cartridges, Tips, Tip-Holders, Tubes, DNase I, Buffer RLT	959034
EZ1 RNA Universal Tissue Kit — for purification of total RNA from all types of human tissue on EZ1 workstations		
EZ1 RNA Universal Tissue Kit (48)	For 48 preps: Reagent Cartridges, Tips, Tip-Holders, Tubes, QIAzol Lysis Reagent	956034
MagAttract RNA Tissue Mini M48 Kit — for purification of total RNA from easy-to-lyse human tissues on the BioRobot M48 workstation		
MagAttract RNA Tissue Mini M48 Kit (192)	For 192 preps: MagAttract Suspension E, DNase I, Buffers	959236
MagAttract RNA Universal Tissue M48 Kit — for purification of total RNA from all types of human tissue on the BioRobot M48 workstation		
MagAttract RNA Universal Tissue M48 Kit (192)	For 192 preps: MagAttract Suspension E, DNase I, QIAzol Lysis Reagent, Buffers	956336
QIASymphony RNA Kit — for purification of total RNA from cells and tissues on the QIASymphony SP		
QIASymphony RNA Kit (192)	For 192 preps: 2 Reagent Cartridges, and Enzyme Racks	931636
RNeasy Plant Mini Kit — for purification of total RNA from plants and fungi		
RNeasy Plant Mini Kit (20)	For 20 preps: RNeasy Spin Columns, QIAshredder Spin Columns, Collection Tubes, Buffers	74903
RNeasy 96 Kit — for purification of total RNA from cells in 96-well format		
RNeasy 96 Kit (4)	For 4 x 96 preps: RNeasy 96 Plates, Elution Microtubes CL, Caps, S-Blocks, Airpore Tape Sheets, Buffers	74181

Product	Contents	Cat. no.
RNeasy Protect Bacteria Kits — for stabilization and purification of total RNA from bacteria		
RNeasy Protect Bacteria Mini Kit (50)	For 50 preps: RNeasy Protect Bacteria Reagent, RNeasy Mini Kit	74524
RNeasy Protect Bacteria Midi Kit (10)	For 10 preps: RNeasy Protect Bacteria Reagent, RNeasy Midi Kit	75552
AllPrep DNA/RNA/Protein Mini Kit — for simultaneous purification of DNA, RNA, and protein from cells and tissues		
AllPrep DNA/RNA/Protein Mini Kit (50)	For 50 preps: AllPrep DNA Spin Columns, RNeasy Spin Columns, Collection Tubes, Buffers	80004
AllPrep DNA/RNA Kits — for simultaneous purification of DNA and RNA from cells and tissues		
AllPrep DNA/RNA Micro Kit (50)	For 50 preps: AllPrep DNA Spin Columns, RNeasy MinElute Spin Columns, Collection Tubes, Carrier RNA, Buffers	80284
AllPrep DNA/RNA Mini Kit (50)	For 50 preps: AllPrep DNA Spin Columns, RNeasy Spin Columns, Collection Tubes, Buffers	80204
QIAamp DNA Mini Kit — for purification of genomic, mitochondrial, bacterial, parasite, or viral DNA		
QIAamp DNA Mini Kit (50)	For 50 preps: QIAamp Spin Columns, Collection Tubes, Proteinase K, Buffers	51304
EZ1 DNA Tissue Kit — for automated purification of genomic DNA from 1–6 human samples on EZ1 workstations		
EZ1 DNA Tissue Kit (48)	For 48 preps: Reagent Cartridges, Tips, Tip-Holders, Tubes, Proteinase K, Buffer G2	953034
MagAttract DNA Mini M48 Kit — for automated purification of genomic DNA from 6–48 human samples on the BioRobot M48 workstation		
MagAttract DNA Mini M48 Kit (192)	For 192 preps: MagAttract Suspension B, Proteinase K, Buffers	953336

Product	Contents	Cat. no.
QIAasymphony DNA Kits — for purification of DNA from a wide range of sample types on the QIAasymphony SP		
QIAasymphony DNA Mini Kit (192)	For 192 preps of 200 μ l each: 2 Reagent Cartridges, and Enzyme Racks	931236
QIAasymphony DNA Midi Kit (96)	For 96 preps of 1000 μ l each: 2 Reagent Cartridges, and Enzyme Racks	931255
DNeasy Blood & Tissue Kit — for purification of total DNA from animal blood and tissues, and from cells, yeast, bacteria, or viruses		
DNeasy Blood & Tissue Kit (50)	For 50 preps: DNeasy Spin Columns, Collection Tubes, Proteinase K, Buffers	69504
DNeasy 96 Blood & Tissue Kit — for purification of total DNA from animal blood and tissues, and from cells, yeast, bacteria, or viruses in 96-well format		
DNeasy 96 Blood & Tissue Kit (4)	For 4 x 96 preps: DNeasy 96 Plates, Collection Microtubes, Caps, S-Blocks, Elution Microtubes RS, AirPore Tape Sheets, Proteinase K, Buffers	69581
DNeasy Plant Kits — for purification of total DNA from plants and fungi		
DNeasy Plant Mini Kit (50)	For 50 preps: DNeasy Spin Columns, QIAshredder Spin Columns, Collection Tubes, RNase A, Buffers	69104
DNeasy Plant Maxi Kit (6)	For 6 preps: DNeasy Spin Columns, QIAshredder Spin Columns, Collection Tubes, RNase A, Buffers	68161
DNeasy 96 Plant Kit — for purification of total DNA from plants in 96-well format		
DNeasy 96 Plant Kit (6)	For 6 x 96 preps: DNeasy 96 Plates, Collection Microtubes, Caps, S-Blocks, Elution Microtubes RS, AirPore Tape Sheets, RNase A, Reagent DX, Buffers	69181

Product	Contents	Cat. no.
MagAttract 96 DNA Plant Core Kit — for manual or automated purification of total DNA from plants in 96-well format		
MagAttract 96 DNA Plant Core Kit (6)	For 6 x 96 preps: MagAttract Suspension A, RNase A, Buffers	67161
BioSprint 15 DNA Plant Kit — for automated purification of total DNA from plant tissue on the BioSprint 15 workstation		
BioSprint 15 DNA Plant Kit (60)	For 60 preps: MagAttract Suspension G, Rod Covers, Tube Strips, RNase A, Buffers	941514
BioSprint 96 DNA Plant Kit — for automated purification of total DNA from plant tissue on the BioSprint 96 workstation		
BioSprint 96 DNA Plant Kit (576)	For 576 preps: MagAttract Suspension G, Rod Covers, Microplates MP, S-Blocks, RNase A, Buffer RPW	941557

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Notes

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QIAzol Lysis Reagent is a subject of US Patent No. 5,346,994 and foreign equivalents.

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