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QuickExtract™ FFPE RNA Extraction Kit

Cat. Nos. QFR82805 and QFR82050

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1. Introduction

The QuickExtract™ FFPE RNA Extraction Kit is a fast, simple, and inexpensive method for preparing RNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples for RT-PCR amplification. The QuickExtract FFPE RNA Extraction kit requires only heat treatment to melt the paraffin, lyse the cells, decrease the formalin-induced cross-linking in the sample, and degrade compounds that may inhibit amplification. An optional DNase treatment may improve certain downstream applications. Following heat treatment, the sample RNA is ready for RT-PCR. An optional protocol also allows for simultaneous extraction of both RNA and DNA.

2. Product Specifications

Storage: Store the kit at –20°C in a freezer without a defrost cycle. Minimize the number of freeze/thaw cycles to the QuickExtract FFPE RNA Extraction Solution. Thawed extraction solution can be stored at 4°C for 1 month or refrozen in small aliquots.

Storage Buffer: RNase-Free DNase I is supplied in a 50% glycerol solution containing 10 mM Tris-HCl (pH 7.5), 10 mM $CaCl_2$, and 10 mM $MgCl_2$.

Unit Definition: One Molecular Biology Unit (MBU) of RNase-Free DNase I converts 1 µg of pUC19 DNA into oligodeoxynucleotides in 10 minutes at 37°C.

Quality Control: The QuickExtract FFPE RNA Extraction Kit is function-tested by assaying for a PCR product from cDNA produced from RNA extracted from a slide-mounted, FFPE tissue slice.

Contaminating Activity Assays: All components of the QuickExtract FFPE RNA Extraction Kit are free of detectable RNase activity, and all of the components except DNase I are free of detectable exo- and endonuclease activities.

3. Kit Contents and Specifications

Cat. #	Concentration	Quantity
QuickExtract™ FFPE RNA Extraction Kit Contents		
The QuickExtract FFPE RNA Extraction Kit is available in two sizes (5 ml and 50 ml), sufficient to perform 50 and 500 standard FFPE RNA extractions respectively.		
5 ml (50 Extractions)		
QFR82805		
QuickExtract™ FFPE RNA Extraction Solution		5 ml
Buffer 1		100 µl
RNase-Free DNase I	@ 1 MBU/µl	100 µl
Stop Solution		100 µl
50 ml (500 Extractions)		
QFR82050		
QuickExtract™ FFPE RNA Extraction Solution		50 ml
Buffer 1		1 ml
RNase-Free DNase I	@ 1 MBU/µl	1 ml
Stop Solution		1 ml

4. Related Products

The following products are also available:

- QuickExtract™ FFPE DNA Extraction Kit
- MMLV Reverse Transcriptase 1st-Strand cDNA Synthesis Kit
- MonsterScript™ 1st-Strand cDNA Synthesis Kit
- MMLV High Performance Reverse Transcriptase

Notes on Use of the QuickExtract FFPE RNA Extraction Kit

1. Perform QuickExtract FFPE RNA extractions in an RNase-free work environment.
   - Always wear gloves when handling samples containing RNA and kit components.
   - Do not pick up any kit component with an ungloved hand.
   - Keep all kit components tightly sealed when not in use.
   - Keep all tubes containing RNA tightly sealed during the incubation steps.
2. The extraction protocols are scaleable. If using a larger or smaller amount of tissue, adjust the reagent volume accordingly.
3. Nucleic acids isolated from preserved, paraffin-embedded tissues are generally of poor quality. The degree of degradation of these samples limits analysis to mainly techniques involving amplification. See recommendations on page 4.

5. Protocols

A. FFPE Tissue Slices from Microscope Slides

1. Add 100 µl of QuickExtract FFPE RNA Extraction Solution to the paraffin-embedded tissue section on the slide (0.8-1.0 cm^2 tissue section). Scrape with a sterile blade to remove the tissue section from the slide and transfer the solution and tissue to a small microcentrifuge tube. Alternatively, the tissue section can be scraped and added to the solution in the tube, but prewetting the slide facilitates transfer of the tissue slice.
2. Briefly centrifuge the tube to collect the solution and tissue at the bottom of the tube. If some tissue remains on the wall of the tube, begin heating the sample (Part A, Step 3) to melt the paraffin, then mix by vortexing and briefly centrifuge to collect the melted sample at the bottom of the tube.
3. Heat the tube in a thermocycler for 30 minutes at 56°C, and then for 2 minutes at 98°C. If desired, mix the sample by vortexing once during the incubation to aid in extraction, then briefly centrifuge the sample and continue the incubation.
4. The RNA can be used directly in a cDNA synthesis reaction or stored frozen at –70°C.
5. The DNA can be removed with an optional DNase I treatment (see Part C).

B. Paraffin-Embedded Tissues

1. Remove a section of tissue using a clean microtome blade. Trim off any excess paraffin.
2. Place 10-50 mg of tissue or up to three 5-10 µm thick paraffin sections into a small microcentrifuge tube containing 100 µl of QuickExtract FFPE RNA Extraction Solution.

   Note: *The amount of extraction solution used can be adjusted to produce more concentrated extracted RNA. Thin slices are more important than the amount of tissue.*
3. Follow Part A, Steps 2 through 4 of the FFPE Tissue Slices from Microscope Slides protocol (above).

C. Optional DNase I Treatment

1. Add 2 µl of Buffer 1 and 2 µl of RNase-free DNase I to the extracted RNA. Mix the sample and incubate the tube at 37°C for 10 minutes.
2. Add 2 µl of Stop Solution to the tube and incubate at 65°C for 10 minutes.
3. Store the tubes on ice or frozen until the sample is to be used for cDNA synthesis.

D. Simultaneous DNA and RNA Extractions

The QuickExtract FFPE RNA Extraction Kit extracts total nucleic acids (TNA) from FFPE tissues.

1. Add 100 µl of QuickExtract FFPE RNA Extraction Solution to the paraffin-embedded tissue section on the slide (0.8-1.0 cm2 tissue section). Scrape with a sterile blade to remove the tissue section from the slide and transfer the solution and tissue to a small microcentrifuge tube. Alternatively, the tissue section can be scraped and added to the solution in the tube, but prewetting the slide facilitates transfer of the tissue slice.

   Note: *If using a larger or smaller amount of tissue, adjust the reagent volume accordingly.*
2. Briefly centrifuge the tube to collect the solution and tissue at the bottom of the tube. If some tissue remains on the wall of the tube, begin heating the sample (Part D, Step 3) to melt the paraffin, then mix by vortexing and briefly centrifuge to collect the melted sample at the bottom of the tube.
3. Heat the tube in a thermocycler for 30 minutes at 56°C. If desired, mix the sample by vortexing once during the incubation to aid in extraction, then briefly centrifuge the sample and continue the incubation.
4. At the end of the 30-minute incubation, vortex the sample and divide it into two tubes (one each for RNA and DNA extraction).

   Note: *Do not heat the RNA sample longer than 30 minutes.*
5. Follow the protocol below for each tube.

RNA tube

a) Heat the RNA tube to 98°C for 2 minutes.

b) The RNA can be used directly in a cDNA synthesis reaction or stored frozen at –70°C.
Note: *The sample can be placed on ice while the DNA sample is being processed.*

c) The DNA can be removed with an optional DNase I treatment (see Part C).

DNA tube

a) Heat the DNA tube in a thermocycler for an additional 30 minutes at 56°C, and then for 2 minutes at 98°C.

b) Quantitate the DNA yield by fluorimetry using Hoechst dye 33258 to avoid an overestimation given by A_{260} readings.

c) Store the DNA at –20°C, or at –70°C for archival purposes.

PCR Amplification Recommendations for cDNA and DNA

1. For cDNA synthesis, 2-10 µl of extracted RNA can be used directly with any reverse transcriptase and a standard 20-µl protocol. Up to 50% of the reaction volume can be extracted RNA.
2. For standard and fast end-point PCR cycling profiles, 1-5 µl of cDNA or 1-10 µl of extracted DNA can be used directly. Profiles should include 40 amplification cycles to ensure successful amplification.
3. Primers should be designed so that PCR amplicons will be less than 300 bases in length. The average fragment size for RNA recovered from FFPE tissues has been documented at approximately 200 bases, and the average size DNA that is extracted from FFPE tissues as 300-400 bp.[1,2] Therefore, the shorter the amplicon, the greater the chance of successful amplification. Real-time PCR amplicons should be less than 150 bp in length.
4. Extracted RNA and DNA has been used successfully in standard and fast end-point PCR, random amplification of polymorphic DNA (RAPD) PCR, mitochondrial PCR, and real-time PCR. The resulting amplicons can be used for single-nucleotide polymorphism (SNP) detection or DNA sequencing.
5. When using extracts in real-time RT-PCR with SYBR® Green I Dye, or other similar dye detection, DNase treatment of the extract before cDNA synthesis is recommended.

6. References

1. Godfrey, T.I. *et al.,* (2000) *J. Mol. Diagn.* **2**, 84.
2. Lehmann, U. and Kreipe, H. (2001) *Methods* **25**, 409.

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