September 2011

Allprotect® Tissue Reagent Handbook

For immediate stabilization of DNA, RNA, and proteins in animal and human tissues, and subsequent storage

QIAGEN®

Sample & Assay Technologies

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Allprotect Tissue Reagent	**(100 ml)**
Catalog no.	**76405**
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* The actual volume of reagent in the bottle is greater than 100 ml. This compensates for the uptake of reagent by the pump and for the discarding of reagent at the start of each day. The pump will dispense more than 100 ml reagent.

Storage

The bottle of Allprotect Tissue Reagent should be stored dry and upright at room temperature (15–25°C). The bottle should be closed immediately after use by using the bottle lid or by locking the Allprotect Reagent Pump. Under these conditions, the reagent is stable for at least 6 months after the bottle is first opened. Storage of Allprotect Tissue Reagent at lower temperatures may increase viscosity. Before using the reagent, equilibrate it to room temperature.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Allprotect Tissue Reagent is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Allprotect Tissue Reagent is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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

Product Warranty and Satisfaction Guarantee

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

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

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding Allprotect Tissue Reagent 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 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/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

Data collection for systems biology requires standardization of the sample preparation procedure. This includes having a method for immediate and effective stabilization of all analytes in the biological sample upon harvesting. Stabilization of RNA and proteins is an absolute prerequisite for reliable gene expression analysis. Immediate stabilization of RNA in biological samples is necessary because, directly after harvesting the samples, changes in the gene expression pattern occur due to specific and nonspecific RNA degradation as well as to transcriptional induction. Such changes need to be avoided for all reliable quantitative gene expression analyses, including microarray analysis, quantitative RT-PCR, such as TaqMan® and LightCycler® technology, and other nucleic acid-based technologies. Proteins in biological samples need to be stabilized immediately, since the protein profile changes significantly within the first few hours after sample harvesting. Preservation of DNA in harvested samples is also important to prevent intracellular DNases from degrading the DNA.

Allprotect Tissue Reagent provides immediate and convenient preservation of DNA, RNA, and proteins in animal and human tissues, enabling reliable results in gene expression analysis as well as protein and DNA analyses. After harvesting, tissues are immediately submerged in Allprotect Tissue Reagent, which rapidly permeates the tissues to stabilize and protect cellular DNA, RNA, and proteins in situ. The reagent preserves DNA, RNA, and proteins for up to 1 day at 37°C, 7 days at 15–25°C, or 6 months at 2–8°C, allowing storage, transportation, and processing of samples without ice or dry ice (alternatively, the samples can be archived at –20°C or –80°C). During storage or transport in Allprotect Tissue Reagent, even at elevated temperatures (e.g., room temperature or 37°C), the cellular nucleic acids and proteins remain intact and undegraded. Allprotect technology allows large numbers of samples to be easily processed and replaces inconvenient, dangerous, and equipment-intensive methods, such as snap-freezing of samples in liquid nitrogen, storage at –80°C, cutting and weighing on dry ice, or immediate processing of harvested samples.

Note: Allprotect Tissue Reagent is intended for use with tissues only, and is not suitable for stabilizing DNA, RNA, and proteins in cultured cells, whole blood, plasma, or serum.

This handbook provides a detailed protocol for stabilization of DNA, RNA, and proteins in harvested animal and human tissues. For simultaneous purification of DNA, RNA, and proteins from stabilized tissues, we recommend the AllPrep® DNA/RNA/Protein Mini Kit (cat. no. 80004). QIAGEN kits for individual purification of DNA, RNA, and proteins are also available. For more details, see Table 1, page 7, and ordering information, page 22.

Table 1. QIAGEN kits compatible with Allprotect Tissue Reagent

Analytes purified	Recommended kit
Genomic DNA, total RNA, and total protein	AllPrep DNA/RNA/Protein Mini Kit*
Genomic DNA and total RNA	AllPrep DNA/RNA Mini Kit
Genomic DNA	DNeasy® Blood & Tissue Kit QIAamp® DNA Mini Kit
Total RNA	RNeasy® Micro Kit (for small tissue samples) RNeasy Mini Kit (for easy-to-lyse tissues) RNeasy Plus Mini Kit† (for easy-to-lyse tissues) RNeasy Fibrous Tissue Mini Kit (for fibrous tissues) RNeasy Lipid Tissue Mini Kit (for fatty tissues)
Total protein	Qproteome Mammalian Protein Prep Kit‡

* Protein purified from Allprotect stabilized tissue is denatured and suitable for SDS-PAGE and western blotting.

† Kit includes gDNA Eliminator columns.

‡ Protein purified from Allprotect stabilized tissue is suitable for all downstream applications.

The range of QIAGEN kits for purification of DNA, RNA, and proteins is continuously expanding. Visit www.qiagen.com to find out about the latest kits.

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.

- Storage containers or tubes for individual tissue samples
- 5 mm diameter tissue punches (optional): Stiefel® Biopsy Punch from Stiefel Laboratories, Inc. (www.biopsypunch.com) or BIOPUNCH® from Fray Products Corp. (www.frayproducts.com)*
- Instrument for tissue disruption and homogenization: TissueRuptor or TissueLyser (see ordering information, page 22)
- Kit for DNA, RNA, and/or protein purification (see "Introduction", page 6)

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

Important Notes

Stabilization of DNA, RNA, and proteins

Nucleic acids and proteins in harvested tissue are not protected until the tissue is completely submerged in a sufficient volume of Allprotect Tissue Reagent. After harvesting, the tissue should be **immediately** placed in **at least 10 volumes of pure reagent (or approximately 100 µl reagent per 10 mg tissue)**. Larger volumes can be used if necessary or desired. Smaller volumes may lead to nucleic acid and protein degradation during storage. Storage containers should be wide enough so that the reagent covers the entire tissue. Storage containers or tubes with large diameters may require more reagent to completely cover the tissue. We recommend completely filling the storage container or tube with reagent to ensure the tissue always remains submerged. The procedures for tissue harvesting and stabilization should be carried out as quickly as possible.

Tissue size is critical for successful stabilization with Allprotect Tissue Reagent. To ensure rapid and reliable stabilization of DNA, RNA, and proteins even in the inner parts of solid tissues, the sample must be cut into slices **less than 0.5 cm thick**. The slices can be any convenient size, provided one dimension of the sample is <0.5 cm. If the slices are thicker than 0.5 cm, the reagent will diffuse too slowly into the interior of the sample and nucleic acid and protein degradation will occur. For convenience, we recommend using 5 mm diameter tissue punches to cut out cylinders of 5 mm diameter from any tissue. These tissue cylinders can be directly transferred to tubes containing Allprotect Tissue Reagent.

The following guide may help you to determine the amount of Allprotect Tissue Reagent required for DNA, RNA, and protein stabilization:

- A cube of rat kidney with a 5 mm edge length ($[5\ mm]^3 = 125\ mm^3 = 125\ µl$) weighs 150–175 mg and requires at least 1.5–1.75 ml of the reagent.
- A 3 mm cube ($[3\ mm]^3 = 27\ mm^3 = 27\ µl$) of most animal tissues weighs 30–35 mg and requires at least 300–350 µl of the reagent.
- A tissue cylinder of 5 mm diameter and 5 mm length ($[5/2\ mm]^2 \times \pi \times 5\ mm = 98\ mm^3 \approx 100\ µl$) of most animal tissues weighs 50–150 mg and requires at least 500–1500 µl of the reagent.

Although weighing tissues is generally more accurate, nucleic acids and proteins in unstabilized tissues will degrade during weighing. In some cases, it may be more convenient to quickly estimate the weight of tissue pieces. Average weights of various entire adult mouse organs and the corresponding amounts of Allprotect Tissue Reagent to use are given in Table 2.

Table 2. Tissue weights and amounts of Allprotect Tissue Reagent

Mouse organ	Weight (mg)	Minimum amount of Allprotect Tissue Reagent (ml)	Number of strokes from Allprotect Reagent Pump
Kidney	180–250	1.8–2.5	4–5
Spleen	100–160	1–1.6	2–4
Lung	190–210	1.9–2.1	4–5
Heart	100–170	1–1.7	2–4
Liver	1000–1800	10–18	20–36

Purification of DNA, RNA, and proteins

Before using a QIAGEN kit to purify DNA, RNA, and/or proteins from tissues stabilized with Allprotect Tissue Reagent, carefully read the handbook supplied with the kit. The handbook provides guidelines on determining the amount of starting material and on choosing the appropriate method for disruption and homogenization of tissues. Optimal yield and purity depend on using the correct amount of starting material and on efficient disruption and homogenization.

Weighing tissue is the most accurate way to quantify the amount of starting material. Storage in Allprotect Tissue Reagent does not dissolve or disrupt the structure of tissue samples. Stabilized tissue can be removed from the reagent (e.g., by using forceps) for weighing and cutting at room temperature. The tissue pieces can then be used for DNA, RNA, and/or protein purification or returned to the reagent for continued storage.

After storage in Allprotect Tissue Reagent, tissues become harder than fresh or thawed tissues. However, disruption and homogenization of this tissue is usually not a problem. For optimal results, we recommend disrupting and homogenizing tissues using the TissueLyser, which is a bead mill that allows simultaneous processing of multiple samples (see ordering information, page 22). Alternatively, tissues can be disrupted and homogenized using the TissueRuptor or another rotor–stator homogenizer. Since stabilized tissues are harder than fresh or frozen tissues, extended disruption times may be needed with rotor–stator homogenization. However, prolonged disruption using a metal probe can cause overheating of the probe, leading to RNA and protein degradation. We therefore recommend using the TissueRuptor, which uses a plastic disposable probe.

Tissues stored in Allprotect Tissue Reagent at –20°C or at –80°C can be thawed prior to cutting and weighing at room temperature. There is no need to cut and weigh the tissues on dry ice. RNA remains intact for up to 15 freeze–thaw cycles, but as certain proteins are sensitive to freezing and thawing, we suggest not to exceed 5 freeze–thaw cycles. However, we recommend testing the effect of freeze–thaw cycles on sensitive protein applications (e.g., enzyme assays) and on your proteins of interest.

Protocol: Stabilization of DNA, RNA, and Proteins in Harvested Tissues

This protocol describes how to stabilize and store animal and human tissues in pure Allprotect Tissue Reagent. For DNA, RNA, and/or protein purification from the stabilized tissues using a QIAGEN kit, refer to the handbook supplied with the kit.

Important points before starting

- For effective stabilization, **always keep tissues completely submerged in Allprotect Tissue Reagent**. For details, read "Important Notes" (page 9).
- Allprotect Tissue Reagent is for single use only. Do not reuse.
- Only fresh, unfrozen tissues can be stabilized using Allprotect Tissue Reagent. Previously frozen tissues thaw too slowly in the reagent, preventing the reagent from diffusing into the tissues quickly enough to prevent nucleic acid and protein degradation.

Things to do before starting

- Remove the lid of the bottle of Allprotect Tissue Reagent, and screw in the Allprotect Reagent Pump. Immediately after use, close the bottle by locking the pump, and store the bottle dry and upright. We strongly recommend using the pump, as the reagent is extremely viscous and difficult to pipet.

Procedure

1. **Before excising the tissue sample, estimate the volume (or weight) of the sample to be stabilized in Allprotect Tissue Reagent.**
2. **Determine the appropriate volume of Allprotect Tissue Reagent for preserving the tissue. At least 10 volumes of the reagent (or approximately 100 µl reagent per 10 mg of tissue) are required. Using the Allprotect Reagent Pump, dispense at least the minimum amount of reagent into an appropriate collection vessel.**

   Note: Be sure to completely submerge the tissue in Allprotect Tissue Reagent. For details, see "Important Notes", page 9.

   Open the pump by turning it counterclockwise. Immediately after use, close and lock the pump by turning it clockwise. Each stroke of the pump dispenses approximately 0.5 ml reagent.

   Note: If using Allprotect Tissue Reagent for the first time today, discard the reagent from the first stroke of the pump (however, there is no need to do this if using the pump for the very first time). The actual volume of reagent in the bottle is greater than 100 ml to compensate for discarded reagent.

3. **Excise the tissue sample from the animal. If necessary, cut it into slices less than 0.5 cm thick, or use 5 mm diameter tissue punches to cut out samples of optimal size. Perform this step as quickly as possible and proceed immediately to step 4.**

   Note: For effective nucleic acid and protein stabilization, the tissue sample must be less than 0.5 cm thick. Tissue punches provide a convenient method of obtaining tissue samples of optimal size.

4. **Completely submerge the tissue piece(s) in the collection vessel containing Allprotect Tissue Reagent from step 2.**

   The tissue piece(s) can be transferred using forceps.

   Note: The tissue sample must be **immediately** submerged in Allprotect Tissue Reagent to protect DNA, RNA, and proteins.

5. **Store the tissue submerged in Allprotect Tissue Reagent for up to 6 months at 2–8°C, up to 7 days at 15–25°C, or up to 1 day at 37°C.**

   For archival storage at –20°C, first incubate the tissue overnight in the reagent at 2–8°C. Then transfer the tissue, in the reagent, to –20°C for storage.

   For archival storage at –80°C, first incubate the tissue overnight in the reagent at 2–8°C. Then remove the tissue from the reagent, and transfer it to –80°C for storage.

   Note: Lower temperatures are recommended for longer storage (e.g., 2–8°C instead of room temperature or 37°C; or –20°C or –80°C for longer storage). If analyzing proteins using downstream applications more sensitive than SDS-PAGE and western blotting, we recommend storage at lower temperatures (i.e., 2–8°C) for shorter durations, and testing the storage conditions for each protein of interest.

   If transporting tissue samples in Allprotect Tissue Reagent, ensure that the tissues always remain submerged in the reagent by keeping the tubes upright during transport or by completely filling the tubes with reagent.

6. **After storage, purify DNA, RNA, and/or proteins using a QIAGEN kit.**

   Be sure to remove tissues from Allprotect Tissue Reagent using forceps prior to disruption and homogenization in the DNA, RNA, and/or protein purification procedure. Remove excess Allprotect Tissue Reagent on the surface of the tissues (e.g., by dabbing or rolling the tissues over a paper towel). Trace amounts of reagent remaining on the tissues do not interfere with DNA, RNA, and/or protein purification and common downstream applications.

   Tissues stored in Allprotect Tissue Reagent at –20°C do not freeze. The low temperature increases the viscosity of the reagent. Samples stored at –20°C or –80°C can be equilibrated to room temperature prior to cutting and weighing.

For optimal disruption and homogenization of tissues, we recommend using either the TissueRuptor or the TissueLyser. Since stabilized tissues are harder than fresh or frozen tissues, extended disruption times may be needed, especially for larger samples or if working with the TissueRuptor.

Guidelines on using the TissueLyser to disrupt and homogenize Allprotect stabilized tissues are provided in the protocols on page 14 (for use with RNA, DNA/RNA, or DNA/RNA/protein purification), page 16 (for use with DNA purification), and page 18 (for use with protein preparation). For guidelines on using the TissueRuptor, refer to the *TissueRuptor Handbook*. Protocols for DNA, RNA, and/or protein purification from stabilized tissues using a QIAGEN kit can be found in the handbook supplied with the kit.

Other methods for disruption and homogenization as well as for DNA, RNA and/or protein purification are possible, but should be individually optimized depending on the tissue type, lysis buffer, target of interest, and downstream application. This is especially important when working with proteins.

Protocol: Disruption and Homogenization of Stabilized Tissues for RNA, DNA/RNA, or DNA/RNA/Protein Purification

This protocol gives guidelines for disrupting and homogenizing Allprotect stabilized tissues using the TissueLyser for RNA purification using the RNeasy Mini Kit, DNA/RNA purification using the AllPrep DNA/RNA Mini Kit, or DNA/RNA/protein purification using the AllPrep DNA/RNA/Protein Mini Kit.

Important points before starting

- Carefully read the *RNeasy Mini Handbook, AllPrep DNA/RNA Mini Handbook, or AllPrep DNA/RNA/Protein Mini Handbook*, especially the "Safety Information" and "Important Notes" sections and, if working with RNA for the first time, Appendix A.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.

Things to do before starting

- β-Mercaptoethanol (β-ME)* must be added to Buffer RLT or Buffer RLT Plus before use. Add 10 µl β-ME per 1 ml Buffer RLT or Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT or Buffer RLT Plus containing β-ME can be stored at room temperature (15–25°C) for up to 1 month.
- Buffer RLT or Buffer RLT Plus may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.

Procedure

1. **Add 600 µl Buffer RLT or Buffer RLT Plus per 2 ml microcentrifuge tube.**

   Note: Ensure that β-ME is added to Buffer RLT or Buffer RLT Plus before use (see "Things to do before starting").

2. **Add one stainless steel bead (5 mm mean diameter) per tube.**
3. **Remove each tissue sample from Allprotect Tissue Reagent using forceps. Remove residual reagent (e.g., by dabbing or rolling the tissue over a paper towel).**

   If tissues were stored at –20°C or –80°C, they can be first thawed to room temperature.

* 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.

Trace amounts of reagent left on the tissues due to reagent viscosity do not interfere with RNeasy or AllPrep purification.

4. **Determine the amount of each tissue sample. Do not use more than the amount recommended in the RNeasy or AllPrep handbook.**

   If necessary, cut the tissues on a clean surface to obtain the appropriate amount.

   Weighing tissue is the most accurate way to determine the amount. For guidelines, see the RNeasy or AllPrep handbook.

   DNA, RNA, and proteins in Allprotect stabilized tissues remain protected during cutting and weighing of tissues at room temperature (15–25°C). It is not necessary to cut the tissues on ice or dry ice or in a refrigerated room. Remaining tissues can be stored in Allprotect Tissue Reagent. Alternatively, previously stabilized tissues can be stored at –80°C without the reagent.

5. **Place the tissue samples into the tubes from step 2.**

6. **Place the tubes in the TissueLyser Adapter Set 2 x 24.**

7. **Operate the TissueLyser for 2 min at 25 Hz.**

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

   Note: Incomplete homogenization leads to significantly reduced DNA and RNA yields and can cause clogging of AllPrep and RNeasy spin columns.

   Note: Prolonged homogenization and/or higher homogenization frequencies result in greater DNA fragmentation.

8. **Rearrange the tubes so that the outermost tubes are innermost and the innermost tubes are outermost. Operate the TissueLyser for another 2 min at 25 Hz.**

   Rearranging the tubes allows even homogenization.

9. **Centrifuge the tubes (containing homogenate and bead) for 3 min at full speed. Carefully pipet the supernatants (i.e., cleared lysates) into new microcentrifuge tubes.**

   Do not reuse the stainless steel beads.

10. **Use the cleared lysates for RNA purification using the RNeasy Mini Kit, DNA/RNA purification using the AllPrep DNA/RNA Mini Kit, or DNA/RNA/protein purification using the AllPrep DNA/RNA/Protein Mini Kit.**

    Follow the protocol for animal tissues in the RNeasy or AllPrep handbook. If using the RNeasy Mini Kit, be sure to add ethanol before loading the lysates onto the RNeasy spin columns. If using an AllPrep Kit, the lysates are loaded directly onto the AllPrep spin columns.

Protocol: Disruption and Homogenization of Stabilized Tissues for DNA Purification

This protocol gives guidelines for disrupting and homogenizing Allprotect stabilized tissues using the TissueLyser for DNA purification using the DNeasy Blood & Tissue Kit or QIAamp DNA Mini Kit.

Important points before starting

- Carefully read the handbook supplied with the DNeasy Blood & Tissue Kit or QIAamp DNA Mini Kit, especially the "Safety Information" and "Important Notes" sections.
- Perform all steps of the procedure at room temperature (15–25°C).

Things to do before starting

- Buffer ATL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.

Procedure

1. **Add 180 µl Buffer ATL per 2 ml microcentrifuge tube.**
2. **Add one stainless steel bead (5 mm mean diameter) per tube.**
3. **Remove each tissue sample from Allprotect Tissue Reagent using forceps. Remove residual reagent (e.g., by dabbing or rolling the tissue over a paper towel).**

   If tissues were stored at –20°C or –80°C, they can first be thawed to room temperature.

   Trace amounts of reagent left on the tissues due to reagent viscosity do not interfere with DNeasy or QIAamp purification.
4. **Determine the amount of each tissue sample. We recommend starting with 10 mg tissue. If the procedure works satisfactorily, up to 25 mg tissue can be used in future procedures.**

   If necessary, cut the tissues on a clean surface to obtain the appropriate amount.

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

   DNA, RNA, and proteins in Allprotect stabilized tissues remain protected during cutting and weighing of tissues at room temperature (15–25°C). It is not necessary to cut the tissues on ice or dry ice or in a refrigerated room. Remaining tissues can be stored in Allprotect Tissue Reagent. Alternatively, previously stabilized tissues can be stored at –80°C without the reagent.
5. **Place the tissue samples into the tubes from step 2.**
6. **Place the tubes in the TissueLyser Adapter Set 2 x 24.**

7. **Operate the TissueLyser for 20 s at 15 Hz.**

   Note: Prolonged homogenization and/or higher homogenization frequencies result in greater DNA fragmentation.

8. **Briefly centrifuge the tubes (containing homogenate and bead) to remove drops from inside the lid.**

9. **Add 40 µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C for 1 h in a shaker incubator at full speed.**

   Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1 h. Shaking the samples facilitates lysis and results in shorter lysis times and more intact DNA.

10. **Briefly centrifuge the tubes to remove drops from inside the lid.**

    Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml; cat. no. 19101),* mix by vortexing, and incubate for 2 min at room temperature before continuing.

11. **Use the lysates for DNA purification using the DNeasy Blood & Tissue Kit or QIAamp DNA Mini Kit.**

    Follow the spin-column protocol for animal tissues in the handbook supplied with the DNeasy Blood & Tissue Kit or QIAamp DNA Mini Kit. Start at the step where Buffer AL is added to the samples.

    Do not reuse the stainless steel beads.

* 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.

Protocol: Disruption and Homogenization of Stabilized Tissues for Total Protein Preparation

This protocol gives guidelines for disrupting and homogenizing Allprotect stabilized tissues using the TissueLyser for total protein purification using the Qproteome Mammalian Protein Prep Kit.

Important points before starting

- Carefully read the *Qproteome Mammalian Protein Preparation Handbook*, especially the "Safety Information" section.
- During the procedure, work quickly.

Procedure

1. **Add 10 µl of 100x Protease Inhibitor Solution and, optionally, 1 U Benzonase® per 1 ml Mammalian Cell Lysis Buffer. Transfer 400 µl of this solution to each 2 ml microcentrifuge tube.**

   Note: Use of higher volumes of lysis buffer is possible, but this can lead to reduced homogenization efficiency and thus lower protein yields.

   Use of lower volumes (e.g., 200 µl) is also possible and leads to higher protein concentrations. We recommend starting with 200 µl Mammalian Cell Lysis Buffer per 10 mg tissue.

2. **Add one stainless steel bead (5 mm mean diameter) per tube.**

3. **Remove each tissue sample from Allprotect Tissue Reagent using forceps. Remove residual reagent (e.g., by dabbing or rolling the tissue over a paper towel).**

   If tissues were stored at –20°C or –80°C, they can first be thawed to room temperature.

   Trace amounts of reagent left on the tissues due to reagent viscosity do not interfere with purification using the Qproteome Mamalian Protein Prep Kit.

4. **Determine the amount of each tissue sample. We recommend starting with up to 20 mg tissue. If the procedure works satisfactorily, larger amounts of tissue can be used in future procedures.**

   If necessary, cut the tissues on a clean surface to obtain the appropriate amount.

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

   DNA, RNA, and proteins in Allprotect stabilized tissues remain protected during cutting and weighing of tissues at room temperature (15–25°C). It is not necessary to cut the tissues on ice or dry ice or in a refrigerated room. Remaining tissues can be stored in Allprotect Tissue Reagent. Alternatively, previously stabilized tissues can be stored at –80°C without the reagent.

5. **Place the tissue samples into the tubes from step 2.**
6. **Place the tubes in the TissueLyser Adapter Set 2 x 24.**
7. **Operate the TissueLyser for 2 min at 20 Hz.**

   Note: The time depends on the tissue being processed and can be extended until the tissue is completely homogenized. If extending the homogenization time, rearrange the tubes so that the outermost tubes are innermost and the innermost tubes are outermost (this allows even homogenization).

   Note: Incomplete homogenization leads to significantly reduced protein yields.

   Note: Prolonged homogenization and/or higher homogenization frequencies can cause mechanical and heat stress for the proteins.
8. **Centrifuge the tubes (containing homogenate and bead) for 20 min at 14,000 x *g* in a microcentrifuge precooled to 4°C. Carefully pipet the supernatants (i.e., total protein fractions) into new microcentrifuge tubes precooled to 4°C.**

   Do not reuse the stainless steel bead.
9. **For some applications, the total protein fraction may need to be concentrated. For details, see the *Qproteome Mammalian Protein Preparation Handbook*.**

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or molecular biology applications (for contact information, see back cover or visit www.qiagen.com).

		Comments and suggestions
Degradation of RNA, DNA, or proteins		
a)	Harvested tissue not immediately stabilized	Submerge the tissue in the appropriate volume of Allprotect Tissue Reagent immediately after harvesting.
b)	Too much tissue for proper stabilization	Reduce the amount of tissue, or increase the amount of Allprotect Tissue Reagent.
c)	Tissue too thick for stabilization	Use 5 mm diameter tissue punches to cut out tissue samples of the correct size, or cut large samples into slices less than 0.5 cm thick.
d)	Tissue not fully submerged in Allprotect Tissue Reagent	Ensure that the tissue remains fully submerged in Allprotect Tissue Reagent. Smaller tissues may tend to stick to the lid or the side of the container.
e)	Frozen tissue used for stabilization	Use only fresh, unfrozen tissue for stabilization in Allprotect Tissue Reagent.
f)	Storage duration in Allprotect Tissue Reagent exceeded	Allprotect stabilized tissue can be stored for up to 1 day at 37°C, up to 7 days at 15–25°C, or up to 6 months at 2–8°C, and can be archived at –20°C or –80°C. We recommend lower temperatures whenever possible.
g)	RNA degradation during RNA purification	Although all QIAGEN buffers for RNA purification have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during RNA purification or later handling. See the handbook supplied with the QIAGEN RNA purification kit for general remarks on handling RNA.

		Comments and suggestions
h)	DNA degradation during DNA purification	Prior to proteinase K digestion, disrupt the tissue samples using the TissueLyser as described in the protocol on page 16. Double the amount of proteinase K, and reduce proteinase K digestion time to 1 h.
Low yield of RNA, DNA, or proteins		
a)	Insufficient disruption and homogenization	Use the TissueLyser to disrupt and homogenize tissues. For details, see the protocols on pages 14, 16, and 18. Alternatively, use the TissueRuptor or another rotor–stator homogenizer to disrupt and homogenize tissues. Since Allprotect stabilized tissues are harder than fresh or frozen tissues, disruption times may need to be extended when using a rotor–stator homogenizer. In subsequent preparations, reduce the amount of starting material.
b)	Too much sample material	In subsequent preparations, reduce the amount of starting material.
c)	Carryover of too much Allprotect Tissue Reagent into the lysis buffer	Carryover of a larger volume of Allprotect Tissue Reagent into the lysis buffer dilutes the lysis buffer and results in reduced yields. Remove the tissue sample from the Allprotect Tissue Reagent using forceps, and remove residual reagent on the tissue surface (e.g., by dabbing or rolling the tissue over a paper towel).
d)	RNA not purified with an optimal kit	If processing fiber-rich tissues such as heart or muscle, use the RNeasy Fibrous Tissue Mini Kit. If processing fatty tissues such as brain or adipose tissue, use the RNeasy Lipid Tissue Mini Kit.
e)	Components of protein extraction buffer interfere with protein quantification, leading to inconsistent results	Perform a precipitation step (e.g., using acetone)* to remove these interfering components, or use another protein quantification method.

* 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.

Ordering Information

Product	Contents	Cat. no.
Allprotect Tissue Reagent (100 ml)	100 ml Allprotect Tissue Reagent, Allprotect Reagent Pump	76405
Accessories		
AllPrep DNA/RNA/Protein Mini Kit — for simultaneous purification of genomic DNA, total RNA, and total protein from the same cell or tissue sample		
AllPrep DNA/RNA/ Protein Mini Kit (50)	50 AllPrep DNA Mini Spin Columns, 50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	80004
AllPrep DNA/RNA Mini Kit — for simultaneous purification of genomic DNA and total RNA from the same cell or tissue sample		
AllPrep DNA/RNA Mini Kit (50)	50 AllPrep DNA Mini Spin Columns, 50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	80204
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)	50 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes	69504
DNeasy Blood & Tissue Kit (250)	250 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes	69506
QIAamp DNA Mini Kit — for purification of genomic, mitochondrial, bacterial, parasite, or viral DNA		
QIAamp DNA Mini Kit (50)	50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes	51304
QIAamp DNA Mini Kit (250)	250 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes	51306

Ordering Information

Product	Contents	Cat. no.
RNeasy Micro Kit — for purification of concentrated total RNA from small amounts of tissue or small numbers of cells		
RNeasy Micro Kit (50)	50 RNeasy MinElute® Spin Columns, Collection Tubes, RNase-Free DNase I, Carrier RNA, RNase-Free Reagents and Buffers	74004
RNeasy Mini Kit — for purification of total RNA from animal cells or tissues, or yeast		
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74104
RNeasy Mini Kit (250)*	250 RNeasy Mini Spin Columns, Collection Tubes, RNase-free Reagents and Buffers	74106
RNeasy Plus Mini Kit — for purification of total RNA from cultured cells and tissues using gDNA Eliminator columns		
RNeasy Plus Mini Kit (50)	50 RNeasy Mini Spin Columns, 50 gDNA Eliminator Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74134
RNeasy Fibrous Tissue Mini Kit — for purification of total RNA from fiber-rich tissues		
RNeasy Fibrous Tissue Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes, Proteinase K, RNase-Free DNase I, RNase-Free Reagents and Buffers	74704
RNeasy Lipid Tissue Mini Kit — for purification of total RNA from fatty tissues		
RNeasy Lipid Tissue Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes, QIAzol® Lysis Reagent, RNase-Free Reagents and Buffers	74804

* Larger kit formats available; please inquire.

Ordering Information

Product	Contents	Cat. no.
Qproteome Mammalian Protein Prep Kit — for total protein preparations from mammalian cells and tissues		
Qproteome Mammalian Protein Prep Kit	For approximately 100 protein preparations from cultured mammalian cells: Buffer, Reagents, Protease Inhibitor Solution, Benzonase	37901
TissueLyser II and TissueLyser LT — for efficient disruption of a wide range of biological samples		
TissueLyser II (100-120/ 220-240 V, 50/60 Hz)	Universal laboratory mixer-mill disruptor	85300
TissueLyser LT (100-240 V AC, 50-60 Hz).	Universal laboratory small bead mill disruptor	85600
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser	69982
TissueLyser Adapter Set 2 x 96	2 sets of Adapter Plates and 2 racks for use with Collection Microtubes (racked) on the TissueLyser II	69984
TissueLyser LT Adaptor, 12-Tube	Adapter for disruption of up to 12 samples in 2 ml microcentrifuge tubes on the TissueLyser LT	69980
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with the TissueLyser system	69989
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
TissueRuptor — for low-throughput disruption of a wide range of biological samples using disposable probes		
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor	990890
TissueRuptor (120 V, 60 Hz, US/JP)	Handheld rotor–stator homogenizer, 120 V, 60 Hz (for North America and Japan), 5 TissueRuptor Disposable Probes	9001271

Ordering Information

Product	Contents	Cat. no.
TissueRuptor (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	9001272
TissueRuptor (230 V, 50/60 Hz, UK)	Handheld rotor–stator homogenizer, 230 V, 50/60 Hz (for UK and Ireland), 5 TissueRuptor Disposable Probes	9001273
TissueRuptor (230 V, 50/60 Hz, AUS)	Handheld rotor–stator homogenizer, 230 V, 50/60 Hz (for Australia), 5 TissueRuptor Disposable Probes	9001274

QIAGEN also provides products for stabilization of RNA in:

- Animal and human tissues — RNA*later*® TissueProtect Tubes and RNA*later* RNA Stabilization Reagent
- Mammalian cells — RNAprotect® Cell Reagent
- Human blood — PAXgene® Blood RNA Tubes
- Human saliva — RNAprotect Saliva Reagent
- Bacteria — RNAprotect Bacteria Reagent

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 or your local distributor.

Notes

Notes

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Belgium ▪ Orders 0800-79612 ▪ Fax 0800-79611 ▪ Technical 0800-79556

Brazil ▪ Orders 0800-557779 ▪ Fax 55-11-5079-4001 ▪ Technical 0800-557779

Canada ▪ Orders 800-572-9613 ▪ Fax 800-713-5951 ▪ Technical 800-DNA-PREP (800-362-7737)

China ▪ Orders 86-21-3865-3865 ▪ Fax 86-21-3865-3965 ▪ Technical 800-988-0325

Denmark ▪ Orders 80-885945 ▪ Fax 80-885944 ▪ Technical 80-885942

Finland ▪ Orders 0800-914416 ▪ Fax 0800-914415 ▪ Technical 0800-914413

France ▪ Orders 01-60-920-926 ▪ Fax 01-60-920-925 ▪ Technical 01-60-920-930 ▪ Offers 01-60-920-928

Germany ▪ Orders 02103-29-12000 ▪ Fax 02103-29-22000 ▪ Technical 02103-29-12400

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