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# AllPrep® DNA/RNA FFPE Handbook

For simultaneous purification of genomic DNA and total RNA (including small RNAs) from formalin-fixed, paraffin-embedded tissue sections

Sample to Insight

QIAGEN

# Contents

# Kit Contents

| **AllPrep DNA/RNA FFPE Kit** | **(50)** |
|---|---|
| **Catalog no.** | **80234** |
| RNeasy® MinElute® Spin Columns (pink) | 50 |
| QIAamp® MinElute Spin Columns | 50 |
| Collection Tubes (1.5 ml) | 100 |
| Collection Tubes (2 ml) | 200 |
| Buffer PKD | 15 ml |
| Proteinase K | 2 x 1.4 ml |
| Buffer RLT* | 45 ml |
| Buffer FRN*† (concentrate) | 14 ml |
| Buffer RPE‡ (concentrate) | 11 ml |
| RNase-Free DNase I (lyophilized) | 1500 units§ |
| Buffer RDD | 2 x 2 ml |
| RNase-Free Water (for use with RNase-Free DNase I) | 1.9 ml |
| Buffer ATL | 14 ml |
| Buffer AL* | 12 ml |
| Buffer AW1*‡ (concentrate) | 19 ml |
| Buffer AW2‡¶ (concentrate) | 13 ml |
| Buffer ATE | 20 ml |
| RNase-Free Water | 10 ml |
| Quick-Start Protocol | 1 |

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for safety information.

† Before using for the first time, add 3 volumes of isopropanol (96–100%) as indicated on the bottle and as described on page 17 to obtain a working solution.

‡ Before using for the first time, add ethanol (96–100%) as indicated on the bottle and as described on page 18 to obtain a working solution.

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

¶ Contains sodium azide as a preservative.

# Storage

RNase-Free DNase I, Buffer RDD, RNeasy MinElute spin columns, and QIAamp MinElute spin columns should be immediately stored at 2–8°C upon arrival. The remaining buffers can be stored at room temperature (15–25°C). Under these conditions, the kit components can be kept for at least 9 months without any reduction in performance, if not otherwise stated on the label.

Proteinase K is supplied in a specially formulated storage buffer and is stable for at least 1 year after delivery when stored at room temperature. If longer storage is required or if ambient temperatures often exceed 25°C, we recommend storage at 2–8°C.

# Intended Use

The AllPrep DNA/RNA FFPE Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

QIAcube® Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

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.

# Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of AllPrep DNA/RNA FFPE Kit is tested against predetermined specifications to ensure consistent product quality.

# Safety Information

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

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Buffer RLT and Buffer FRN contain guanidine thiocyanate, and Buffer AL and Buffer AW1 contain guanidine hydrochloride. Guanidine salts can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

# Introduction

The AllPrep DNA/RNA FFPE Kit is specially designed for simultaneous purification of genomic DNA and total RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. DNA and RNA are released sequentially by differential solubilization of the same precious FFPE sample. After solubilization, both nucleic acids are treated separately to remove formaldehyde cross-links and then purified. In contrast to other procedures where either the biological sample or the purified total nucleic acids is divided into two before being processed separately, with the AllPrep FFPE procedure, pure DNA and RNA are obtained from the entire sample.

Due to fixation and embedding conditions, nucleic acids in FFPE samples are usually heavily fragmented and chemically modified by formaldehyde. Therefore, nucleic acids isolated from FFPE samples are often of a lower molecular weight than those obtained from fresh or frozen samples. The degree of fragmentation depends on the type and age of the sample, and on the conditions for fixation, embedding, and storage of the sample. Although formaldehyde modification cannot be detected in standard quality control assays, such as gel electrophoresis or lab-on-a-chip analysis, it does strongly interfere with enzymatic analyses.

While the AllPrep DNA/RNA FFPE Kit is optimized to reverse as much formaldehyde modification as possible without further DNA and RNA degradation, nucleic acids purified from FFPE samples should not be used in downstream applications that require high-molecular–weight DNA or full-length RNA. Some applications may require modifications to allow the use of fragmented nucleic acids (e.g., designing small amplicons for PCR and RT-PCR). For cDNA synthesis, gene-specific primers should be used instead of oligo-dT primers. If it is not possible to use gene-specific primers, random primers should be used.

## Automated purification of nucleic acids on QIAcube Instruments

Purification of nucleic acids can be fully automated on QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using the AllPrep DNA/RNA FFPE Kit for purification of high-quality nucleic acids.

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

**QIAcube Connect.**

## Principle and procedure

The AllPrep DNA/RNA FFPE procedure integrates well-established QIAamp and RNeasy technologies for DNA and RNA purification with QIAGEN's novel technology for selective removal of DNA and RNA from a single FFPE sample. Specially optimized lysis conditions allow the differential release of DNA and RNA from the same FFPE sample and avoid the need for overnight proteinase K incubation.

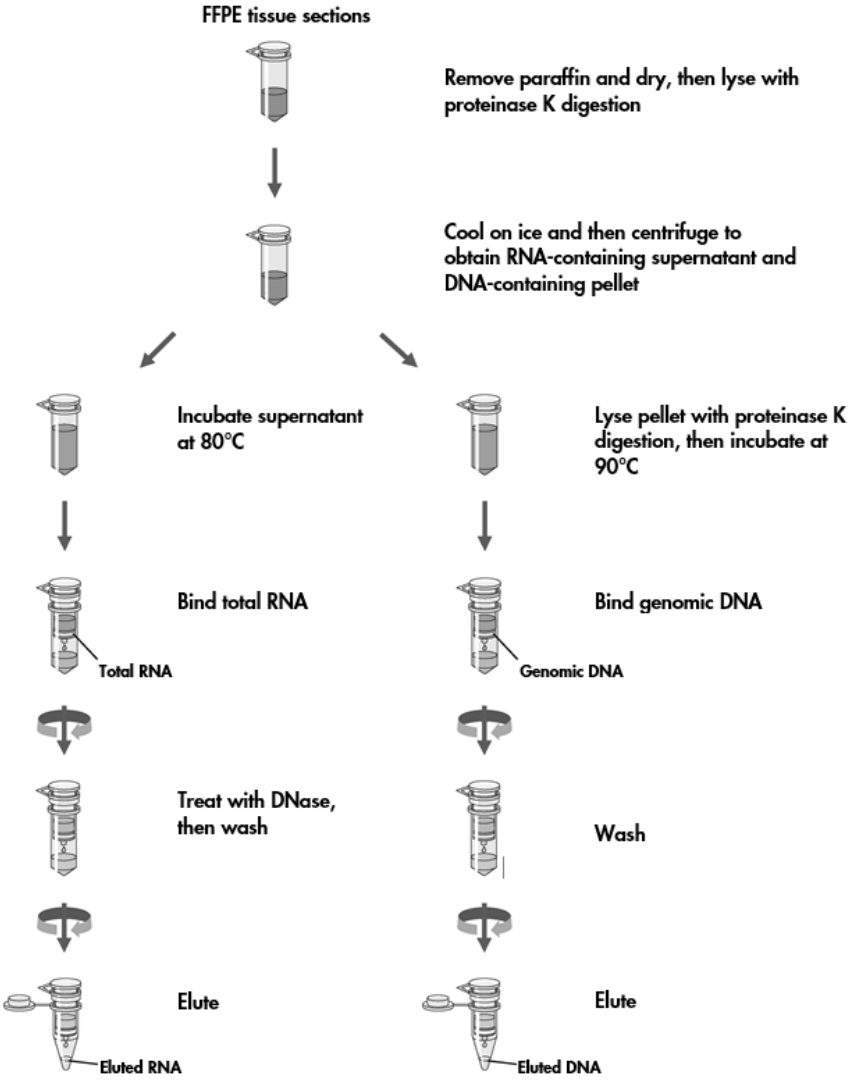
Freshly cut FFPE tissue sections are incubated in an optimized lysis buffer that contains proteinase K. Under these conditions, RNA is released into solution, whereas genomic DNA and other insoluble material are precipitated. The sample is then centrifuged to give an RNA-containing supernatant and a DNA-containing pellet, which then undergo separate purification procedures.

The RNA-containing supernatant is incubated at 80°C to partially reverse formalin cross-linking. This incubation step helps to improve RNA yield and quality, as well as RNA performance in downstream enzymatic assays. The supernatant is then mixed with Buffer RLT and ethanol to provide appropriate binding conditions for RNA. The sample is applied to an RNeasy MinElute spin column, where RNA binds specifically to the silica membrane. The bound RNA is treated with DNase to digest contaminating genomic DNA, washed with Buffer FRN and Buffer RPE to remove contaminants and then eluted in 14–30 µl of RNase-free water. Small RNAs, such as microRNA, are either present or absent in the purified RNA, depending on the volume of ethanol used earlier in the procedure to adjust RNA binding conditions.

The DNA-containing pellet is lysed further in the presence of proteinase K and then incubated at 90°C. Incubation at this high temperature partially reverses formalin cross-linking, helping to improve DNA yield and quality and DNA performance in downstream applications. The sample is mixed with Buffer AL and ethanol to provide optimal DNA binding conditions and then applied to a QIAamp MinElute spin column. Genomic DNA binds specifically to the silica membrane and is washed with Buffer AW1, Buffer AW2, and ethanol to remove contaminants. Pure DNA is then eluted in 30–100 µl of Buffer ATE.

The purified RNA and DNA samples are ready to use in downstream applications. Alternatively, they can be stored at –30 to –15°C.

## AllPrep DNA/RNA FFPE Procedure



## Solutions for FFPE research

QIAGEN's dedicated products for FFPE samples enable easy deparaffinization and efficient recovery of DNA, RNA, miRNA, and protein (see ordering information, page 41). Solutions for reliable downstream analysis include dedicated chemistry for PCR amplification of small fragments.

QIAGEN's comprehensive FFPE portfolio provides:

- Maximum data output with minimum sample consumption
- Prep technologies that reverse cross-links for higher yields
- DNA, RNA, and protein purification without further compromising analyte integrity
- Optimized chemistries for analysis of lower quality FFPE analytes
- FFPE research data you can trust

# 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.

- Sterile, RNase-free pipet tips (to avoid cross-contamination, we recommend pipet tips with aerosol barriers)
- 1.5 ml Safe-Lock microcentrifuge tubes (available from Brinkmann, cat. no. 022363204, or Eppendorf, cat. no. 0030 120.086) or 1.5 ml SafeSeal microcentrifuge tubes (Sarstedt, cat. no. 72.706)*
- If purifying RNA containing miRNAs: 2 ml Safe-Lock microcentrifuge tubes (available from Brinkmann, cat. no. 022363352, or Eppendorf, cat. no. 0030 120.094) or 2 ml SafeSeal microcentrifuge tubes (Sarstedt, cat. no. 72.695)*
- Microcentrifuge (with rotor for 2 ml tubes)
- Vortexer
- 96–100% ethanol†
- 96–100% isopropanol
- For deparaffinization of FFPE tissue sections:
  - Deparaffinization Solution (cat. no. 19093), or
  - 99–100% heptane, methanol and 96–100% ethanol, or
  - 99–100% xylene and 96–100% ethanol
  - Thermal mixer, heated orbital incubator, heating block, or water bath capable of incubation at 56°C, 80°C, and 90°C
- Optional: RNase A (100 mg/ml, cat. no. 19101)

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

# Important Notes

## Starting material

Standard formalin-fixation and paraffin-embedding procedures always result in significant fragmentation and cross-linking of nucleic acids. To limit the extent of nucleic acid fragmentation and cross-linking, be sure to:

- Fixate tissue samples in 4–10% formalin as quickly as possible after surgical removal
- Use a maximum fixation time of 24 hours (longer fixation times lead to over-fixation and more severe nucleic acid fragmentation, resulting in poor performance in downstream assays)
- Thoroughly dehydrate samples prior to embedding, as residual formalin can inhibit proteinase K digestion
- Use low-melting paraffin for embedding, as high temperatures during embedding can cause nucleic acid fragmentation
- Store FFPE samples at low temperatures (2–8°C); storage at room temperature (15–25°C) can lead to nucleic acid degradation

The starting material for nucleic acid purification should be freshly cut sections of FFPE tissue, each with a thickness of 10–20 µm. Thicker sections may result in lower nucleic acid yields, even after prolonged incubation with proteinase K. Thinner sections can be used but are more difficult to pellet. Up to 4 sections, each with a thickness of 10 µm and a surface area of up to 150 $mm^2$, or 2 sections each with a thickness of 20 µm and a surface area of up to 150 $mm^2$, can be combined in one preparation.

Avoid using too much starting material, as this affects lysis efficiency and purification and can lead to reduced yields and nucleic acid fragmentation.

If there is no information about the nature of your starting material or if the surface area of the sample is high, we recommend starting with one 10–20 µm thick section per preparation.

Do not overload the QIAamp and RNeasy MinElute spin columns, as this will significantly reduce DNA/RNA yield and quality.

## Deparaffinization

Prior to nucleic acid purification, the paraffin in an FFPE sample needs to be removed to allow exposure of the sample to proteinase K. One of the following deparaffinization procedures should be used.

### Deparaffinization using Deparaffinization Solution

Deparaffinization Solution (cat. no. 19093) dissolves paraffin efficiently and allows deparaffinization without further washing steps. Deparaffinization Solution is colored to enable easy visibility for removal of supernatant during the procedure. The volume of Deparaffinization Solution needed for deparaffinization depends on the amount of sample material. One set of Deparaffinization Solution (2 x 8 ml, cat. no. 19093) is sufficient for deparaffinization of 50 samples consisting of up to two 10 µm sections or one 20 µm section. For larger samples, the volume of Deparaffinization Solution required is doubled.

### Deparaffinization using heptane and methanol

Deparaffinization with heptane and methanol is very efficient. After precipitation of the sample and removal of the supernatant, residual heptane is removed by washing with ethanol. This procedure usually gives more compact pellets than other procedures and allows good results in DNA and RNA applications.

### Deparaffinization using xylene

Paraffin is first dissolved in xylene. After precipitation of the sample and removal of the supernatant, residual xylene is removed by washing with ethanol. This procedure is commonly used when purifying nucleic acids from FFPE samples for use in different applications.

## Copurification of nucleic acids

Separation of RNA from DNA in the AllPrep protocol is highly effective. This may result in lower $A_{260}$ values when measuring the concentration of DNA purified using the AllPrep DNA/RNA FFPE Kit compared to other methods. As $A_{260}$ values measure both DNA and RNA, lower $A_{260}$ values from DNA purified using the AllPrep DNA/RNA FFPE Kit may indicate high DNA purity and the absence of contaminating RNA. Higher $A_{260}$ values from DNA purified using alternative methods may indicate the presence of significant amounts of contaminating RNA.

DNA purified using the AllPrep DNA/RNA FFPE Kit may contain trace amounts of RNA, depending on the FFPE sample used. Residual RNA may inhibit some downstream enzymatic reactions, although it does not affect PCR. If RNA-free genomic DNA is required, RNase A should be added to the sample, as indicated in the protocol. The protocol describes the use of a 100 mg/ml RNase A stock solution.

RNA purified using the AllPrep DNA/RNA FFPE Kit is virtually free of DNA contamination, as the RNA purification procedure includes a DNase digestion step.

## Eluting pure nucleic acids

For downstream applications that require small starting volumes (e.g., some PCR assays), a more concentrated eluate may increase assay sensitivity. QIAamp MinElute spin columns allow a minimum elution volume of 20 µl for concentrated DNA eluates. Similarly, RNeasy MinElute spin columns allow elution of RNA in a volume of 14–30 µl.

For downstream applications that require a larger starting volume, the elution volume for QIAamp MinElute spin columns can be increased to 100 µl. However, an increase in elution volume will decrease the concentration of DNA in the eluate.

The volume of eluate recovered may be up to 5 µl less than the volume applied to the QIAamp or RNeasy MinElute spin column. For example, an elution volume of 20 µl results in ≥15 µl eluate. The volume of eluate recovered depends on the nature of the sample.

## Handling of QIAamp and RNeasy MinElute spin columns

Due to the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling QIAamp and RNeasy MinElute spin columns to avoid cross-contamination between sample preparations:

- Carefully apply the sample or solution to the spin column. Pipet the sample into the spin column without wetting the rim.
- Always change pipet tips between liquid transfers. We recommend the use of aerosol-barrier pipet tips.
- Avoid touching the spin column membrane with the pipet tip.
- After all pulse-vortexing steps, briefly centrifuge the microcentrifuge tubes to remove drops from the inside of the lids.
- Open only one spin column at a time, and take care to avoid generating aerosols.
- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

## Preparation of buffers

### Preparing Buffer RLT

Before starting the procedure, check whether precipitate has formed in Buffer RLT. If necessary, dissolve by warming with gentle agitation.

### Preparing Buffer FRN

Before using Buffer FRN for the first time, check whether a precipitate has formed. If necessary, dissolve by warming with gentle agitation. After equilibration to room temperature (15–25°C), add 42 ml isopropanol (96–100%) to the entire concentrate (14 ml). Tick the check box on the bottle label to indicate that isopropanol has been added.

**Note**: Before starting the procedure, mix reconstituted Buffer FRN by shaking.

### Preparing DNase I stock solution

Prepare DNase I stock solution by dissolving the lyophilized DNase I (1500 Kunitz units) in 550 µl RNase-free water. In some cases, the vial of DNase may appear to be empty. This is due to lyophilized enzyme sticking to the septum. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex.

Insoluble material may remain when dissolving DNase. This does not affect DNase performance. Due to the production process, insoluble material may be present in the lyophilized DNase. However, rigorous QC tests are carried out to ensure that DNase activity remains consistent from lot to lot.

**Note**: Do not vortex reconstituted DNase I. DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.

For long-term storage of DNase I, remove the stock solution from the vial, divide it into single-use aliquots, and store at –30 to –15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

### Preparing Buffer RPE

Add 4 volumes (44 ml) ethanol (96–100%) to the bottle containing 11 ml Buffer RPE concentrate. Tick the check box on the bottle label to indicate that ethanol has been added.

**Note**: Before starting the procedure, mix reconstituted Buffer RPE by shaking.

### Preparing Buffer ATL

Before starting the procedure, check whether precipitate has formed in Buffer ATL. If necessary, dissolve by heating to 70°C with gentle agitation.

### Preparing Buffer AL

Before starting the procedure, check whether precipitate has formed in Buffer AL. If necessary, dissolve by heating to 70°C with gentle agitation.

### Preparing Buffer AW1

Add 25 ml ethanol (96–100%) to the bottle containing 19 ml Buffer AW1 concentrate. Tick the check box on the bottle label to indicate that ethanol has been added.

**Note**: Before starting the procedure, mix reconstituted Buffer AW1 by shaking.

### Preparing Buffer AW2

Add 30 ml ethanol (96–100%) to the bottle containing 13 ml Buffer AW2 concentrate. Tick the check box on the bottle label to indicate that ethanol has been added.

**Note**: Before starting the procedure, mix reconstituted Buffer AW2 by shaking.

# Protocol: Purification of Genomic DNA and Total RNA (Including Small RNAs) from FFPE Tissue Sections

## Important points before starting

- If using the AllPrep DNA/RNA FFPE Kit for the first time, read "Important Notes" (page 13).
- If working with RNA for the first time, read Appendix A (page 33).
- Buffer RLT, Buffer FRN, Buffer AL, and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for safety information.
- Unless otherwise indicated, perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps using a microcentrifuge placed at 15–25°C. If using a refrigerated microcentrifuge, set the temperature to 20–25°C; otherwise, significant cooling below 15°C may occur.
- In the procedure below, ■ indicates specific steps for purification of total RNA that does not include small RNAs, and ▲ indicates specific steps for purification of total RNA that does include small RNAs.

## Things to do before starting

- If using Buffer FRN, Buffer RPE, Buffer AW1, Buffer AW2, and RNase-Free DNase I for the first time, reconstitute them as described in "Preparation of buffers" (page 17).
- If necessary, warm and gently agitate Buffer RLT, Buffer ATL, and Buffer AL to redissolve any precipitates that may have formed.
- Equilibrate all buffers to room temperature (15–25°C). Mix reconstituted Buffer FRN, Buffer RPE, Buffer AW1, and Buffer AW2 by shaking.

- Set a thermal mixer or heated orbital incubator to 56°C for use in step 6. Incubation at 56°C (steps 6 and 26) and at 80°C (step 10) can be done with or without agitation. Incubation at 90°C (step 27) must be done without agitation. If a thermal mixer or heated orbital incubator is not available, a heating block or water bath can be used instead.

## Procedure

1. Using a scalpel, trim excess paraffin off the sample block.
2. Cut sections 10–20 µm thick.

   Do not use more than four 10 µm sections of 150 $mm^2$ surface area or two 20 µm sections of 150 $mm^2$ surface area.

   If the sample surface has been exposed to air, discard the first 2–3 sections.
3. Immediately place the sections in a 1.5 ml Safe-Lock microcentrifuge tube (not supplied), and close the lid.
4. Remove the paraffin according to step 4a, 4b, or 4c.

   4a. Deparaffinization using Deparaffinization Solution:
   - Add Deparaffinization Solution: for two 10 µm sections or one 20 µm section, add 320 µl Deparaffinization Solution; for more sample material, add 640 µl Deparaffinization Solution.
   - Vortex vigorously for 10 s, and centrifuge briefly to bring the sample to the bottom of the tube.
   - Incubate at 56°C for 3 min, then allow to cool at room temperature (15–25°C), and centrifuge at full speed for 2 min.
   - Carefully remove the supernatant by pipetting without disturbing the pellet. Carefully remove any residual Deparaffinization Solution using a fine pipet tip.
   - Keep the lid open, and incubate for 10 min at 37°C to dry the pellet. Proceed to step 5.

   4b. Deparaffinization using heptane (may provide more compact sample pellets in step 8):

- Add 500 µl heptane, vortex vigorously for 10 s, and incubate for 10 min at room temperature.
- Add 25 µl methanol, vortex vigorously for 10 s, and centrifuge for 2 min at 9000 x *g*.
- Carefully remove the supernatant by pipetting without disturbing the pellet. Carefully remove any residual heptane/methanol using a fine pipet tip.
- Add 1 ml ethanol (96–100%) to the pellet, mix by vortexing, and centrifuge at full speed for 2 min.
- Carefully remove the supernatant by pipetting without disturbing the pellet. Carefully remove any residual ethanol using a fine pipet tip.
- Keep the lid open, and incubate at room temperature or at up to 37°C for 10 min or until all residual ethanol has evaporated. Proceed to step 5.

4c. Deparaffinization using xylene:

- Add 1 ml xylene, vortex vigorously for 10 s, and centrifuge at full speed for 2 min.
- Carefully remove the supernatant by pipetting without disturbing the pellet.
- Add 1 ml ethanol (96–100%) to the pellet, mix by vortexing, and centrifuge at full speed for 2 min.
  The ethanol extracts residual xylene from the sample.
- Carefully remove the supernatant by pipetting without disturbing the pellet. Carefully remove any residual ethanol using a fine pipet tip.
- Keep the lid open, and incubate at room temperature or at up to 37°C. Incubate for 10 min or until all residual ethanol has evaporated. Proceed to step 5.

5. Resuspend the pellet by adding 150 µl Buffer PKD and flicking the tube to loosen the pellet. Add 10 µl proteinase K and mix by vortexing.
6. Incubate at 56°C for 15 min.

   Depending on the sample material, the sample may not be completely lysed. This does not affect the procedure. Proceed to step 7.

7. Incubate on ice for 3 min.

   Complete cooling is important for efficient precipitation in step 8.

8. Centrifuge for 15 min at 20,000 x *g*.

9. Carefully transfer the supernatant, without disturbing the pellet, to a new ■ 1.5 ml or ▲ 2 ml Safe-Lock microcentrifuge tube for RNA purification in steps 10–24. Keep the pellet for DNA purification in steps 25–35.

   **Note**: Depending on the amount and nature of the FFPE sample, the pellet may be very small or difficult to see. If the pellet is aspirated with the supernatant, allow the pellet to drop slowly to the bottom of the tube and use the pipet tip to reattach the pellet to the tube. Alternatively, centrifuge the supernatant again.

   The DNA-containing pellet can be stored for 2 h at room temperature, for up to 1 day at 2–8°C or for longer periods at –30 to –15°C.

## Purification of total RNA

10. Incubate the supernatant from step 9 at 80°C for 15 min.

    This incubation step partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented RNA.

    If using only one heating block, keep the supernatant at room temperature until the heating block has reached 80°C. To ensure maximum RNA yields, the supernatant must be incubated at 80°C only for exactly 15 min.

11. Briefly centrifuge the tube to remove drops from the inside of the lid.

12. Add 320 µl Buffer RLT to adjust binding conditions, and mix by vortexing or pipetting.

13. Add ■ 720 µl or ▲ 1120 µl ethanol (96–100%), and mix well by vortexing or pipetting. Proceed immediately to step 14.

    Precipitates may be visible after addition of ethanol. This does not affect the procedure.

14. Transfer 700 µl of the sample, including any precipitate that may have formed, to an RNeasy MinElute spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.*

    Reuse the collection tube in step 15.

15. Repeat step 14 until the entire sample has passed through the RNeasy MinElute spin column.

    Reuse the collection tube in step 16.

16. Add 350 µl Buffer FRN to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.*

    **Note**: Buffer FRN is supplied as a concentrate. Ensure that isopropanol is added before use as described in "Preparing Buffer FRN" (page 17).

    Reuse the collection tube in step 17.

17. Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.

    **Note**: DNase I is supplied lyophilized and should be reconstituted as described in "Preparing DNase I stock solution" (page 17).

    **Note**: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.

18. Add the DNase I incubation mix (80 µl) directly to the RNeasy MinElute spin column membrane, and place on the benchtop (20–30°C) for 15 min.

    **Note**: Be sure to add the DNase I incubation mix directly to the membrane. DNase digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.

19. Add 500 µl Buffer FRN to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Save the flow-through for use in step 20.

    Do not discard the flow-through, as it contains RNA including small RNAs.

* Flow-through contains Buffer RLT and is therefore not compatible with bleach. See page 5 for safety information.

20. Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Apply the flow-through from step 19 to the spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.*

    Reuse the collection tube in step 21.

21. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.

    Reuse the collection tube in step 22.

    **Note**: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added before use as described in "Preparing Buffer RPE" (page 18).

22. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the collection tube with the flow-through.

23. Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid, and centrifuge at full speed for 5 min. Discard the collection tube with the flow-through.

    To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

    It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

* Flow-through contains Buffer FRN and is therefore not compatible with bleach. See page 5 for safety information.

24. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14–30 µl RNase-free water directly to the spin column membrane. Close the lid gently, and incubate for 1 min at room temperature. Centrifuge at full speed for 1 min to elute the RNA.

    Elution with smaller volumes of RNase-free water leads to higher total RNA concentrations, but lower RNA yields.

    The dead volume of the RNeasy MinElute spin column is 2 µl: elution with 14 µl RNase-free water results in a 12 µl eluate.

## Purification of genomic DNA

25. Resuspend the pellet from step 9 in 180 µl Buffer ATL, add 40 µl proteinase K, and mix by vortexing.

    The pellet should be equilibrated to room temperature prior to resuspension.

26. Incubate at 56°C for 1 h.

27. Incubate at 90°C for 2 h without agitation.

    This incubation step partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented DNA.

    If using only one heating block, keep the sample at room temperature until the heating block has reached 90°C.

    **Note**: Agitation during this incubation step leads to lower DNA yields.

28. Briefly centrifuge the microcentrifuge tube to remove drops from the inside of the lid.

    **Optional**: In contrast to commonly used DNA preparation methods, DNA purified using the AllPrep DNA/RNA FFPE procedure contains only trace amounts of RNA, since RNA is efficiently separated from DNA during the procedure. If RNA-free genomic DNA is required, allow the sample to cool to room temperature and then add 4 µl RNase A (100 mg/ml). Incubate for 2 min at room temperature before proceeding to step 29.

29. Add 200 µl Buffer AL, and mix thoroughly by vortexing. Then add 200 µl ethanol (96–100%), and mix thoroughly again by vortexing or pipetting.

    It is essential that the sample, Buffer AL, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples.

    A white precipitate may form on addition of Buffer AL and ethanol. This precipitate does not interfere with the AllPrep procedure.

30. Transfer the entire sample to a QIAamp MinElute spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm). Discard the collection tube with the flow-through.*

    If the sample has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the QIAamp MinElute spin column is empty.

31. Place the QIAamp MinElute spin column in a new 2 ml collection tube (supplied). Add 700 µl Buffer AW1 to the spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.*

    Reuse the collection tube in step 32.

    **Note**: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added before use as described in "Preparing Buffer AW1" (page 18).

32. Add 700 µl Buffer AW2 to the QIAamp MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.

    Reuse the collection tube in step 33.

    **Note**: Buffer AW2 is supplied as a concentrate. Ensure that ethanol is added before use as described in "Preparing Buffer AW2" (page 18).

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 5 for safety information.

33. Add 700 µl ethanol (96–100%) to the QIAamp MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the collection tube with the flow-through.

34. Place the QIAamp MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column, and centrifuge at full speed for 5 min. Discard the collection tube with the flow-through.

    To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

    It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during DNA elution,

35. Place the QIAamp MinElute spin column in a new 1.5 ml collection tube (supplied). Add 30–100 µl Buffer ATE directly to the spin column membrane. Close the lid gently, and incubate for 1 min at room temperature. Centrifuge at full speed for 1 min to elute the DNA.

    **Important**: Ensure that Buffer ATE is equilibrated to room temperature. If using small elution volumes (<50 µl), pipet Buffer ATE onto the center of the membrane to ensure complete elution of bound DNA.

    QIAamp MinElute spin columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. The volume of eluate will be up to 5 µl less than the volume of Buffer ATE applied to the column.

    Incubating the QIAamp MinElute spin column loaded with Buffer ATE for 5 min at room temperature before centrifugation can increase DNA yield.

# Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **http://www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have either about the information and protocols in this handbook, or about sample and assay technologies (for contact information, visit **www.qiagen.com**).

| | | Comments and suggestions |
|---|---|---|
| **Precipitated DNA forms a loose pellet after centrifugation** | | |
| a) | Supernatant removed too vigorously | If the pellet is aspirated with the supernatant, allow the pellet to drop slowly to the bottom of the tube and use the pipet tip to reattach the pellet to the tube. Alternatively, centrifuge the supernatant again. |
| b) | Not enough starting material | Increase the amount of starting material. Use thicker sections (20 µm) instead of several thinner sections. |
| c) | Insufficient deparaffinization | Repeat the purification procedure, using heptane for deparaffinization. |
| d) | Sample not cooled prior to precipitation | After the first proteinase K digestion, the sample needs to be cooled on ice to allow efficient precipitation (step 7 of the protocol). If the sample is not cooled, insoluble DNA may not be precipitated and may be lost into the supernatant. |
| **Clogged RNeasy or QIAamp MinElute spin column** | | |
| a) | Too much starting material | Reduce the amount of starting material. It is essential to use the correct amount of starting material (see page 13). |
| b) | Centrifugation temperature too low | The centrifugation temperature should be 15–25°C. Some centrifuges may cool to below 15°C even when set at 20°C. This can cause formation of precipitates that can clog the QIAamp or RNeasy MinElute spin column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol-containing sample to 37°C before transferring it to the QIAamp or RNeasy MinElute spin column (steps 14 and 30 of the protocol). |

| | | Comments and suggestions |
|---|---|---|
| **Low nucleic acid yield** | | |
| a) | Poor quality of starting material | Samples that were fixed for over 20 h or stored for very long periods may contain very little usable nucleic acids.<br>Sections that were mounted on microscope slides may yield very little usable nucleic acids due to prolonged exposure to air. |
| b) | Too much starting material | Using too much material for lysis and overloading RNeasy and QIAamp MinElute spin columns significantly reduces nucleic acid yields and causes nucleic acid fragmentation. Reduce the amount of starting material (see page 13). |
| c) | Insufficient deparaffinization, or sample contains too much paraffin | During deparaffinization (step 4 of the protocol), be sure to remove the supernatant carefully without disturbing the pellet. If processing samples containing large amounts of paraffin, repeat the deparaffinization step one more time. |
| d) | Insufficient drying of sample after deparaffinization | After deparaffinization, be sure to evaporate residual solvent (see step 4 of the protocol). |
| e) | Insufficient sample lysis | Proteinase K was stored at high temperatures for a prolonged period. Repeat the procedure using new samples and fresh proteinase K. Make sure that the samples were thoroughly dehydrated prior to embedding. Residual formalin can inhibit digestion with proteinase K.<br>Loosen the pellet by flicking the tube prior to addition of proteinase K. |
| f) | Low-percentage ethanol used instead of 96–100% ethanol | Repeat the purification procedure with new samples using 96–100% ethanol. Do not use denatured alcohol, which contains other substances, such as methanol or methylethylketone. |
| g) | Buffer FRN, Buffer RPE, Buffer AW1, or Buffer AW2 prepared incorrectly | Be sure to dilute the buffer concentrates with the correct volume of ethanol or isopropanol as described in “Preparation of buffers” (page 17). |
| h) | DNA or RNA still bound to spin column membrane | Repeat the elution step, but incubate the QIAamp or RNeasy MinElute spin column on the benchtop for 10 min with Buffer ATE (for DNA) or RNase-free water (for RNA) before centrifuging. |
| **Low RNA yield** | | |
| a) | Loss of supernatant after DNA precipitation | Ensure that the RNA-containing supernatant is removed completely after DNA precipitation (step 9 of the protocol). RNA in supernatant remaining on the DNA-containing pellet will be copurified with the DNA. |
| b) | No rebinding of RNA after DNase digestion | Ensure that the flow-through after on-column DNase digestion is reapplied to the RNeasy MinElute spin column (steps 19 and 20 of the protocol). Otherwise, RNA, especially small RNAs, may be lost. |

| | | Comments and suggestions |
|---|---|---|
| c) | RNA-containing supernatant stored too long at ambient temperature | After removal of the RNA-containing supernatant (step 9 of the protocol), proceed immediately with RNA purification or store the supernatant for up to 24 h at 2–8°C or up to one week at –30 to –15°C. Equilibrate the pellet to room temperature (15–25°C) before starting RNA purification. |
| **Low DNA yield** | | |
| a) | Prolonged initial proteinase K digestion | Ensure that the first proteinase K digestion step (step 6 of the protocol) does not exceed 15 min. Otherwise, genomic DNA will be released into the supernatant and be copurified with the RNA. |
| b) | Loss of pellet after DNA precipitation | Ensure that the DNA-containing pellet remains in the tube after removal of the RNA-containing supernatant (step 9 of the protocol). If the pellet or parts of the pellet are removed with the supernatant, DNA will be copurified with the RNA. See also "Precipitated DNA forms a loose pellet after centrifugation" (page 15). |
| c) | DNA-containing pellet stored too long at ambient temperature | After removal of the RNA-containing supernatant (step 9 of the protocol), store the DNA-containing pellet for 2 h at room temperature (15–25°C) or for up to 1 day at 2–8°C. For longer storage, freeze the pellet at –30 to –15°C. Equilibrate the pellet to room temperature before starting DNA purification (step 25 of the protocol). |
| d) | Insufficient lysis with proteinase K | Proteinase K digestion of the DNA-containing pellet (step 26 of the protocol) was too short: be sure to incubate for 1 h at 56°C. To increase DNA yield, an overnight incubation can be performed. Exceeding an overnight incubation may result in greater DNA fragmentation. |
| e) | Agitation during the 90°C incubation | During the incubation at 90°C (step 27 of the protocol), do not agitate, shake, or vortex the sample. If using a thermal mixer, make sure it is not rotating. |
| f) | Yield measured by spectrophotometry lower than alternative DNA purification methods | Separation of RNA from DNA in the AllPrep protocol is highly effective. This may result in lower $A_{260}$ values when measuring the concentration of DNA purified using the AllPrep DNA/RNA FFPE Kit compared to other methods. As $A_{260}$ values measure both DNA and RNA, lower $A_{260}$ values from DNA purified using the AllPrep DNA/RNA FFPE Kit may indicate high DNA purity and the absence of contaminating RNA. Higher $A_{260}$ values from DNA purified using alternative methods may indicate the presence of significant amounts of contaminating RNA. |
| **Low $A_{260}/A_{280}$ value** | | |
| a) | Water used to dilute nucleic acid for $A_{260}/A_{280}$ measurement | Use 10 mM Tris·Cl, pH 7.5, not water, to dilute the sample before measuring purity (see Appendix B, page 35). |

| | | Comments and suggestions |
|---|---|---|
| **DNA contamination in RNA eluate** | | |
| a) | Too much starting material | Removal of DNA by on-column DNase digestion is very efficient. However, the efficiency of DNA removal may be reduced when processing very large amounts of tissues rich in DNA (e.g., thymus). If the RNA eluate contains significant DNA contamination, repeat the purification procedure using fewer tissue sections. |
| b) | Tissue has high DNA content | When processing very large amounts of tissues rich in DNA (e.g., thymus), the DNA may not be completely digested. Repeat the purification procedure using fewer tissue sections.<br>Alternatively, perform DNase digestion of the RNA eluate using the RNase-Free DNase Set (cat. no. 79254). Then clean up the RNA according to QIAGEN Supplementary Protocol RY26, which can be downloaded at **www.qiagen.com/literature**.<br>If using the RNA in real-time two-step RT-PCR, synthesize the cDNA using the QuantiTect® Reverse Transcription Kit (cat. no. 205311), which integrates reverse transcription with elimination of genomic DNA contamination. If using the RNA in real-time one-step RT-PCR, we recommend the QuantiFast® Probe RT-PCR *Plus* Kit (cat. no. 204482), which includes an integrated genomic DNA removal step. |
| **Nucleic acids do not perform well in downstream assays** | | |
| a) | Nucleic acids fragmented or blocked due to formaldehyde modification | Although the incubation steps in the AllPrep DNA/RNA FFPE procedure remove some of the formaldehyde modifications, DNA and RNA purified from FFPE sections are not an optimal template for enzymatic reactions. We recommend using only random primers or gene-specific primers for cDNA synthesis. We also recommend keeping amplicons as short as possible for PCR. |
| b) | Ethanol carryover | Before eluting DNA or RNA, be sure to centrifuge the QIAamp or RNeasy MinElute spin column at full speed for 5 min using a new collection tube to completely dry the membrane. |
| c) | Wash buffers not mixed well after storage | The salts and alcohol in Buffer FRN, Buffer RPE, Buffer AW1, and Buffer AW2 may have separated out after long-term storage. Be sure to mix the buffers thoroughly before use. |
| d) | Salt carryover during DNA/RNA elution | Ensure that Buffer RPE and Buffer AW2 are at room temperature (15–25°C). |

| | | Comments and suggestions |
|---|---|---|
| e) | Reverse transcription with insufficient amount of RNA | Most reverse transcriptases are intended for use with approximately 1 µg RNA. If performing reverse transcription with very small amounts of RNA, we recommend using the Sensiscript® RT Kit (cat. no. 205211), which is specially designed for highly sensitive reverse transcription using <50 ng RNA.<br>If performing one-step RT-PCR or quantitative, real-time RT-PCR, we recommend using the QIAGEN OneStep RT-PCR Kit or QuantiFast/QuantiTect Kits, respectively. These kits allow amplification of a wide range of RNA amounts, from as little as 1 pg per reaction. For details, visit **www.qiagen.com/PCR**. |
| **RNA does not perform well in downstream assays for small RNAs** | | |
| a) | Incorrect volume of ethanol used in RNA purification procedure | Be sure to use the higher volume of ethanol in the RNA purification procedure, as indicated by the ▲ symbol. A higher volume of ethanol is required for efficient binding of small RNAs to the RNeasy MinElute spin column membrane. |
| b) | No rebinding of RNA after DNase digestion | Ensure that the flow-through after on-column DNase digestion is reapplied to the RNeasy MinElute spin column (steps 19 and 20 of the protocol). Otherwise, RNA, especially small RNAs, may be lost. |
| c) | Buffer FRN prepared incorrectly | Be sure to dilute the buffer concentrate with the correct volume of isopropanol as described in "Preparation of buffers" (page 17). |

# Appendix A: General Remarks on Handling RNA

## Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. To create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

## General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

To remove RNase contamination from bench surfaces, nondisposable plasticware, and laboratory equipment (e.g., pipets and electrophoresis tanks), general laboratory reagents can be used. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 34), or rinse with chloroform* if the plasticware is chloroform-resistant. To decontaminate electrophoresis tanks, clean with detergent (e.g., 0.5% SDS),* rinse with RNase-free water, rinse with ethanol (if the tanks are ethanol-resistant), and allow to dry.

* 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.

## Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

## Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate), as described in "Solutions" below.

## Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and $CO_2$. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues has been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

**Note**: AllPrep buffers are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

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

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

## Storage of RNA

Purified RNA may be stored at –30 to –15°C or –70°C in RNase-free water. Under these conditions, no degradation of RNA is detectable after 1 year.

## Quantification of RNA

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

## Spectrophotometric quantification of RNA

To ensure significance, $A_{260}$ readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml ($A_{260}$=1 → 44 µg/ml). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 36), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

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

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

"Solutions", page 34). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample = 100 µl
Dilution = 10 µl of RNA sample + 490 µl of 10 mM Tris·Cl,* pH 7.0 (1/50 dilution)
Measure absorbance of diluted sample in a 1 ml cuvette (RNase-free)
$A_{260}$ = 0.2
Concentration of RNA sample = 44 µg/ml x $A_{260}$ x dilution factor
= 44 µg/ml x 0.2 x 50
= 440 µg/ml
Total amount = concentration x volume in milliliters
= 440 µg/ml x 0.1 ml
= 44 µg of RNA

## Purity of RNA

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

For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration ($A_{260}$ reading of 1 = 44 µg/ml RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "Spectrophotometric quantification of RNA", page 35).

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

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

## DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. While the AllPrep DNA/RNA FFPE procedure will remove the vast majority of cellular DNA, trace amounts may still remain, depending on the amount and nature of the sample.

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

To prevent any interference by DNA in real-time RT-PCR applications, such as with Applied Biosystems® and Rotor-Gene® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiTect Primer Assays from QIAGEN are designed for SYBR® Green-based real-time RT-PCR analysis of RNA sequences (without detection of genomic DNA) where possible (see **www.qiagen.com/GeneGlobe**). For real-time RT-PCR assays where amplification of genomic DNA cannot be avoided, the QuantiTect Reverse Transcription Kit provides fast cDNA synthesis with integrated removal of genomic DNA contamination (cat. no. 205311).

## Integrity of RNA

The integrity and size distribution of total RNA purified with the AllPrep DNA/RNA FFPE Kit can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining* or by using the QIAxcel Advanced system or Agilent 2100 Bioanalyzer. For intact RNA, the respective ribosomal RNAs should appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S rRNA should be approximately 2:1. However, due to effects of formalin fixation, embedding, and storage, RNA from FFPE specimens will usually show varying degrees of degradation.

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

# Appendix C: Storage, Quantification, and Determination of Quality of Genomic DNA

## Storage of DNA

For long-term storage, purified DNA in Buffer ATE can be stored at to –30 to –15°C. Avoid any contamination, as this may lead to DNA degradation. We recommend storing samples in aliquots to avoid repeated freezing and thawing.

## Quantification of DNA

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

## Spectrophotometric quantification of DNA

DNA concentration can be determined by measuring the absorbance at 260 nm ($A_{260}$) in a spectrophotometer using a quartz cuvette. For greatest accuracy, readings should be between 0.1 and 1.0. Using a standard 1 cm path length, an absorbance of 1 unit at 260 nm corresponds to 50 µg genomic DNA per ml ($A_{260}$ =1 → 50 µg/ml). This relation is valid only for measurements made at neutral pH. Therefore, samples should be diluted in a low-salt buffer with neutral pH (e.g., Tris·Cl, pH 7.0).* Use the buffer in which the DNA is diluted to zero the spectrophotometer. An example of the calculation involved in DNA quantification is shown below:

* 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.

Volume of DNA sample = 100 µl
Dilution = 20 µl of DNA sample + 180 µl of buffer (1/10 dilution)
Measure absorbance of diluted sample in a 0.2 ml cuvette
$A_{260}$ = 0.2
Concentration of DNA sample = 50 µg/ml x $A_{260}$ x dilution factor
= 50 µg/ml x 0.2 x 10
= 100 µg/ml
Total amount = concentration x volume of sample in milliliters
= 100 µg/ml x 0.1 ml
= 10 µg of DNA

RNA concentration can also be determined by measuring the absorbance at 260 nm. If the eluate contains both DNA and RNA, a fluorometer must be used to quantify the DNA.

## Determination of DNA purity

The ratio of the readings at 260 nm and 280 nm ($A_{260}/A_{280}$) provides an estimate of DNA purity with respect to contaminants that absorb UV light, such as protein. The $A_{260}/A_{280}$ ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting $A_{260}/A_{280}$ ratio can vary greatly. Lower pH results in a lower $A_{260}/A_{280}$ ratio and reduced sensitivity to protein contamination. * For accurate $A_{260}/A_{280}$ values, we recommend measuring absorbance in a slightly alkaline buffer (e.g., 10 mM Tris·Cl, pH 7.5). Make sure to zero the spectrophotometer with the appropriate buffer.

Pure DNA has an $A_{260}/A_{280}$ ratio of 1.7–1.9. Scanning the absorbance from 220–320 nm will show whether there are contaminants affecting absorbance at 260 nm. Absorbance scans should show a peak at 260 nm and an overall smooth shape.

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

## Determination of DNA length

The precise length of genomic DNA can be determined by pulsed-field gel electrophoresis (PFGE) through an agarose gel. The DNA should be concentrated by alcohol* precipitation and reconstituted by gentle agitation in approximately 30 µl TE buffer, pH 8.0,* for at least 30 minutes at 60°C. Avoid drying the DNA pellet for more than 10 minutes at room temperature (15–25°C), since over-dried genomic DNA is very difficult to redissolve. Load 3–5 µg of DNA per well. Standard PFGE conditions are as follows:

- 1% agarose* gel in 0.5x TBE electrophoresis buffer*
- Switch intervals = 5–40 s
- Run time = 17 h
- Voltage = 170 V

* 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.

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| AllPrep DNA/RNA FFPE Kit | 50 RNeasy MinElute Spin Columns, 50 QIAamp MinElute Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers | 80234 |
| **QIAcube Connect – for fully automated nucleic acid extraction with QIAGEN spin-column kits** | | |
| QIAcube Connect* | Instrument, connectivity package, 1 year warranty on parts and labor | Inquire |
| Starter Pack, QIAcube | Filter tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder | 990395 |
| **Accessories** | | |
| Collection Tubes (2 ml) | 1000 Collection Tubes (2 ml) | 19201 |
| QIAGEN Proteinase K (2 ml) | 2 ml (>600 mAU/ml, solution) | 19131 |
| QIAGEN Proteinase K (10 ml) | 10 ml (>600 mAU/ml, solution) | 19133 |
| RNase-Free DNase Set (50) | 1500 units RNase-free DNase I, RNase-free Buffer RDD, and RNase-free water for 50 RNA minipreps | 79254 |
| RNase A (17,500 U) | 2.5 ml (100 mg/ml; 7000 units/ml, solution) | 19101 |
| Buffer AL (216 ml) | 216 ml Lysis Buffer | 19075 |
| Buffer ATL (200 ml) | 200 ml Tissue Lysis Buffer for 1000 preps | 19076 |

| Product | Contents | Cat. no. |
|---|---|---|
| Buffer RLT (220 ml) | 220 ml RNeasy Lysis Buffer | 79216 |
| Buffer RPE (concentrate, 55 ml) | 55 ml concentrated wash buffer for use with RNeasy, miRNeasy, and AllPrep Kits; requires addition of ethanol | 1018013 |
| Deparaffinization Solution | 2 x 8 ml Deparaffinization Solution | 19093 |
| **Related products** | | |
| QIAamp DNA FFPE Tissue Kit (50) | For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Buffers, Collection Tubes (2 ml) | 56404 |
| REPLI-g® FFPE Kit (25)† | For 25 x 50 µl whole genome amplification reactions: DNA Polymerase, Buffers and Reagents | 150243 |
| EpiTect® Plus FFPE Bisulfite Kit (48) | For 48 preps: 48 MinElute DNA Spin Columns, Bisulfite Mix, DNA Protect Buffer, Carrier RNA, Buffers, Deparaffinization Solution, Lysis Buffers FTB | 59144 |
| RNeasy FFPE Kit (50) | For 50 preps: 50 RNeasy MinElute Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers | 73504 |
| miRNeasy FFPE Kit (50) | For 50 preps: 50 RNeasy MinElute Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers | 217504 |
| Qproteome® FFPE Tissue Kit (20)† | For 20 protein preparations from FFPE tissue samples: Extraction Buffer, Collection Tubes, Collection Tube Sealing Clips | 37623 |

| Product | Contents | Cat. no. |
|---|---|---|
| RT² FFPE PreAMP Primer Mixes | Pathway-Focused Primer Mixes for all RT² Profiler PCR Arrays | Varies |

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training, and preventive subscription. Contact your local sales representative to learn about your options.

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

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.

# Document Revision History

| Date | Description of changes |
|---|---|
| December 2014 | Previous release |
| December 2017 | Updated DNA elution volume: 30–100 µl instead of 20–100 µl.<br>Updated RNase contamination removal recommendation.<br>Updated to Sample to Insight branding. |
| January 2020 | Updated text, ordering information and intended use for QIAcube Connect. |
| August 2020 | Corrected the storage condition for Buffer RDD, which should be stored at 2–8°C as it is part of the RNase-Free DNase Set. Removed discontinued products (Qproteome FFPE Tissue 2D-PAGE Kit, QuantiFast Probe Assays, and QuantiFast Probe RT-PCR Plus Kit [80]) in the "Ordering Information" section. |

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