June 2022

RNeasy® DSP FFPE Kit
Instructions for Use (Handbook)

50

Version 2

IVD

For In Vitro Diagnostic Use
For use with RNeasy DSP FFPE Kit

CE

REF 73604

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Sample to Insight

Contents

Intended Use

The RNeasy DSP FFPE Kit is a system intended for the manual purification of total RNA from formalin-fixed, paraffin embedded (FFPE) tissues.

It deploys an optimized silica spin-column-based protocol, and includes enzymatic removal of residual DNA.

The RNeasy DSP FFPE Kit is intended for in vitro diagnostic use

Intended User

The product is intended to be used by professional users, such as technicians and physicians that are trained in molecular biological techniques.

Description and Principle

Summary and explanation

The RNeasy DSP FFPE Kit is specially designed for purification of total RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. By isolating RNA molecules longer than 70 nucleotides, the kit provides recovery of usable RNA fragments for applications such as RT-PCR.

Due to fixation and embedding conditions, nucleic acids in FFPE samples are usually 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. For standardization of pre-examination processes for FFPE tissue, we recommend to proceed according to ISO 20166-1:2018 "Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue — Part 1: Isolated RNA".

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

Staining of FFPE sections may also impair RNA quality and performance in downstream applications. This is especially true for many immunohistochemical staining protocols

Principles of the procedure

The RNeasy DSP FFPE procedure uses well-established RNeasy technology for RNA purification. Specially optimized lysis conditions allow total RNA to be effectively purified from FFPE tissue sections. The DNase I digestion step efficiently removes DNA contamination, including highly fragmented molecules.

First, all paraffin is removed FFPE tissue sections by treating with Deparaffinization Solution. Next, samples are incubated in an optimized lysis buffer, which contains proteinase K to release RNA from the sections. A short incubation at a higher temperature partially reverses formalin crosslinking of the released nucleic acids, improving RNA yield and quality, as well as RNA performance in downstream enzymatic assays. This is followed by DNase I treatment that is optimized to eliminate genomic DNA, including very small DNA fragments that are often present in FFPE samples after prolonged formalin fixation and/or long storage times. Next, the lysate is mixed with Buffer RBC. Ethanol is added to provide appropriate binding conditions for RNA, and the sample is then applied to an RNeasy MinElute spin column, where the total RNA binds to the membrane and contaminants are efficiently washed away. RNA is then eluted in a minimum of 14 µl of RNase-free water.

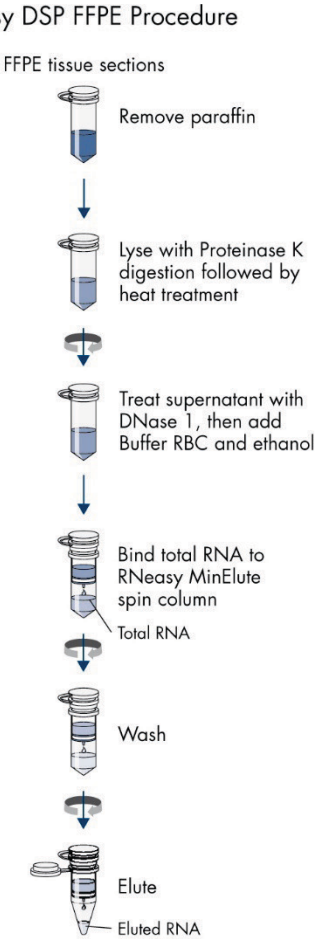

Figure 1. RNA purification procedure from FFPE tissue using the RNeasy DSP FFPE Kit.

Materials Provided

Kit contents

RNeasy DSP FFPE Kit			(50)
Catalog no.			73604
Number of preps			50
	Identity	**Symbols**	**Quantity**
RNeasy MinElute Spin	RNeasy MinElute® Spin Columns (pink) (each in a 2 ml Collection Tube)	COL	50
ET	Elution Tubes (1.5 ml)	ELU TUBE	50
LT	Lysis Tubes (2 ml)	LYS TUBE	150
WT	Wash Tubes (2 ml)	WASH TUBE	250
DPS	Deparaffinization Solution	DEPAR SOL	20 ml
RBC	Buffer RBC*	BIND BUF	45 ml
PKD	Buffer PKD	PROTK DIL	15 ml
PK	Proteinase K	PROTK	1.25 ml
DN	RNase-Free DNase I (lyophilized)	DNase	1
RNFW	RNase-Free Water	ELU DIL	3 x 1.5 ml
DBB	DNase Booster Buffer	DNase BUF	2 ml
RPE	Buffer RPE† (concentrate)	WASH BUF CONC	11 ml
HB, v2	RNeasy DSP FFPE Kit Handbook		1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 11 for safety information.

† Before using for the first time, add 4 volumes of ethanol (96–100%, non-denatured) as indicated on the bottle, and described on page 16 to obtain a working solution.

Components of the kit

The principal components of the kit are explained below.

Table 1. Reagents supplied containing active ingredients

Reagent		Components	Volume
Symbol	**Name**		
DPS	Deparaffinization Solution	Hexadecane	≥90% to ≤100% w/w
RBC	Buffer RBC	Guanidine hydrochloride	≥30% to 70% w/w
PKD	Buffer PKD	None	–
PK	Proteinase K	Proteinase K	≥1% to <3% w/w
DN	RNase-Free DNase I (lyophilized)	DNase	≥90% to ≤100% w/w
RNFW	RNase-Free Water	None	–
DBB	DNase Booster Buffer	None	–
RPE	Buffer RPE (concentrate)	None	–

To minimize the risk of any negative impact on diagnostic results generated after RNA isolation, adequate controls for downstream applications should be used.

Materials Required but Not Provided

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), available from the product supplier.

Make sure that instruments have been checked and calibrated according to the manufacturer´s recommendations.

- Sterile, RNase-free pipet tips and pipettes
- Microcentrifuge (with rotor for 2 ml tubes)
- Vortexer
- 96–100% ethanol (do not use denatured alcohol, which contains other substances such as methanol or methylethylketone)
- Disposable gloves
- Heating block with shaking function capable of incubation at 56°C and 80°C

Warnings and Precautions

Please be aware that you may be required to consult your local regulations for reporting serious incidents that have occurred in relation to the device to the manufacturer and the regulatory authority in which the user and/or the patient is established.

All intended mitigations were implemented when possible at this stage of product development and were systematically reviewed. Based on Risk Management, the overall residual risk is judged acceptable and the use of the device is judged safe. There are no residual risks for the RNeasy DSP FFPE Kit.

For in vitro diagnostic use.

Read all instructions carefully before using the kit.

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 PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

WARNING 	Risk of personal injury DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Buffers in the RNeasy DSP FFPE kit contain sodium azide. If buffers of the kit are spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious

agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Emergency information

CHEMTREC
USA & Canada 1-800-424-9300
Outside USA & Canada +1 703-527-3887

Precautions

The following hazard and precautionary statements apply to components of the RNeasy DSP FFPE Kit.

PKD, RPE, RNFW, DBB

Contains: Sodium azide. Warning! May be harmful if swallowed. Call a POISON CENTER or doctor/physician if you feel unwell.

Deparaffinization Solution

Contains: hexadecane. Danger! May be fatal if swallowed and enters airways. Causes skin irritation. Causes serious eye irritation. May cause respiratory irritation. May cause long lasting harmful effects to aquatic life. Wear protective gloves/protective clothing/ eye protection/face protection. IF exposed or concerned: Immediately call a POISON CENTER or doctor/physician. Do NOT induce vomiting. Store locked up. Dispose of contents/ container to an approved waste disposal plant.

Proteinase K

Contains: Proteinase K. Danger! Causes mild skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. Wear respiratory protection. IF exposed or concerned: Call a POISON CENTER or doctor/physician. Remove person to fresh air and keep

comfortable for breathing. Dispose of contents/container to an approved waste disposal plant.

DNase I

Contains: DNase. Danger! May cause an allergic skin reaction. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. Wear respiratory protection. If exposed or concerned: Call a POISON CENTER or doctor/physician. Remove person to fresh air and keep comfortable for breathing.

Buffer RBC

Contains: guanidine hydrochloride. Warning! Harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

DNAse Booster Buffer

Warning! Causes mild skin irritation. Wear protective gloves/protective clothing/eye protection/face protection.

Reagent Storage and Handling

RNase-Free DNase I and RNeasy MinElute spin columns should be stored at 2–8°C immediately upon arrival. The buffers can be stored at room temperature (15–25°C). Under these conditions, the kit can be stored as stated by the expiration date on the box label without any reduction in performance.

Do not use the RNeasy DSP FFPE Kit once it has expired.

In-use stability

The Kit can be used for 10 months after first use or until the expiry date.

Kit components

Expiration dates for each reagent are indicated on the individual component labels. Under correct storage conditions, the product will maintain performance for the stability time as long as the same batches of components are used.

For long-term storage of DNase I after reconstitution, remove the stock solution from the vial, divide it into single-use aliquots and store at –15 to –30°C for up to 10 months. Thawed aliquots can be stored at 2–8°C for up to 8 weeks. Do not refreeze the aliquots after thawing.

Avoid exposure of the reagents to UV light (e.g., used for decontamination) as exposure may cause accelerated aging.

Procedure

Important points before starting

Starting material

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

- Use tissue samples less than 5 mm thick to allow complete penetration by formalin
- Fix tissue samples in 4–10% neutral-buffered formalin as quickly as possible after surgical removal
- Use a maximum fixing time of 24 hours (longer fixing times lead to over-fixing and more severe nucleic acid fragmentation, resulting in poor performance in downstream assays)
- Thoroughly dehydrate samples prior to embedding
- Use low-melting paraffin for embedding

The starting material for RNA purification should be cut sections of FFPE tissue, each with a thickness of up to 20 µm. Thicker sections may result in lower nucleic acid yields, even after prolonged incubation with proteinase K. Up to 4 sections, each with a thickness of up to 10 µm, can be combined in one preparation. More than 4 sections can be combined if the total sum of the thickness of the sections is 40 µm or less (e.g., eight 5 µm thick sections).

For tissues with particularly high DNA content we recommend using fewer sections per preparation to avoid DNA contamination of the purified RNA.

If there is no information about the nature of your starting material, we recommend starting with no more than 2 sections per preparation. Depending on RNA yield and purity, it may be

possible to use up to 4 sections in subsequent preparations. However, overloading of the RNeasy MinElute spin column might significantly reduce RNA yield and quality.

Preparation of buffers

Preparing DNase I stock solution

Prepare DNase I stock solution by dissolving the lyophilized DNase I in 550 µl RNase-free water. 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.

In some cases, the vial of DNase I 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. Instead, dissolve DNase I using a needle and syringe as described below.

Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.

Note: Please take care that the complete volume of RNase-free water is injected into the vial.

Insoluble material may remain after dissolving DNase I. Due to the production process, insoluble material may be present in the lyophilized DNase I. This does not affect DNase I performance.

For long-term storage of DNase I, remove the stock solution from the vial, divide it into single-use aliquots and store at –15 to –30°C for up to 10 months. Thawed aliquots can be stored at 2–8°C for up to 8 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.

Things to do before starting

- If using the RNeasy DSP FFPE Kit for the first time, read "Important points before starting" (page 15)
- If working with RNA for the first time, read "Appendix: General Remarks on Handling RNA" (page 30).
- Buffer RBC contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See page 11 for safety information.
- Unless otherwise indicated, perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly; do not stop in between.
- 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 the volumes to use if processing 1–2 sections per sample, while ● indicates the volumes to use if processing 3–4 sections per sample.
- If using Buffer RPE and RNase-Free DNase I for the first time, reconstitute them as described in "Preparation of buffers" (page 16).
- Equilibrate all buffers to room temperature (15–25°C). Mix reconstituted Buffer RPE by shaking.
- Set a thermal mixer to 56°C for use in step 5 and step 9. To reduce waiting time, set a second thermal mixer to 80°C for use in step 9.
- **Note**: Do not stop the purification procedure in between as increased incubation times might lead to loss or degradation of RNA. The average processing time of up to 12 samples in parallel is approximately 130 minutes.

Protocol: Purification of total RNA from FFPE tissue sections

1. Using a scalpel, trim excess paraffin off the sample block.
2. Cut sections 5–20 µm thick.

   If the sample surface has been exposed to air, discard the first 2–3 sections.
3. Immediately place the sections in a ▲ 1.5 or ● 2 ml microcentrifuge tube, and close the lid.
4. Add ▲ 160 or ■ 320 µl Deparaffinization Solution, vortex vigorously for 10s and centrifuge briefly to bring the sample to the bottom of the tube.
5. Incubate at 56°C for 3 min then allow to cool down for 5 min at room temperature.

   If too little Deparaffinization Solution is used or if too much paraffin is carried over with the sample, the Deparaffinization Solution may become waxy or solid after cooling. If this occurs, add additional Deparaffinization Solution in 160 µl steps and repeat step 5.
6. Add ▲ 150 or ● 240 µl Buffer PKD, and mix by vortexing for 3 s.
7. Centrifuge for 1 min at 11,000 *x g*.
8. Add 10 µl proteinase K to the lower, clear phase, and mix by pipetting gently 10 times up and down (do not mix separated phases).
9. Incubate at 56°C for 15 min at 1100 rpm, then at 80°C for 15 min at 1100 rpm.

   If using only one heating block, leave the sample at room temperature after the 56°C incubation until the heating block has reached 80°C.

   Note: Complete digestion of tissue by proteinase K is not required for maximum RNA yield; however, the 80°C incubation step is crucial.

   Important: Ensure that the heating block has reached 80°C before starting the 15 min incubation. The 15 min incubation at 80°C is critical for reversal of formaldehyde crosslinks and optimal RNA performance in downstream applications such as real-time RT-PCR.
10. Centrifuge briefly and transfer ▲ 145 or ● 230 µl of the lower, uncolored phase into a new 1.5 ml microcentrifuge tube.

11. Incubate on ice for 3 min. Then, centrifuge for 15 min at 20,000 *x g*.
12. Transfer the supernatant to a new 2 ml microcentrifuge, tube taking care not to disturb the pellet.

    The pellet contains insoluble tissue debris, including crosslinked DNA.
13. Add DNase Booster Buffer equivalent to a tenth of the total sample volume (▲ 14.5 or ● 23 µl) and 10 µl DNase I stock solution. Mix by inverting the tube. 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 16.

    Note: DNase I is especially sensitive to denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.
14. Incubate at room temperature for 15 min.
15. Add ▲ 320 or ● 500 µl Buffer RBC to adjust binding conditions, and mix the lysate thoroughly by vortexing for 3 seconds and centrifuge briefly.
16. Add ▲ 720 µl or ■ 1200 µl ethanol (96–100%) to the sample. Do not centrifuge. Proceed immediately to step 17.

    Precipitates may be visible after addition of ethanol. This does not affect the procedure.
17. Mix well by pipetting 5 times up and down and 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. Close the lid gently, and centrifuge for 15 s at ≥8000 *x g*. Discard the collection tube with the flow-through* and place the column into a new collection tube (supplied).
18. Repeat step 17 (without additional mixing) until the entire sample has passed through the RNeasy MinElute spin column.
19. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8,000 *x g*. Discard the collection tube with the flow-through* and place the column into a new collection tube (supplied).

* Flow-through contains Buffer RBC and is therefore not compatible with bleach. See page 8 for safety information.

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

20. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 2 min at ≥8,000 *x g* to wash the spin column membrane. Discard the collection tube with the flow-through* and place the column into a new collection tube (supplied).

    Note: After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

21. 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 because residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

22. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14–32 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.

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

    Note: For expected low RNA yields, a Low Bind tube is recommended for elution (not supplied). The mean dead volume of the RNeasy MinElute spin column is 2 µl: elution with 14 µl RNase-free water results in approximately 12 µl eluate.

* Flow-through contains Buffer RBC and is therefore not compatible with bleach. See page 8 for safety information.

23. Store RNA eluates at –60 to –90°C or at –15 to –30°C for up to 12 weeks.

    Note: Eluate stability will depend on the content and type of isolated RNA, elution volume, and storage conditions. We recommend that users establish the eluate stability as needed for their particular requirements.

Quality Control

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

Limitations

System performance has been established in performance evaluation studies purifying human RNA from formalin-fixed, paraffin-embedded samples.

It is the user´s responsibility to validate system performance for any procedures used in their laboratory that are not covered by the QIAGEN performance evaluation studies.

To minimize the risk of a negative impact on the diagnostic results, adequate controls for downstream applications should be used.

Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

Performance Characteristics

The applicable performance characteristics can be found under the resource tab of the product page on www.qiagen.com.

Disposal

The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed properly. Refer to your local safety regulations for proper disposal procedures.

For more information, please consult the appropriate safety data sheets (SDSs). These are available online in PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit components.

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: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
Clogged RNeasy 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 15).
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 RNeasy MinElute spin column. If this happens, set the centrifugation temperature to 25°C.
Low RNA yield		
a)	Poor quality of starting material	Samples that were fixed for more than 24 hours or were stored for very long periods may contain very little usable RNA. Sections that were mounted on microscope slides may yield very little usable RNA due to prolonged exposure to air.
b)	Too much starting material	Overloading the RNeasy MinElute spin column significantly reduces nucleic acid yields. Reduce the amount of staring material (see page 15).
c)	RNA still bound to RNeasy MinElute spin column membrane	Repeat RNA elution, but incubate the RNeasy Min Elute spin column on the benchtop for 10 minutes with RNFW before centrifugation.
d)	Wrong storage of buffers/reagents	RNeasy MinElute spin columns as well as DNase I need to be stored at 2–8°C upon arrival of the kit. Check the correct storage temperature as exposure to higher temperatures for longer time periods might lead to loss of functionality.

		Comments and suggestions
Low A_{260}/A_{280} value		
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.
DNA contamination in downstream experiments		
a)	Too much starting material	For some tissue types, the efficiency of DNA removal may be reduced when processing very high amounts. If the eluted RNA contains substantial DNA contamination, try processing fewer tissue sections per preparation.
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. Check if the DNase I has been stored correctly as described in "Reagent Storage and Handling" and "Preparing DNase I stock solution".
c)	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 a reverse transcriptase that is specially designed for highly sensitive reverse transcription.
RNA does not perform well in downstream assays/applications		
a)	RNA fragmented or blocked due to formaldehyde modification	The 80°C incubation in the RNeasy DSP FFPE procedure is crucial for optimal RNA performance in reverse transcription and other enzymatic downstream applications. Ensure that the incubation temperature is maintained at 80°C throughout the entire 15 minute incubation time. Although the 80°C incubation removes some of the formaldehyde modifications, RNA purified from FFPE sections is 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 (<500 nucleotides).
b)	Ethanol carryover	During the second wash with Buffer RPE, be sure to centrifuge at ≥8000 x *g* for 2 minutes at 15–25°C to dry the RNeasy MinElute spin column membrane. After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow-through. Then, place the column in a new collection tube and centrifuge at full speed for 5 minutes.
c)	Salt carryover during RNA elution	Ensure that Buffer RPE was reconstituted by adding the correct volume of ethanol and that the buffer is at room temperature (15–25°C).

		Comments and suggestions
d)	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 a reverse transcriptase which is specially designed for highly sensitive reverse transcription.

Symbols

The following symbols appear in the instructions for use or on the packaging and labeling:

Symbol	Symbol definition
 <N>	Contains reagents sufficient for <N> reactions
	Use by
	This product fulfills the requirements of the European Regulation 2017/746 for in vitro diagnostic medical devices.
	In vitro diagnostic medical device
	Upon arrival
	DN
	RNeasy MinElute Spin
	Catalog number
	Lot number
	Material number (i.e., component labeling)
	Components (i.e., a list of what is included)
	Contains (contents)

Symbol	Symbol definition
NUM	Number (i.e., vials, bottles)
GTIN	Global Trade Item Number
Rn	R is for revision of the Instructions for Use (Handbook) and n is the revision number
	Temperature limitation
	Manufacturer
	Consult instructions for use
	Caution
PROTK	Proteinase K
Sodium azide	Sodium azide
UDI	Unique device identifier

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Appendix: 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 plastic ware 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 non-disposable 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, non-disposable plasticware, and laboratory equipment (e.g., pipets and electrophoresis tanks), use of RNaseZap® (cat. no. AM9780) from Ambion® is recommended. RNase contamination can alternatively be removed using general laboratory reagents. To decontaminate plastic ware, rinse with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water (see "Solutions", page 32) or rinse with chloroform if the plastic ware 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.

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 plastic ware 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 have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

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

Ordering Information

Product	Contents	Cat. no.
RNeasy DSP FFPE Kit (50)	50 RNeasy MinElute Spin Columns, Elution Tubes, Wash Tubes, Lysis Tubes, RNase-free Reagents and Buffers	73604

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Document Revision History

Revision	Description
R1, June 2022	Update to Kit Version 2 for compliance to IVDR. No changes to protocols or performance compared to Kit Version 1 Update of Warnings and Precautions (Addition of residual risks, emergency information) Addition of Disposal section

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