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TissueLyser Handbook

For high-throughput disruption of biological samples

WWW.QIAGEN.COM

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Storage

The TissueLyser should be stored upright, in a dry environment, at room temperature (15–25°C).

Product Use Limitations

The TissueLyser is intended as general-purpose device. No claim or representation is intended for its use to identify any specific organism or for a specific clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of the TissueLyser for any particular use, since their performance characteristics have not been validated for any specific organism. The TissueLyser may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

All due care and attention should be exercised in the handling of many of the materials described in this text. 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.

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 page).

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 molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the TissueLyser 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 call one of the QIAGEN Technical Service Departments or local distributors (see back page).

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/ts/msds.asp 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

The TissueLyser provides rapid and efficient disruption of up to 192 biological samples, including plant and animal 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 the grinding vessels.

Disruption is critically important in order to release the nucleic acids from the sample material. Homogenization of the material acts to shear the high-molecular-weight cellular proteins and 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 both disrupts and homogenizes sample material in one simple and reliable step.

The TissueLyser is easily programmed to provide variable speeds from 3 to 30 Hz (180–1800 oscillations/minutes) and run times from 10 seconds to 99 minutes.

Applications

The TissueLyser enables fast and uniform disruption of plant and animal tissues, bacteria, and yeast in various sample volumes in several formats. QIAGEN offers Adapter Sets for 2 x 96 collection microtubes (1.2 ml) or 2 x 24 microcentrifuge tubes (2.0 ml) (see ordering information, page 22). For more specific application details, see Tables 1 and 2 (pages 11 and 12). For disruption of large sample amounts (midi and maxi format) grinding jars are also available from other vendors (Retsch). The TissueLyser provides efficient disruption of biological material in each sample vessel for reproducible, high-quality results in downstream applications such as the purification of total DNA or RNA from a variety of animal and plant tissues.

General Remarks about Sample Disruption and Homogenization

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, non-cellular 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 yield and quality of your DNA and RNA preparation.

Disruption and homogenization of starting materials

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

Homogenization: Homogenization is necessary to reduce the viscosity of the cell lysates produced by disruption. Homogenization shears the high-molecular-weight cellular proteins and carbohydrates to create a homogeneous lysate. Incomplete homogenization results in inefficient binding of DNA or RNA to the QIAGEN column and therefore significantly reduced yields.

Disruption and homogenization using the TissueLyser

In bead-milling, cells and tissues can be disrupted by rapid agitation in the presence of beads. QIAGEN offers kits and protocols that are based on two different methods for disruption and homogenization using the TissueLyser: samples can be disrupted and homogenized at room temperature in lysis buffer, or after freezing the sample-containing disruption vessel in liquid nitrogen. In the latter method, the lysis buffer is added after disruption of the sample. In special cases (e.g., the disruption of teeth or plant seeds), the sample can also be disrupted at room temperature without the addition of lysis

buffer, although this increases the risk of nucleic acid degradation by nucleases. 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 agitator (speed and duration)
- consistency of sample
- type of disruption vessel

Bead selection

For disruption of small sample amounts, the optimal beads to use are 0.1–0.6 mm (mean diameter) glass beads for bacteria, 0.5 mm glass beads for yeast and unicellular animal cells, and 3–7 mm stainless steel or tungsten carbide beads for plant and animal tissues. 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 and protocols not addressed in this handbook must be determined empirically. For disruption of large samples (midi and maxi format), grinding jars with steel beads are available from Retsch (www.retsch.de).

Note: Do not use Buffer RLT in conjunction with tungsten carbide beads. Buffer RLT reacts chemically with tungsten carbide causing damage to the bead surface.

* Nitric acid is extremely corrosive and can cause severe burns. Use in a fume hood and take appropriate safety measures

Introduction to Disruption Protocols and Nucleic Acid Purification Kits

For small sample amounts, lysis buffer and bead(s) suitable for the application, are added to disposable grinding vessels and assembled into the Adapter Sets. The bead should be placed in the disruption vessel prior to adding the sample or lysis buffer. The Adapter Sets are then fixed into the clamps (arms) of the TissueLyser. Disruption is carried out in high-speed (20–30 Hz) shaking steps.

When disrupting frozen samples using the TissueLyser Adapter Sets, the disposable disruption microtubes, containing the sample and bead, should be pre-chilled in liquid nitrogen and then assembled into the Adapter Sets.

Note: Do not freeze the adapter sets in liquid nitrogen.

For processing of samples in midi and maxi format, grinding jars made of Teflon® (for disruption in liquids) or stainless steel (for disruption in liquid nitrogen or for special samples, e.g., teeth or woody plant tissues) are used. Grinding jar sets, with steel beads, are available from Retsch (www.retsch.de).

Optimized QIAGEN kit protocols, which use the TissueLyser for sample material homogenization, are available for the purification of RNA and DNA from a wide range of biological samples (see Table 1 and 2 respectively, pages 11 and 12).

Typical TissueLyser Protocol

Homogenization of tissue(s) or cells using the TissueLyser is typically achieved by placing the sample material into the recommended container, together with a bead. Disruption can be undertaken in the presence or absence of buffer depending upon the specific protocol. Disruption for 2 x 3 minutes at 20–30 Hz is usually sufficient to release RNA. Shorter disruption times are recommended for DNA purification, in order to prevent DNA shearing. When using the TissueLyser Adapter Set 2 x 24 or 2 x 96, samples on the inside of the adapter rack move more slowly than samples on the outside. To prevent variation in sample homogenization, the Adaptor Set should be removed from the TissueLyser and disassembled after the first disruption step. For the second disruption step, the Adaptor Set should be reassembled so that the microtube order is reversed. Rotating the racks of collection microtubes in this way ensures that all samples are thoroughly and equally disrupted.

IMPORTANT: Merely rotating the entire plate sandwich so that the QIAGEN logos are upside down when reinserted into the TissueLyser is not sufficient, since the same samples that were outermost during the initial disruption will remain outermost in the second disruption step.

For optimal operation the TissueLyser should always be balanced. A balance can be provided by assembling a second Adaptor Set using a rack of collection microtubes without samples or buffers, but containing the disruption beads, and fixing this Adaptor Set into the empty clamp. If using grinding jars then the balance should consist of a second grinding jar containing a stainless steel ball.

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 page). Supplementary protocols can be identified by their reference number, which is made up of two letters followed by two numbers (e.g., RY15 — Isolation of total RNA from animal tissues using the RNeasy 96 Kit; vacuum/spin procedure).

Note: All protocols for use with the Mixer Mill can be used on the TissueLyser, without modification.

Table 1. Kit Selection Guide for RNA Purification

Starting material	QIAGEN kit	Kit format and amount of starting material per prep	Page no.
Standard human and animal tissue (e.g., kidney, liver, and lung)	RNeasy® Mini Kit	Spin column; up to 30 mg tissue	13
	RNeasy Protect Mini Kit	Spin column; up to 30 mg tissue with stabilization	13
	RNeasy 96 Kit	96-well plate; up to 15 mg tissue	13
	EZ1 RNA Tissue Mini Kit	1–6 samples; automated; up to 10 mg tissue	14
	MagAttract® RNA Tissue M48 Kit	6–48 samples; automated; up to 10 mg tissue	14
Fibrous human and animal tissue (e.g., heart and muscle)	RNeasy Fibrous Mini Kit	Spin column; up to 30 mg tissue	14
	RNeasy Fibrous Midi Kit	Spin midi column; up to 250 mg tissue	14
Lipid human and animal tissue (e.g., adipose tissue and brain)	RNeasy Lipid Tissue Mini Kit	Spin column; up to 100 mg tissue	15
Plant (e.g., leaf)	RNeasy Plant Mini Kit	Spin column; up to 30 mg tissue	16
	RNeasy 96 Kit	96-well plate; up to 25 mg tissue	16
	EZ1 RNA Tissue Mini Kit	1–6 samples; automated; up to 10 mg tissue	17
	MagAttract RNA Tissue Mini M48 Kit	6–48 samples; automated; up to 10 mg tissue	17
Bacteria (Gram-positive and -negative)	RNeasy Protect Bacteria Mini Kit	Spin column; up to 2.5×10^8 cells	17
	RNeasy Protect Bacteria Midi Kit	Spin column; up to 1.5×10^9 cells	17

Table 2. Kit Selection Guide for DNA Purification

Starting material	QIAGEN kit	Kit format and amount of starting material per prep	Page no.
Standard animal tissue (e.g., kidney, liver, and lung)	DNeasy® Tissue Kit	Spin column; up to 25 mg tissue	18
	QIAamp® DNA Mini Kit	Spin column; up to 30 mg tissue	18
	EZ1 DNA Tissue	1–6 samples; automated; up to 10 mg tissue	18
	MagAttract DNA Mini M48 Kit	6–48 samples; automated; up to 10 mg tissue	18
Plant (e.g., leaf)	DNeasy Plant Mini Kit	Spin column; up to 100 mg tissue	19
	DNeasy Plant Maxi Kit	Maxi spin column; up to 1 g tissue	19
	DNeasy 96 Plant Kit	96-well plate; up to 50 mg tissue	20
	MagAttract 96 DNA Plant Kit	96-well plate; up to 100 mg tissue	20

RNA Purification from Human and Animal Tissues

Standard tissues

QIAGEN kit	Sample disruption and nucleic acid purification protocol
RNeasy Mini Kit	*RNeasy Mini Handbook*
RNeasy Protect Mini Kit	*RNeasy Mini Handbook*
RNeasy 96 Kit	RY15 — Isolation of total RNA from animal tissues using the RNeasy 96 Kit; vacuum/spin procedure
EZ1 RNA Tissue Mini Kit	*EZ1 RNA Mini Handbook*
MagAttract RNA Tissue Mini M48 Kit	*MagAttract RNA Tissue Mini M48Handbook*

General protocol

Homogenization using the TissueLyser is carried out by adding a stainless steel bead (3–7 mm diameter) to the sample, followed by 350 µl Buffer RLT. The sample material is disrupted for 2 x 3 minutes at 20–30 Hz. The duration of homogenization can be extended until no tissue debris is visible.

Note: Do not use Buffer RLT in conjunction with tungsten carbide beads. Buffer RLT reacts chemically with tungsten carbide causing damage to the bead surface.

RNeasy Mini Kit, RNeasy 96 Kit, and RNeasy Mini Protect Kit

The RNeasy Mini and 96 Kits and RNeasy Mini Protect Kit allow the rapid purification of up to 100 µg of RNA from many standard animal tissues including kidney, liver, lung, pancreas, spleen, testes, and thymus. Tissue is disrupted and homogenized in Buffer RLT (supplied with the kit). Buffer RLT contains guanidine isothiocyanate, a highly denaturing substance that immediately inactivates RNases. After disruption and homogenization using the TissueLyser, the lysate is centrifuged, the supernatant is removed, and the RNA is purified using RNeasy silica-gel-based membrane technology in a simple bind-wash-elute procedure.

Note: After storage in RNA*later*™ RNA Stabilization Reagent (provided in the RNeasy Mini Protect Kit), tissues become slightly harder than fresh or thawed

tissues. Disruption and homogenization of this tissue, however, is usually not a problem. We recommend increasing the amount of lysis buffer to 600 µl and prolonging the homogenization time up to 2 x 5 minutes.

MagAttract RNA Tissue Mini M48 Kit and EZ1 RNA Tissue Mini Kit

Automated RNA purification can be performed on the BioRobot® M48 workstation using the MagAttract RNA Tissue M48 Kit and BioRobot EZ1workstation using the EZ1 RNA Tissue Kit. The TissueLyser protocol for sample disruption is the same as that given on the previous page.

Fibrous tissues

QIAGEN kit	Sample disruption and nucleic acid purification protocol
RNeasy Fibrous Mini Kit	*RNeasy Fibrous Tissue Mini Handbook*
RNeasy Fibrous Midi Kit	*RNeasy Fibrous Tissue Midi Handbook*

RNeasy Fibrous Tissue Mini and Midi Kits

RNeasy Fibrous Mini and Midi Kits contain proteinase K in order to digest fibrous proteins. Samples are disrupted and homogenized using the TissueLyser (see “general protocol”, page 13) in Buffer RLT (supplied with the kit), which contains guanidine isothiocyanate, a highly denaturing substance that immediately inactivates RNases. After lysis and treatment with proteinase K the RNA is purified using RNeasy silica-gel-based membrane technology in a simple bind-wash-elute procedure. The RNeasy Fibrous Mini and Midi Kits yield up to 100 µg and 1 mg of RNA respectively.

Note: Fibrous tissues are sometimes not completely homogenized after processing in the TissueLyser; small amounts of debris however have no effect on the RNA purification procedure, and usually the tissue will be completely digested after the proteinase K digest. Since the RNase-inactivating buffer, Buffer RLT, must be diluted to permit proteinase K digestion, this protocol should not be used for tissues rich in RNases, such as pancreas or intestine.

Fatty tissues

QIAGEN kit	Sample disruption and nucleic acid purification protocol
RNeasy Lipid Tissue Mini Kit	*RNeasy Lipid Tissue Mini Handbook*

RNeasy Lipid Tissue Mini Kit

Homogenization using the TissueLyser is performed by placing a 5 mm stainless steel bead, the sample for disruption then 1 ml of QIAzol Lysis Reagent (included in RNeasy Lipid Tissue Kits) into a disruption microtube. Homogenization for 2 x 2 minutes at 20 Hz is generally sufficient for soft tissues such as adipose tissue. For tissues that are more difficult to disrupt the vibration speed can be increased to 25 Hz and the duration of homogenization can be extended.

The RNeasy Lipid Tissue Mini Kit is designed for optimal lysis of tissues rich in fat, such as brain and adipose tissues, and purification of high-quality total RNA. The sample is disrupted and homogenized, using the TissueLyser, in QIAzol Lysis Reagent (supplied with the kit), which is a monophasic solution of phenol and guanidine isothiocyanate, designed to facilitate lysis of fatty tissues and inhibit RNases. After disruption and homogenization using the TissueLyser, chloroform is added, the lysate is centrifuged and the aqueous phase containing the RNA is removed. The RNA is purified using RNeasy silica-gel-based membrane technology in a simple bind-wash-elute procedure. The RNeasy Lipid Tissue Mini Kit enables the purification of up to 100 μg of total RNA.

RNA Purification from Plant Tissues

QIAGEN kit	Sample disruption and nucleic acid purification protocol
RNeasy Plant Mini Kit	RY08 — Isolation of total RNA from plant tissues using the RNeasy Plant Mini Kit
RNeasy 96 Kit	RY11 — Isolation of total RNA from plants using the RNeasy 96 Kit
EZ1 RNA Tissue Mini Kit	*EZ1 RNA Mini Handbook*
MagAttract RNA Tissue Mini M48 Kit	*MagAttract RNA Tissue Mini M48 Handbook*

RNeasy Plant Mini Kit and RNeasy 96 Kit

Homogenization using the TissueLyser is carried out by adding a stainless steel bead (3–7 mm diameter), the tissue sample, then 450 µl Buffer RLT or Buffer RLC (RNeasy Plant Mini Kit) to a disruption microtube. Disruption for 2 x 1.5 minutes at 30 Hz is usually sufficient, however, the duration of homogenization can be extended until no tissue debris is visible.

The RNeasy Plant Mini Kit and RNeasy 96 Kit allow low- or high-throughput RNA extraction from fresh or frozen plant tissue samples, without organic extraction or precipitation. The sample tissue is disrupted and homogenized in Buffer RLT or RLC, which contain guanidine isothiocyanate or guanidine hydrochloride respectively. These highly denaturing substances immediately inactivate RNases. The lysed sample is applied to wells of the RNeasy 96 plate or the RNeasy column (after being passed through a QIAshredder). Total RNA binds efficiently to the silica-gel-based membrane and contaminants are washed away. The RNeasy Plant Mini Kit and RNeasy 96 Kit can process up to 100 mg of plant tissue and yield up to 100 µg of RNA.

Note: Do not use Buffer RLT in conjunction with tungsten carbide beads. Buffer RLT reacts chemically with tungsten carbide causing damage to the bead surface.

For initial experiments, do not use more than 25 mg plant material per sample. With optimization, it may be possible to use larger amounts of starting material. The RNA content of plant tissues can vary due to tissue type, developmental stage, growth conditions used, and other factors.

Soft, fresh tissues from plants such as *Nicotiana* and *Arabidopsis* can often be disrupted by homogenization in lysis buffer. Hard plant tissues (e.g., woody plant materials) may require freezing and disruption under frozen conditions.

Note: If using frozen plant tissue, perform the disruption using liquid nitrogen, and do not add Buffer RLT until the samples have been disrupted.

MagAttract RNA Tissue Mini M48 Kit and EZ1 RNA Tissue Mini Kit

Automated RNA purification can be performed on the BioRobot M48 workstation using the MagAttract RNA Tissue M48 Kit and BioRobot EZ1workstation using the EZ1 RNA Tissue Kit. Further details can be found in the *MagAttract RNA Tissue Mini M48* and *EZ1 RNA Mini Handbooks*.

RNA Purification from Bacteria

QIAGEN kit	Sample disruption and nucleic acid purification protocol
RNeasy Protect Bacteria Mini Kit	RY14 — Stabilization and isolation of total RNA from Gram-positive bacteria using RNeasy Protect Bacteria Kits and *RNAprotect™ Bacteria Reagent Handbook*
RNeasy Protect Bacteria Midi Kit	RY14 — Stabilization and isolation of total RNA from Gram-positive bacteria using RNeasy Protect Bacteria Kits and *RNAprotect™ Bacteria Reagent Handbook*

RNeasy Protect Bacteria Mini and Midi Kits

QIAGEN offers several protocols, which combine enzymatic and/or mechanical disruption of bacterial cell walls. Bead milling will lyse 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 organisms. Detailed protocols are described in the *RNAprotect Bacteria Reagent Handbook*.

The RNeasy Protect Bacteria Kits contain RNAprotect Bacteria Reagent, which stabilizes RNA preventing degradation, prior to protein digestion with lysozyme and proteinase K. Further RNA purification is achieved using the RNeasy Mini or Midi Spin Columns (provided in the kit). Typically, cells are disrupted in Buffer RLT for 5 minutes at 30 Hz using acid-washed glass beads (150–600 mm, available from Sigma, cat. nos. G1145, G1277, and G8772). The cell lysate is then applied to an RNeasy column, which binds the RNA, allowing contaminants to be efficiently washed away. The resulting high-quality RNA accurately represents the expression profile of living bacteria.

DNA Purification from Human and Animal Tissues

QIAGEN kit	Sample disruption and nucleic acid purification protocol
DNeasy Tissue Kit	DY11 — Isolation of DNA from soft tissue using the TissueLyser and QIAamp DNA Mini Kit
QIAamp DNA Mini Kit	QA31 — Isolation of DNA from soft tissue using the TissueLyser and DNeasy Tissue Kit
EZ1 DNA Tissue Kit	MA23 — Isolation of DNA from soft tissue using the TissueLyser and EZ1 DNA Tissue Kit
MagAttract DNA Mini M48 Kit	MA22 — Isolation of DNA from soft tissue using the TissueLyser and MagAttract Mini M48 Kit

QIAamp DNA Mini Kit and DNeasy Tissue Kit

Using the TissueLyser up to 10 mg of tissue can be disrupted in a 2 ml microcentrifuge tube with a 5 mm stainless steel bead. Tissue is disrupted for 20 seconds at 30 Hz in Buffer ATL (QIAamp DNA Mini Kit and DNeasy Tissue Kit).*

Following disruption, the addition of proteinase K aids cell lysis while RNase A addition enables the purification of RNA-free genomic DNA. The lysed sample is either applied to a silica-gel-based membrane or mixed with MagAttract particles (MagAttract DNA Mini M48 Kit and EZ1 DNA Tissue Kit, see below), which selectively binds the DNA, and contaminants are removed by washing.

MagAttract DNA Mini M48 Kit and EZ1 DNA tissue Kit

Automated DNA purification can be performed on the BioRobot M48 workstation using the MagAttract DNA Mini M48 Kit and BioRobot EZ1workstation using the EZ1 DNA tissue Kit. Further details can be found in the *MagAttract DNA M48 Handbook* and the *BioRobot EZ1 Genomic DNA Kit Handbook*.

Note: These protocols have only been tested with “soft” tissues (e.g., liver, spleen, thymus, heart, kidney, brain) and may not work with “hard” tissues (e.g., bone, teeth, skin). The protocol can be used for both fresh and frozen tissues, and for tissues stabilized with RNA*later* RNA Stabilization Reagent.

* Longer disruption times may shear genomic DNA.

DNA Purification from Plant Tissues

QIAGEN kit	Sample disruption and nucleic acid purification protocol
DNeasy Plant Mini Kit	*DNeasy Plant Mini Handbook*
DNeasy Plant Maxi Kit	*DNeasy Plant Maxi Handbook*
DNeasy 96 Plant Kit	*DNeasy 96 Plant Handbook*
MagAttract® 96 DNA Plant Kit	*MagAttract 96 DNA Plant Handbook*

DNeasy Plant Mini and Maxi Kits

Up to 100 mg or 1 g of tissue can be processed using DNeasy Plant Mini Kits or DNeasy Plant Maxi Kits, respectively. The most efficient method of tissue homogenization is to add the bead and sample to the disruption vessel then chill the vessel in liquid nitrogen. Tissue samples processed using the DNeasy Plant Mini Kit should be homogenized in a 2 ml safe-lock microcentrifuge, using a 5 mm stainless steel bead. The microcentrifuge tube, containing the bead and sample, should be frozen in liquid nitrogen or 30 seconds prior to disruption at 30 Hz for 1 minute. After the first disruption step, the microcentrifuge should be removed from the Adapter Set and cooled for an additional 30 seconds in liquid nitrogen. Disruption for a further minute is then carried out. Lyophilized material can be disrupted at room temperature (15–25°C) in the absence of buffer.*

The disruption of large volumes of plant tissue for use with the DNeasy Maxi Kit is achieved using stainless steel grinding jars. The tissue sample and bead are added to the grinding jar, which is then cooled in liquid nitrogen for 1 minute. After disruption at 30 Hz for 1 minute the jar is placed in liquid nitrogen for 1 minute and then further disruption is carried out as before.

Following disruption, the tissue material is quickly transferred into a centrifuge tube and resuspended in Buffer AP1 (supplied with kit). RNA is digested using RNase A (supplied with kit). Proteins and polysaccharides are salt-precipitated and removed using a QIAshredder™ column. The cleared lysate is passed through a DNeasy spin column, which binds the DNA allowing washing and removal of contaminants.

* Protocols for the disruption of plant tissue in Buffer AP1 are available (see *DNeasy Plant Mini Handbook*).

DNeasy 96 Plant Kit and MagAttract 96 DNA Plant Core Kit

This disruption procedure is optimized for use with leaf tissues, but can also be used to isolate DNA from other plant tissues. However, when using tissues other than leaves, the disruption parameters may require optimization to ensure maximum DNA yield and quality.

Plant material and a 3 or 5 mm tungsten carbide or stainless steel bead are added to microtubes. The microtubes are placed into the Adapter Sets, which are fixed into the clamps (arms) of the TissueLyser and disrupted for 2 x 1 minute, at 30 Hz. The order of the microtubes should be reversed after the first disruption step (see page 9). We recommend the use of tungsten carbide beads as these perform better and more consistently than stainless steel beads.

Either fresh or frozen plant tissue samples can be processed using the TissueLyser. Fresh material can be directly disrupted in lysis buffer without using liquid nitrogen. Alternatively, fresh or frozen samples can be disrupted after freezing in liquid nitrogen. When disrupting frozen tissues, the disruption microtube should be placed in liquid nitrogen for 30 seconds following the first disruption step. Disruption of samples in lysis buffer yields DNA ideal for PCR, while disruption of samples in liquid nitrogen yields DNA of a higher molecular weight. We do not recommend disrupting frozen material in lysis buffer as this results in low yields and degraded DNA. Alternatively, lyophilized material can be disrupted at room temperature (15–25°C) without buffer.

TissueLyser Accessories

TissueLyser Single-Bead Dispensers:

TissueLyser Single-Bead Dispenser, 5 mm: for dispensing 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 washing-up liquid. For more information, see the product sheet supplied with the TissueLyser Single-Bead Dispenser.

TissueLyser Single-Bead Dispenser, 7 mm: for dispensing 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 washing-up liquid. For more information, see the product sheet supplied with the TissueLyser Single Bead Dispenser.

TissueLyser Bead Dispensers, 96-well:

TissueLyser 3 mm Bead Dispenser, 96-well: for dispensing 96 beads (3 mm diameter) in parallel into collection microtubes, enabling high-throughput disruption and homogenization

The reservoir holds approximately 1000 beads. The dispenser can be cleaned with water or washing-up liquid. For more information, see the product sheet supplied with the TissueLyser Bead Dispenser, 96-well.

TissueLyser 5 mm Bead Dispenser, 96-well: for dispensing 96 beads (5 mm diameter) in parallel into Collection Microtubes (racked), enabling high-throughput disruption and homogenization

The reservoir holds approximately 300 beads. The dispenser can be cleaned with water or washing-up liquid. For more information, see the product sheet supplied with the TissueLyser Bead Dispenser, 96-well.

TissueLyser Adapter Sets:

TissueLyser Adapter Set 2 x 96: for disrupting 192 (2 x 96) samples in parallel, using QIAGEN Collection Microtubes (racked)

The TissueLyser Adapter Set 2 x 96 is suitable for sample disruption in liquid nitrogen or at room temperature. The adapter can be cleaned with washing-up liquid, microbicides, or up to 96% ethanol. For more information, see the product sheet supplied with the TissueLyser Adapter Set.

TissueLyser Adapter Set 2 x 24: for disrupting 48 (2 x 24) samples in parallel

The 24-well adapter set holds standard 2.0 ml microcentrifuge tubes (e.g., Eppendorf Safe-Lock, cat. no. 0030120.94) and allows sample homogenization in liquid nitrogen or at room temperature. The adapter can be cleaned with washing-up liquid, microbicides, or up to 96% ethanol. For more information, see the product sheet supplied with the TissueLyser Adapter Sets.

Ordering Information

Product	Contents	Cat. no.
TissueLyser (220–240 V, 50/60 Hz)	Universal laboratory mixer-mill disruptor, 220–240 V, 50/60 Hz	85220
TissueLyser (120 V, 50/60 Hz)	Universal laboratory mixer-mill disruptor, 120 V, 50/60 Hz	85210
TissueLyser (100 V, 50/60 Hz)	Universal laboratory mixer-mill disruptor, 100 V, 50/60 Hz	85200
Accessories		
TissueLyser Adapter Set 2 x 96	2 sets of Adapter Plates for use with Collection Microtubes (racked) on the TissueLyser	69984
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2.0 ml microcentrifuge tubes on the TissueLyser	69982
Collection Microtubes (racked)	Nonsterile polypropylene tubes (1.2 ml), 960 in racks of 96	19560
Collection Microtube Caps	Nonsterile polypropylene caps for collection microtubes (1.2 ml) and round-well blocks, 960 in strips of 8	19566
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with the TissueLyser system	69989
Tungsten Carbide Beads, 3 mm (200)	Tungsten Carbide Beads, suitable for use with the TissueLyser system	69997
TissueLyser Single-Bead Dispenser, 5mm	For dispensing individual beads (5 mm diameter)	69965

Product	Contents	Cat. no.
TissueLyser Single-Bead Dispenser, 7mm	For dispensing individual beads (7 mm diameter)	69967
TissueLyser 3 mm Bead Dispenser, 96-Well	For dispensing 96 beads (3 mm diameter) in parallel	69973
TissueLyser 5 mm Bead Dispenser, 96-Well	For dispensing 96 beads (5 mm diameter) in parallel	69975
Related products		
RNeasy Mini Kit — for isolation of up to 100 µg total RNA from animal cells or tissues, yeast and bacteria		
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74104
RNeasy Midi Kit — for isolation of up to 1 mg total RNA from animal cells or tissues, yeast, and bacteria		
RNeasy Midi Kit (10)*†	10 RNeasy Midi Spin Columns, Collection Tubes (15 ml), RNase-free Reagents and Buffers	75142
RNeasy Fibrous Tissue Mini Kit — for isolation of up to 100 µg total RNA from fiber-rich tissues		
RNeasy Fibrous Tissue Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), Proteinase K, RNase-free DNase I, RNase-free Reagents and Buffers	74704

* Larger kit sizes available; please inquire.
† Requires use of a centrifuge capable of attaining 3000–5000 x *g* equipped with a swing-out rotor for 15 ml centrifuge tubes.

Product	Contents	Cat. no.
RNeasy Fibrous Tissue Midi Kit — for isolation of up to 1 mg total RNA from fiber-rich tissues		
RNeasy Fibrous Tissue Midi Kit (10)*	10 RNeasy Midi Spin Columns, Collection Tubes (15 ml), Proteinase K, RNase-free DNase I, RNase-free Reagents and Buffers	75742
RNeasy Lipid Tissue Mini Kit — for isolation of up to 100 µg total RNA from fatty tissues		
RNeasy Lipid Tissue Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, RNase-free Reagents and Buffers	74804
RNeasy Plant Mini Kit — for isolation of up to 100 µg total TNA from plants and fungi		
RNeasy Plant Mini Kit (20)†	20 RNeasy Mini Spin Columns, 20 QIAshredder Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74903
RNeasy Protect Mini Kit — for RNA*later* stabilization and RNeasy purification of up to 100 µg total RNA from animal tissues		
RNeasy Protect Mini Kit (50)†	RNA*later* RNA Stabilization Reagent (50 ml), 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74124
RNeasy Protect Bacteria Mini Kit —for RNAprotect stabilization and RNeasy purification of up to 100 µg total RNA from bacteria		
RNeasy Protect Bacteria Mini Kit (50)	RNeasy Mini Kit (50) and RNAprotect Bacteria Reagent (2 x 100 ml)	74524

* Requires use of a centrifuge capable of attaining 3000–5000 x g equipped with a swing-out rotor for 15 ml centrifuge tubes.
† Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
RNeasy Protect Bacteria Midi Kit —for RNAprotect stabilization and RNeasy purification of up to 1 mg total RNA from bacteria		
RNeasy Protect Bacteria Midi Kit (10)*	RNeasy Midi Kit (10) and RNAprotect Bacteria Reagent (2 x 100 ml)	75552
RNeasy 96 Kit — for high-throughput RNA isolation from animal cells		
RNeasy 96 Kit (4)†	For 4 x 96 total RNA preps: 4 RNeasy 96 Plates, Elution Microtubes CL, Caps, RNase-free Reagents and Buffers	74181
EZ1 RNA Tissue Mini Kit — for automated purification of high-quality total RNA from tissue samples up to 10 mg using the BioRobot EZ1 workstation		
EZ1 RNA Tissue Mini Kit (48)	48 Reagent Cartridges (RNA Tissue), 100 Disposable Tip Holders, 100 Disposable Filter-Tips, 50 Sample Tubes (2 ml), 50 Elution Tubes (1.5 ml), Buffer RLT, RNase-Free DNase I	959134
MagAttract RNA Tissue Mini M48 Kit — for automated purification of genomic DNA from 200 µl lysates of a wide range of human samples using the BioRobot M48 workstation		
MagAttract RNA Tissue Mini M48 Kit (192)	MagAttract Suspension E, Buffers, 4 x RNase-free DNase set, RNase-free water	959336
QIAamp DNA Mini Kit — for isolation of genomic, mitochondrial, bacterial, parasite, or viral DNA		
QIAamp DNA Mini Kit (50)†	For 50 DNA preps: 50 QIAamp Mini Spin Columns, Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304

* Requires use of a centrifuge capable of attaining 3000–5000 x *g* equipped with a swing-out rotor for 15 ml centrifuge tubes.
† Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
DNeasy Tissue Kit — for isolation of up to 40 µg genomic DNA from animal tissues and cells, yeast or bacteria		
DNeasy Tissue Kit (50)*	50 DNeasy Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504
DNeasy Plant Mini Kit — for isolation of up to 30 µg total cellular DNA from plant cells and tissues, or fungi		
DNeasy Plant Mini Kit (50)*	50 DNeasy Mini Spin Columns, 50 QIAshredder Mini Spin Columns, RNase A, Buffers, Collection Tubes (2 ml)	69104
DNeasy Plant Maxi Kit — For isolation of up to 260 µg total cellular DNA from plant cells and tissues, or fungi		
DNeasy Plant Maxi Kit (6)*	6 DNeasy Maxi Spin Columns, 6 QIAshredder Maxi Spin Columns, RNase A, Buffers, Collection Tubes (50 ml)	68161
DNeasy 96 Plant Kit — for isolation of up to 15 µg per well total cellular DNA from plant tissue		
DNeasy 96 Plant Kit (6)†	For 6 x 96 DNA minipreps: 6 DNeasy 96 Plates, Buffers, Reagents, RNase A, S-Blocks, Collection Microtubes (1.2 ml), Caps, AirPore™ Tape Sheets	69181
MagAttract 96 DNA Plant Core Kit — for high-throughput purification of genomic DNA from plant material		
MagAttract 96 DNA Plant Core Kit (6)*†	MagAttract Suspension A and buffers for 6 x 96 minipreps	67161

* Larger kit sizes available; please inquire.
† Requires the QIAGEN 96-Well-Plate Centrifugation system.

Product	Contents	Cat. no.
EZ1 DNA Tissue Kit — for purification of high-quality DNA from tissue using the BioRobot EZ1		
EZ1 DNA Tissue Kit (48)	48 Reagent Cartridges (Tissue), 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes (2.0 ml), 50 Elution Tubes (1.5 ml), Buffer G2, Proteinase K	953034
MagAttract DNA Mini M48 Kit — for automated purification of genomic DNA from 200 µl lysates of a wide range of human samples using the BioRobot M48 workstation		
MagAttract DNA Mini M48 Kit (192)	For 192 DNA preps: MagAttract Suspension B, Buffers, Proteinase K	953336

Notes

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QIAGEN Companies

Please see the back cover for contact information for your local QIAGEN office.

QIAGEN Distributors

Argentina
Tecnolab S.A.
Tel: (011) 4555 0010
Fax: (011) 4553 3331
E-mail: info@tecnolab.com.ar
Web site: www.tecnolab.com.ar

Austria
VWR International GmbH
Tel: (01) 576 00 0
Fax: (01) 576 00 600
E-mail: info@at.vwr.com
Web site: www.vwr.com

Belgium/Luxemburg
Westburg b.v.
Tel: 0800-1-9815
Fax: (31) 33-4951222
E-mail: info@westburg.nl
Web site: www.westburg.nl

Brazil
Uniscience do Brasil
Tel: 011 3622 2320
Fax: 011 3622 2323
E-mail: info@uniscience.com
Web site: www.uniscience.com

China
Gene Company Limited
Tel: (852)2896-6283
Fax: (852)2515-9371
E-mail:
Hong Kong: info@genehk.com
Beijing: gene@public2.bta.net.cn
Shanghai: gene@public.sta.net.cn
Chengdu: gene@public.cd.sc.cn
Guangzhou:
gzyitao@public.guangzhou.gd.cn

Croatia
INEL Medicinska Tehnika d.o.o.
Tel: (01) 2984-898
Fax: (01) 6520-966
E-mail:
inel-medicinska-tehnika@zg.tel.hr

Cyprus
Scientronics Ltd
Tel: 02-357 22 765416
Fax: 02-357 22 764614
E-mail: a.sarpetsas@biotronics.com.cy

Czech Republic
BIO-CONSULT spol. s.r.o.
Tel/Fax: (420) 2 417 29 792
E-mail: bio-cons@login.cz
Web site: www.bio-consult.cz

Denmark
VWR International A/S
Tel: 43 86 87 88
Fax: 43 86 87 90
E-mail: info@dk.vwr.com
Web site: www.vwr.com

Egypt
Clinilab
Tel: 52 57 212
Fax: 52 57 210
E-mail: Clinilab@link.net

Finland
VWR International Oy
Tel: (09) 804 551
Fax: (09) 8045 5200
E-mail: info@fi.vwr.com
Web site: www.vwr.com

Greece
BioAnalytica S.A.
Tel: (210)-640 03 18
Fax: (210)-646 27 48
E-mail: bioanalyt@hol.gr

Hungary
Kasztel-Med Co. Ltd.
Tel: (01) 385 3887
Fax: (01) 381 0695
E-mail: info@kasztel.hu
Web site: www.kasztel.hu

India
Genetix
Tel: (011)-2542 1714
or (011)-2515 9346
Fax: (011)-2546 7637
E-mail: genetix@nda.vsnl.net.in

Israel
Westburg (Israel) Ltd.
Tel: 08-6650813/4
or 1-800 20 22 20 (toll free)
Fax: 08-6650934
E-mail: info@westburg.co.il
Web site: www.westburg.co.il

Korea
LRS Laboratories, Inc.
Tel: (02) 924-86 97
Fax: (02) 924-86 96
E-mail: webmaster@lrslab.co.kr
Web site: www.lrslab.co.kr

Malaysia
RESEARCH BIOLABS SDN. BHD.
Tel: (603)-8070 3101
Fax: (603)-8070 5101
E-mail: biolabs@tm.net.my
Web site: www.researchbiolabs.com

Mexico
Quimica Valaner S.A. de C.V.
Tel: (55) 55 25 57 25
Fax: (55) 55 25 56 25
E-mail: qvalaner@infosel.net.mx

The Netherlands
Westburg b.v.
Tel: (033)-4950094
Fax: (033)-4951222
E-mail: info@westburg.nl
Web site: www.westburg.nl

New Zealand
Biolab Ltd
Tel: (09) 980 6700
or 0800 933 966
Fax: (09) 980 6788
E-mail:
biosciences@nzl.biolabgroup.com
Web site: www.biolabgroup.com/nzl

Norway
VWR International AS
Tel: 22 90 00 00
Fax: 22 90 00 40
E-mail: info@no.vwr.com
Web site: www.vwr.com

Poland
Syngen Biotech Sp.z.o.o.
Tel: (071) 351 41 06
or 0601 70 60 07
Fax: (071) 351 04 88
E-mail: info@syngen.pl
Web site: www.syngen.com.pl

Portugal
IZASA PORTUGAL, LDA
Tel: (21) 424 7312
Fax: (21) 417 2674

Singapore
Research Biolabs Pte Ltd
Tel: 62731066
Fax: 62734914
E-mail: biolabs@singnet.com.sg

Slovak Republic
BIO-CONSULT Slovakia spol. s.r.o.
Tel/Fax: (02) 5022 1336
E-mail: bio-cons@post.sk
Web site: www.bio-consult.cz

Slovenia
MEDILINE d.o.o.
Tel: (01) 830-80-40
Fax: (01) 830-80-70
or (01) 830-80-63
E-mail: mediline@siol.net

South Africa
Southern Cross Biotechnology
(Pty) Ltd
Tel: (021) 671 5166
Fax: (021) 671 7734
E-mail: info@scb.co.za
Web site: www.scb.co.za

Spain
IZASA, S.A.
Tel: (93) 902.20.30.90
Fax: (93) 902.22.33.66
E-mail: suministros@izasa.es

Sweden
VWR International AB
Tel: (08) 621 34 00
Fax: (08) 760 45 20
E-mail: info@se.vwr.com
Web site: www.vwr.com

Taiwan
TAIGEN Bioscience Corporation
Tel: (02) 2880 2913
Fax: (02) 2880 2916
E-mail: taigen@ms10.hinet.net

Thailand
Theera Trading Co. Ltd.
Tel: (02) 412-5672
Fax: (02) 412-3244
E-mail: theetrad@samart.co.th

Turkey
Medek Medikal Ürünler
ve Saglik Hizmetleri A. S.
Tel: (216) 302 15 80
Fax: (216) 302 15 88
E-mail: makialp@med-ek.com

All other countries
QIAGEN GmbH, Germany

Australia ■ **QIAGEN Pty Ltd** ■ ABN 75 072 382 944 ■ PO Box 25 ■ Clifton Hill ■ Victoria 3068
Orders 03-9489-3666 ■ Fax 03-9489-3888 ■ Technical 1-800-243-066

Canada ■ **QIAGEN Inc.** ■ 2800 Argentia Road ■ Unit 7 ■ Mississauga ■ Ontario ■ L5N 8L2
Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

France ■ **QIAGEN S.A.** ■ 3 avenue du Canada ■ LP 809 ■ 91974 COURTABOEUF CEDEX
Orders 01-60-920-920 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930

Germany ■ **QIAGEN GmbH** ■ QIAGEN Strasse 1 ■ 40724 Hilden
Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Italy ■ **QIAGEN S.p.A.** ■ Via Grosio, 10/10 ■ 20151 Milano
Orders 02-33430411 ■ Fax 02-33430426 ■ Technical 02-33430414

Japan ■ **QIAGEN K.K.** ■ Forefront Tower II ■ 13-1, Kachidoki 3 Chome ■ Chuo-ku, Tokyo 104-0054
Telephone 03-5547-0811 ■ Fax 03-5547-0818 ■ Technical 03-5547-0811

Switzerland ■ **QIAGEN AG** ■ Auf dem Wolf 39 ■ 4052 Basel
Orders 061-319-30-30 ■ Fax 061-319-30-33 ■ Technical 061-319-30-31

UK and Ireland ■ **QIAGEN Ltd.** ■ QIAGEN House ■ Fleming Way ■ Crawley ■ West Sussex, RH10 9NQ
Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

USA ■ **QIAGEN Inc.** ■ 28159 Avenue Stanford ■ Valencia ■ CA 91355
Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)

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WWW.QIAGEN.COM

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