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TissueRuptor® II Handbook

For low-throughput disruption of biological samples using disposable probes

Sample to Insight

QIAGEN

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Product Use Limitations

The TissueRuptor II is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of 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.

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 TissueRuptor II and TissueRuptor Disposable Probes 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 QIAGEN Technical Support (see back cover).

Safety Information

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

Introduction

The TissueRuptor II is a handheld rotor–stator homogenizer designed for rapid, efficient and flexible disruption of a variety of biological samples, including plant and animal tissues. Samples are simultaneously disrupted and homogenized by a rotating blade (rotor–stator homogenization). Disposable probes enable flexible sample disruption in a wide range of volumes and formats. Using a separate disposable probe for each sample provides ease of use and minimizes the risk of cross-contamination. The transparent disposable probe also enables visual control of the sample disruption process.

Principle and procedure

Efficient sample disruption is a prerequisite for nucleic acid and protein purification procedures. Incomplete sample disruption can lead to significantly reduced yields and can increase the risk of clogging when using purification columns or magnetic particles. The TissueRuptor II thoroughly disrupts and simultaneously homogenizes single samples in the presence of lysis buffer or liquid nitrogen in 15–120 seconds, depending on the toughness and size of the sample. The blade of the disposable probe rotates at a very high speed causing the sample to be disrupted and homogenized by a combination of turbulence and mechanical shearing. Disruption at full speed for 30 seconds is usually sufficient to release nucleic acids or proteins.

This handbook provides detailed protocols for disruption and homogenization of a variety of sample types. Purification of RNA, DNA, total nucleic acids or protein can then be performed using QIAGEN kits. For more information about QIAGEN kits, see the selection guides on pages 8–10.

Additional applications

In addition to the protocols in this handbook, the TissueRuptor II can be used to homogenize and disrupt other sample types (e.g., homogenization of clotted blood prior to DNA purification using the FlexiGene® DNA Kit).

QIAGEN is continuously developing new protocols for existing products. These supplementary protocols can be obtained by contacting QIAGEN Technical Service (see back cover).

Selection Guide for Manual RNA Purification

	RNeasy Protect Kits	RNeasy Micro Kit	RNeasy Kits	RNeasy Plus Mini Kit	RNeasy Fibrous Tissue Kits	RNeasy Plus Universal Kits	RNeasy® Plant Mini Kit	Oligotex® Kits	AllPrep DNA/RNA Universal Kit	AllPrep® DNA/RNA Mini Kit
Total RNA purification										
Cells			■	■						
Easy-to-lyse tissues (e.g., liver, kidney)	■		■	■	□	■				
Fibrous tissue (e.g., heart, skin, muscle)	□		□	□	■	■			■	
Lipid tissue (e.g., brain, adipose tissue)	□		□	□	□	■			■	
Plant tissue (e.g., maize, arabidopsis)							■			
Small cell or tissue samples		■								
Total RNA and genomic DNA purification										
Cells or tissues									■	■
mRNA purification										
Cells or tissues								■		

■: Recommended kit. □ : Compatible kit.

Selection Guide for Automated RNA Purification

	EZ1 RNA Cell Mini Kit	EZ1 RNA Universal Tissue Mini Kit	EZ1® RNA Tissue Mini Kit	MagAttract RNA Cell Mini M48 Kit	MagAttract RNA Universal Tissue M48 Kit	MagAttract® RNA Tissue M48 Kit
Total RNA purification						
Cells	■	■		■	■	
Easy-to-lyse tissues (e.g., liver, kidney)		■	■		■	■
Fibrous tissue (e.g., heart, skin, muscle)		■			■	
Lipid tissue (e.g., brain, adipose tissue)		■			■	
Small cell or tissue samples	■		■	■		■
Total RNA and genomic DNA purification						
Cells or tissues	■		■	■		■

■: Recommended kit.

Selection Guide for Manual and Automated DNA Purification

	DNeasy® Blood & Tissue Kit	QIAamp® DNA Mini Kit	DNeasy Plant Kits	EZ1 DNA Tissue Kit	BioSprint® 15 DNA Plant Kit
	Manual			**Automated**	
Tissue					
Liver	■	■		■	
Kidney	■	■		■	
Brain	■	■		■	
Spleen	■	■		■	
Lung	■	■		■	
Heart	■	■		■	
Rodent tail	■			■	
Plant tissue					
Maize			■		■
Arabidopsis			■		■

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

For all protocols

- TissueRuptor II, cat. no. 9002755 (North America); cat. no. 9002754 (Japan); cat. no. 9002756 (Europe [excluding UK and Ireland]); cat. no. 9002757 (UK and Ireland); cat. no. 9002758 (Australia)
- Lysis buffer (in the appropriate QIAGEN kit for purification of RNA, DNA, RNA and DNA or protein; see the respective kit handbooks) or liquid nitrogen (for plant material only)
- Suitably sized vessels for disruption
- Pipets and pipet tips

For purification of RNA

Reagents for stabilization of RNA

- For animal tissues: *RNAlater*® RNA Stabilization Reagent (see the *RNAlater handbook*) or liquid nitrogen
- For plant material: liquid nitrogen

Important Notes

Disruption and homogenization using the TissueRuptor II

Efficient disruption and homogenization of the starting material is an absolute requirement for nucleic acid and protein 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 or proteins contained in the sample. 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 nucleic acids or protein to the purification column or magnetic particles and therefore significantly reduced yields.

Using the TissueRuptor II, samples are simultaneously disrupted and homogenized by rapid rotation of the blade of the disposable probe. The transparent probe enables visual control of the sample disruption process.

Cells and tissues are disrupted at room temperature (15–25°C) in lysis buffer. Alternatively, plant material can be disrupted in liquid nitrogen. In this case, lysis buffer must be added to the disrupted sample after the liquid nitrogen has evaporated. Tissue samples of 1 mg to 1 g can be successfully disrupted and homogenized using the TissueRuptor II. Larger samples may require a longer disruption time.

Important: To prevent the rotor from becoming stuck in the stator tube, be sure to choose a suitably sized vessel for disruption. During homogenization and disruption, the tip of the disposable probe must be submerged in lysis buffer or liquid nitrogen to prevent damage to the instrument and probe. Use a minimum volume of 180 µl lysis buffer in a suitably sized vessel (e.g., 2 ml microcentrifuge tube) or keep the sample submerged in liquid nitrogen.

For DNA purification, the disruption time should be kept as short as possible to prevent shearing of DNA. Disruption efficiency is influenced by:

- Ratio of buffer to sample (if lysis buffer is used)
- Amount and consistency of starting material
- Configuration of agitator (speed and duration)
- Type of disruption vessel

Installing a disposable probe

To install a disposable probe, insert the flange end of a probe into the probe adapter, push it towards the motor and twist the probe clockwise to lock it into place (see the *TissueRuptor II User Manual* for more information).

Handling and storage of starting material

Animal tissues

RNA in samples is not protected until the sample material is treated with RNA*later* RNA Stabilization Reagent, flash-frozen or disrupted and homogenized in the presence of RNase-inhibiting or denaturing agents. It is therefore important that samples are immediately immersed in RNA*later* RNA Stabilization Reagent or immediately frozen in liquid nitrogen and stored at –70°C. For more information about stabilizing RNA in animal tissue samples, see the RNA*later Handbook*.

Frozen tissue should not be allowed to thaw during handling or weighing. The relevant procedures should be carried out as quickly as possible.

For DNA purification, best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –70°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. Use of poor-quality starting material will also lead to reduced length and yield of purified DNA.

Important: For efficient disruption, the size of the tissue sample must not be greater than half the diameter of the probe.

Plant material

RNA in plant material is not protected after harvesting until the sample is flash frozen in liquid nitrogen. Frozen tissue should not be allowed to thaw during handling or weighing. The relevant procedures should be carried out as quickly as possible.

For DNA purification, plant tissue should be frozen in liquid nitrogen after harvesting. It can then be stored at –80°C for later processing. Alternatively, tissue can be dried/lyophilized after harvesting to allow storage at room temperature (15–25°C). To ensure DNA quality, samples should be completely dried within 24 hours of collection.

Protocol: Purification of RNA or Total Nucleic Acids from Human and Animal Cells and Tissues

Important points before starting

- Before beginning the procedure, read "Important Notes" on page 12.
- Ensure that you are familiar with operating the TissueRuptor II. Refer to the *TissueRuptor II User Manual* for operating instructions.
- If using a QIAGEN kit for purification of RNA or total nucleic acids, read the supplied handbook carefully before starting.
- After storage in *RNAlater* RNA Stabilization Reagent, tissues become slightly harder than fresh or thawed tissues. However, disruption and homogenization of this stabilized tissue is usually not a problem.
- Fibrous tissue samples may not be completely homogenized after processing with the TissueRuptor II; small amounts of debris do not affect the RNA purification procedure, and the tissue sample will usually be fully digested after treatment with proteinase K in the RNA purification procedure.
- Disruption in lysis buffer may lead to foaming. Use a suitably sized vessel for disruption. If foaming occurs, either let the sample stand at room temperature (15–25°C) for a few minutes or centrifuge the sample briefly before continuing with the purification protocol.
- Disruption for more than 15 seconds may generate heat, depending on the disruption vessel used.

Procedure

1. Place the sample material into a suitably sized vessel and add the appropriate volume of lysis buffer.

   Note: For efficient disruption of tissue samples, the size of the sample must not be greater than half the diameter of the probe. Cut the sample into smaller pieces, if necessary.

2. Place the tip of the disposable probe into the tube containing the sample and lysis buffer. To avoid damage to the TissueRuptor II and probe during sample disruption, make sure that the tip of the probe is submerged in the buffer.

   Important: Use a minimum of 180 µl lysis buffer in a suitably sized vessel.

3. Disrupt the sample for at least 30 s at full speed. The homogenization time can be extended until no debris is visible. Move the tip of the probe within the sample vessel during processing to increase the efficiency of disruption and homogenization.

   Note: For purification of total nucleic acids, keep the disruption time as short as possible. Longer disruption times may lead to shearing of genomic DNA.

   Note: Sample material can be homogenized for up to 5 min without adversely affecting the quality of the RNA.

4. After sample disruption, purify RNA or total nucleic acids using a QIAGEN kit (see selection guides on pages 8–9).

Protocol: Purification of RNA from Plant Tissue

Important points before starting

- Before beginning the procedure, read "Important Notes" on page 12.
- Ensure that you are familiar with operating the TissueRuptor II. Refer to the *TissueRuptor II User Manual* for operating instructions.
- If using the RNeasy Plant Mini Kit for purification of RNA, read the supplied handbook carefully before starting.
- For initial experiments, do not use more than 25 mg plant material per sample. With optimization, it may be possible to increase the amount of starting material used. The RNA content of plant tissues can vary according to tissue type, developmental stage, growth conditions used and other factors.
- Especially hard tissues, such as roots or seeds, could cause the disposable probes to break and may not be well-suited for use with the TissueRuptor II.
- The RNeasy Plant Mini Kit provides two different lysis buffers, Buffer RLT and Buffer RLC, which contain guanidine isothiocyanate (GITC) and guanidine hydrochloride, respectively. In most cases, Buffer RLT is the lysis buffer of choice due to the greater cell disruption and denaturation properties of GITC. However, depending on the amount and type of secondary metabolites in some tissues (such as milky endosperm of maize or mycelia of filamentous fungi), GITC can cause solidification of the sample, making purification of RNA impossible. In these cases, Buffer RLC should be used.
- Disruption in lysis buffer may lead to foaming. Use a suitably sized vessel for disruption. If foaming occurs, either let the sample stand at room temperature (15–25°C) for a few minutes or centrifuge the sample briefly before continuing with the purification protocol.
- Disruption for more than 15 seconds may generate heat, depending on the disruption vessel used.

Procedure

1. To disrupt fresh plant material in lysis buffer, follow step 1a.
   To disrupt fresh or frozen plant material in liquid nitrogen, follow step 1b.

   1a. Place fresh plant material into a suitably sized vessel and add the appropriate volume of Buffer RLT or Buffer RLC. Continue with step 2 of the protocol.

   Note: For efficient disruption of tissue samples, the size of the sample must not be greater than half the diameter of the probe. Cut the sample into smaller pieces, if necessary.

   1b. Place fresh or frozen plant material into a suitably sized vessel and add liquid nitrogen. Continue with step 2 of the protocol.

   Note: For efficient disruption of tissue samples, the size of the sample must not be greater than half the diameter of the probe. Cut the sample into smaller pieces, if necessary.

2. Place the tip of the disposable probe into the tube containing the sample and lysis buffer or liquid nitrogen. To avoid damage to the TissueRuptor II and probe during sample disruption, make sure that the tip of the probe is submerged in the buffer or liquid nitrogen.

   Important: Use a minimum of 180 µl lysis buffer in a suitably sized vessel or keep sample submerged in liquid nitrogen.

3. Disrupt for at least 30 s at full speed. The homogenization time can be extended until no debris is visible. Move the tip of the probe within the sample vessel during processing to increase the efficiency of disruption and homogenization. If processing plant material in lysis buffer, continue with step 5 of the procedure.

   Note: Sample material can be disrupted and homogenized for up to 5 min without adversely affecting the quality of the RNA.

4. After disruption and homogenization in liquid nitrogen, add the appropriate volume of lysis buffer to the sample when the liquid nitrogen has evaporated.
5. After sample disruption, purify total RNA using the RNeasy Plant Mini Kit.

Protocol: Purification of Total DNA from Human and Animal Cells and Tissues

Important points before starting

- Before beginning the procedure, read "Important Notes" on page 12.
- Ensure that you are familiar with operating the TissueRuptor II. Refer to the *TissueRuptor II User Manual* for operating instructions.
- If using a QIAGEN kit for purification of total DNA, read the supplied handbook carefully before starting.
- Disruption has only been tested with soft tissues (e.g., liver and heart). Hard tissues, such as bones or teeth, may not be disrupted properly and could cause the disposable probe to break.
- Disruption in lysis buffer may lead to foaming. Use a suitably sized vessel for disruption. If foaming occurs, either let the sample stand at room temperature (15–25°C) for a few minutes or centrifuge the sample briefly before continuing with the purification protocol.
- Disruption for more than 15 seconds may generate heat, depending on the disruption vessel used.

Procedure

1. Place sample material into a suitably sized vessel and add the appropriate volume of lysis buffer.

   Note: For efficient disruption of tissue samples, the size of the sample must not be greater than half the diameter of the TissueRuptor disposable probe. Cut the sample into smaller pieces, if necessary.

2. Place the tip of the disposable probe into the tube containing the sample and lysis buffer. To avoid damage to the TissueRuptor II and probe during sample disruption, make sure that the tip of the probe is submerged in the buffer.

Important: Use a minimum of 180 µl lysis buffer in a suitably sized vessel.

3. Disrupt for approximately 20 s at full speed. For optimal results, we recommend homogenizing the sample for as short a time as possible. Move the tip of the probe within the sample vessel during processing to increase the efficiency of disruption and homogenization.

   Note: Longer disruption times may lead to shearing of genomic DNA.

4. After sample disruption, purify total DNA using a QIAGEN kit (see selection guide, page 10). If using the DNeasy Tissue Kit or QIAamp DNA Mini Kit for purification of DNA, the proteinase K digestion step can be shortened to 1 hour at 56°C.

Protocol: Purification of Total DNA from Plant Tissue

Important points before starting

- Before beginning the procedure, read “Important Notes” on page 12.
- Ensure that you are familiar with operating the TissueRuptor II. Refer to the *TissueRuptor II User Manual* for operating instructions.
- If using a QIAGEN kit for purification of DNA, read the supplied handbook carefully before starting.
- Especially hard tissues, such as roots or seeds, could cause the disposable probes to break and may not be well-suited for use with the TissueRuptor II.
- Disruption in lysis buffer may lead to foaming. Use a suitably sized vessel for disruption. If foaming occurs, either let the sample stand at room temperature (15–25°C) for a few minutes or centrifuge the sample briefly before continuing with the purification protocol.
- Disruption for more than 15 seconds may generate heat, depending on the disruption vessel used.

Procedure

1. To disrupt fresh plant material in lysis buffer, follow step 1a.
   To disrupt fresh, frozen or lyophilized plant material in liquid nitrogen, follow step 1b.

   Note: For efficient disruption of tissue samples, the size of the sample must not be greater than half the diameter of the probe. Cut the sample into smaller pieces, if necessary.

   1a. Place fresh plant material into a suitably sized vessel and add the appropriate volume of lysis buffer. Continue with step 2 of the protocol.

   Note: We do not recommend disrupting frozen material in lysis buffer as this can result in low yields and degraded DNA.

1b. Place fresh, frozen or lyophilized plant material into a suitably sized vessel and add liquid nitrogen. Continue with step 2 of the protocol.

2. Place the tip of the disposable probe into the tube containing the sample and lysis buffer or liquid nitrogen. To avoid damage to the TissueRuptor II and probe during sample disruption, make sure that the tip of the probe is submerged in the buffer or liquid nitrogen.

   Important: Use a minimum of 180 µl lysis buffer in a suitably sized vessel or keep sample submerged in liquid nitrogen.

3. Disrupt for approximately 20 s at full speed. For optimal results, we recommend homogenizing the sample for as short a time as possible. Move the tip of the probe within the sample vessel during processing to increase the efficiency of disruption and homogenization. If processing plant material in lysis buffer, continue with step 5 of the procedure.

   Note: Longer disruption times may lead to shearing of genomic DNA.

4. After disruption and homogenization in liquid nitrogen, add the appropriate volume of lysis buffer to the sample when the liquid nitrogen has evaporated.

5. After sample disruption, purify total DNA using a QIAGEN kit (see selection guide, page 10).

Protocol: Purification of Protein from Animal Tissues

Important points before starting

- Before beginning the procedure, read "Important Notes" on page 12.
- Ensure that you are familiar with operating the TissueRuptor II. Refer to the *TissueRuptor II User Manual* for operating instructions.
- If using a QIAGEN kit for purification of protein, read the supplied handbook carefully before starting.
- Disruption in lysis buffer may lead to foaming. Use a suitably sized vessel for disruption. If foaming occurs, let the sample stand on ice or at 2–8°C for a few minutes or centrifuge the sample briefly before continuing with the purification protocol.
- Disruption for more than 15 seconds may generate heat, depending on the disruption vessel used.

Procedure

1. Place a 30 mg tissue sample into a suitably sized vessel and add the appropriate volume of lysis buffer.

   Note: For efficient disruption, the size of the tissue sample must not be greater than half the diameter of the probe. Cut the sample into smaller pieces, if necessary.

2. Place the tip of the disposable probe into the tube containing the sample and lysis buffer. To avoid damage to the TissueRuptor II and probe during sample disruption, make sure that the tip of the probe is submerged in the buffer.

   Important: Use a minimum of 180 µl lysis buffer in a suitably sized vessel.

3. Disrupt for 30 s at full speed. Move the tip of the probe within the sample vessel during processing to increase the efficiency of disruption and homogenization.
4. After sample disruption, purify protein using a QIAGEN kit.

Appendix: Cleaning TissueRuptor Disposable Probes

We recommend using each disposable probe once and discarding it after use. This helps to prevent cross-contamination and provides time savings as the probe does not have to be cleaned after homogenization of each sample.

If you wish to reuse the disposable probes, they can be cleaned using one of the methods shown in the table below (see the *TissueRuptor II User Manual* for more information).

Important: Do not use the disposable probes more than 5 times.

Cleaning TissueRuptor Disposable Probes

	Procedure
Sterilization	**Autoclaving:** Probes can be autoclaved up to 5 times (15 minutes, 120°C)
	Formaldehyde: Probes can be incubated for 15 minutes in a solution of 37% formaldehyde*
Biological disinfection	**Ethanol:** Probes can be incubated for 15 minutes in 70% ethanol*
	Formaldehyde: Probes can be incubated for 15 minutes in a solution of 37% formaldehyde

For removal of RNase contamination, we recommend autoclaving the disposable probes or incubating them in formaldehyde.

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

Note: Do not clean disposable probes with solutions containing dichloromethane or phenolic derivatives and do not expose disposable probes to UV radiation.

Disposable probes should not be reused if they show the following signs of degradation:

- Cracking, crazing or brittleness in the clear stator part
- Warping of the tube
- Black particles from the rotor part are visible in the homogenized sample
- The transparent tube becomes opaque

These signs of wear and tear may be the result of overuse, frequent autoclaving, or incorrect autoclaving or cleaning methods.

Ordering Information

Product	Contents	Cat. no.
TissueRuptor II (120 V, 60 Hz, US)	Handheld rotor–stator homogenizer, 120 V, 60 Hz (for North America), 5 TissueRuptor Disposable Probes	9002755
TissueRuptor II (100 V, 50/60 Hz, JP)	Handheld rotor–stator homogenizer, 100 V, 50/60 Hz (for Japan), 5 TissueRuptor Disposable Probes	9002754
TissueRuptor II (230 V, 50/60 Hz, EU/CH)	Handheld rotor–stator homogenizer, 230 V, 50/60 Hz (for Europe [excluding UK and Ireland]), 5 TissueRuptor Disposable Probes	9002756
TissueRuptor II (230 V, 50/60 Hz, UK)	Handheld rotor–stator homogenizer, 230 V, 50/60 Hz (for UK and Ireland), 5 TissueRuptor Disposable Probes	9002757
TissueRuptor II (230 V, 50/60 Hz, AUS)	Handheld rotor–stator homogenizer, 230 V, 50/60 Hz (for Australia), 5 TissueRuptor Disposable Probes	9002758
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor II	990890

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services.

Notes

Notes

www.qiagen.com

Technical Support

www.support.qiagen.com

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