



PureLink™ FFPE Total RNA Isolation Kit

**For rapid purification of total RNA from
formalin-fixed, paraffin-embedded (FFPE)
tissues**

Catalog no. K1560-02

Version B
16 October 2006
25-0875

User Manual

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Experienced Users Procedure

Introduction

This quick reference protocol is included for experienced users of the PureLink™ FFPE Total RNA Isolation Kit.

Perform all centrifugation steps at room temperature.

If you are a first time user, follow the protocol in this manual.

Step	Action
De-paraffinization and Lysis	<ol style="list-style-type: none">1. Place 3-8 pieces of 10 µm sections of formalin-fixed, paraffin-embedded (FFPE) samples into a sterile, RNase-free 1.5 ml microcentrifuge tube.2. Add 300 µl Melting Buffer to the tube.3. Centrifuge for 10-20 seconds at maximum speed.4. Incubate for 10 minutes at 72°C with intermittent gentle mixing every 2-3 minutes by tapping the tube (make sure the tissue stays submerged) to melt the paraffin.5. Tap the tube to collect any liquid remaining on the tube cap.6. Add 20 µl Proteinase K to the tube.7. Incubate at 60°C for 10-60 minutes with occasional mixing. Extend the incubation time by an additional 30-60 minutes and up to 3 hours, until lysis is complete.8. Immediately centrifuge at maximum speed for 1 minute to form a thin paraffin layer at the top, separated from the lysate.9. Penetrate the paraffin layer using a clean pipette tip (1 ml) or a large orifice pipette tip, aspirate the lysate solution, and transfer the lysate to a clean RNase-free tube.
Purification Procedure	<p>Binding Step</p> <ol style="list-style-type: none">1. Add 400 µl Binding Buffer (L3) and 800 µl 100% ethanol to the sample from Step 9, above. Mix well.2. Transfer 700 µl sample with Binding Buffer (L3) and ethanol to a Spin Cartridge in a Collection Tube.3. Centrifuge at 800 x g for 1 minute. Discard the flow through and reinsert the cartridge into the Collection Tube.4. Transfer the remaining sample (~700 µl) from Step 1 to the Spin Cartridge in the Collection Tube.5. Centrifuge the cartridge at 800 x g for 1 minute.6. Discard the flow through. Replace the used Collection Tube with a clean Wash Tube.

Continued on next page

Experienced Users Procedure, Continued

Step	Action
Purification Procedure, continued	<p>Washing Step</p> <ol style="list-style-type: none"> 1. Add 80 ml 100% ethanol to 20 ml Wash Buffer (W5). Mix well and mark the bottle to indicate that ethanol has been added. Store Wash Buffer (W5) with ethanol. 2. Add 500 µl Wash Buffer (W5) with ethanol to the cartridge. Centrifuge the cartridge at maximum speed for 1 minute. Discard the flow through. 3. Repeat Step 2 twice for a total of 3 wash steps. Discard the flow through. Reinsert the cartridge into the Wash Tube. 4. Centrifuge the cartridge at maximum speed for 1 minute to remove any residual Wash Buffer with ethanol. Discard the Wash Tube. <p>Elution Step</p> <ol style="list-style-type: none"> 1. Preheat an aliquot of the RNase-free Water to 65°C. 2. Place the Spin Cartridge into a clean 1.7 ml Recovery Tube. 3. Add 50 µl RNase-Free Water heated to 65°C into the center of the cartridge. 4. Incubate the cartridge for 1 minute. 5. Centrifuge the cartridge at maximum speed for 1 minute. <p>The Recovery Tube contains total RNA.</p> <p>Note: You may perform a second elution with 50 µl RNase-Free Water, if desired. This may increase the total RNA yield by 20-30%. See page 4 for additional information on Elution.</p> <p>Remove and discard the cartridge.</p> <ol style="list-style-type: none"> 6. Place the tube on ice before proceeding to downstream applications, or store the RNA at -80 °C until further use.

Kit Contents and Storage

Shipping and Storage

All components of the PureLink™ FFPE Total RNA Isolation Kit are shipped at room temperature, except DNase I, which is shipped separately on dry ice. Upon receipt, store all components at room temperature. Store the DNase I at -20°C upon arrival.

Contents

The components included in the PureLink™ FFPE Total RNA Isolation Kit are listed below. Sufficient reagents are included in the kit to perform 50 isolations.

Item	Amount
Melting Buffer	15 ml
Proteinase K (20 mg/ml) in storage buffer (proprietary)	1 ml
Binding Buffer (L3)	20 ml
Wash Buffer (W5)	20 ml
RNase-Free Water	4 ml
Spin Cartridges with Collection Tubes	50
Wash Tubes	50
Recovery Tubes	50
DNase I, Amplification Grade	0.1 ml

Product Qualification

The PureLink™ FFPE Total RNA Isolation Kit is functionally qualified by isolating total RNA from 5 pieces of 10 µm sections of formalin-fixed, paraffin-embedded kidney tissue with a tissue surface area of 0.5-1 cm² as described in this manual. The purified RNA is tested for a specified yield of nucleic acid.

In addition, each kit component is free of ribonuclease contamination.

Accessory Products

Additional Products

The following products and a wide variety of additional RT-PCR products, E-Gel® agarose gels, and DNA ladders are available from Invitrogen that may be used with the PureLink™ FFPE Total RNA Isolation Kit. For more details on these products, visit www.invitrogen.com or contact Technical Support (page 17).

Product	Quantity	Catalog no.
Reagents for RT-PCR and qRT-PCR		
SuperScript™ III First-Strand Synthesis System for RT-PCR	50 reactions	18080-051
SuperScript™ III First-Strand Synthesis SuperMix	50 reactions	18080-400
Platinum® PCR SuperMix	100 reactions	11306-016
Platinum® PCR SuperMix High Fidelity	100 reactions	125326-016
SuperScript™ III Two-Step qRT-PCR Kit	100 reactions	111734-050
SuperScript™ III Two-Step qRT-PCR Kit with ROX	100 reactions	11146-100
LUX™ Fluorogenic Primer Sets	50 nmol or 200 nmol	Design and order at www.invitrogen.com/lux
Reagents		
RNase AWAY®	250 ml	10328-011
0.16-1.77 Kb RNA Ladder	75 µg	15623-010
E-Gel® Low Range Quantitative DNA Ladder	100 apps	12373-031
UltraPure™ DEPC-treated Water	1 L	750023
UltraPure™ DNase/RNase-Free Distilled Water	500 ml	10977-015
Quant-iT™ RNA Assay Kit	1000 assays	Q33140
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
DNase I, Amplification Grade	100 units	18068-015

Introduction

Overview

Introduction

The PureLink™ FFPE Total RNA Isolation Kit provides a simple, reliable, and rapid method to isolate high-quality total RNA from formalin-fixed, paraffin-embedded (FFPE) archival tissue specimens. The kit was developed to allow total RNA purification from a wide variety of specimens for retrospective basic and clinical biological studies of gene expression without the use of chemical solvents for deparaffinization. The protocol significantly reduces the time required for total RNA isolation from FFPE tissue without using toxic reagents such as xylene.

The purified RNA is suitable for downstream applications such as reverse transcription, RT-PCR, and real-time quantitative RT-PCR (qRT-PCR). The size of the purified RNA that can be amplified is generally larger than for RNA isolated using organic solvents for deparaffinization.

System Overview

The paraffin from thin sections of a FFPE specimen is melted using heat in the presence of a specially designed Melting Buffer. The tissue is separated from the melted paraffin by centrifugation and digested with Proteinase K. The tissue lysate is further processed by selective binding of RNA to a silica-based membrane in the Spin Cartridge. Impurities are removed by thorough washing with Wash Buffer. The total RNA is eluted in RNase-Free Water.

The expected total RNA yield exceeds 1 µg from 3-8 sections of 10 µm each.

Note: The RNA yield is dependent on tissue type and quality of the specimen.

Continued on next page

Overview, Continued

Advantages

Using the PureLink™ FFPE Total RNA Isolation Kit to isolate total RNA offers the following advantages:

- Environment-friendly procedure without the use of toxic organic solvents
- Heating procedure for deparaffinization partly restores RNA template structure for cDNA synthesis.
- Improved template quality for reverse transcription
- Simple, reliable, and rapid purification procedure
- Higher yields and purity as compared to other commercially available RNA purification systems
- Designed to isolate total RNA from a variety of formalin-fixed, paraffin-embedded specimens
- Minimal genomic DNA contamination of the purified RNA sample and option for DNase digestion
- Reliable performance of the high-quality purified total RNA in downstream applications (see below) for retrospective basic and clinical biological studies

Downstream Applications

Total RNA isolated using the PureLink™ FFPE Total RNA Isolation Kit is suitable for use in the following applications:

- Reverse transcription
- RT-PCR
- Real time quantitative RT-PCR (qRT-PCR)



Note

The actual length of RT-PCR products amplified from RNA purified using the PureLink™ FFPE Total RNA Isolation Kit depends on the age of the FFPE tissue and on the fixation procedure used.

System Specifications

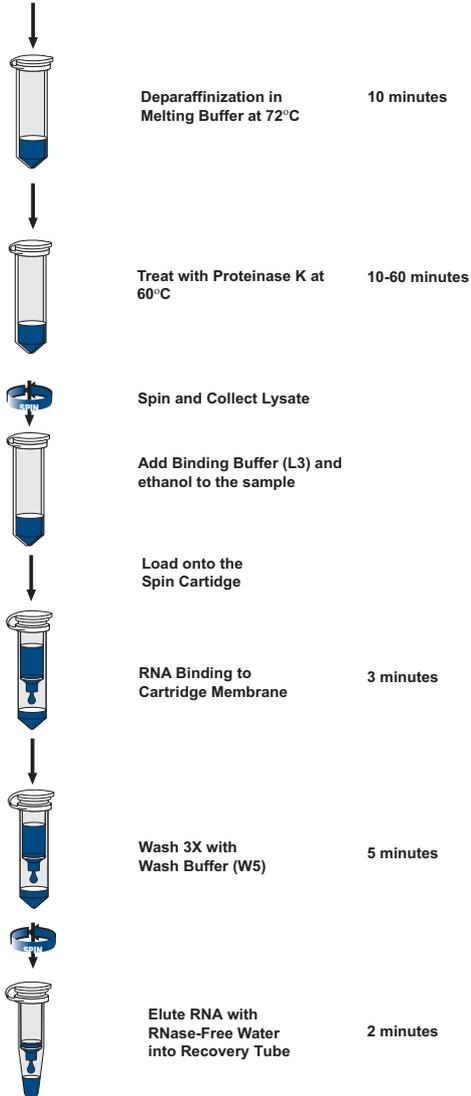
Starting Material:	3-8 pieces of 10 µm sections of FFPE tissue
Column Reservoir Capacity:	700 µl
Wash Tube Capacity:	2.0 ml
Recovery Tube Capacity:	1.7 ml
Centrifuge Compatibility:	Capable of centrifuging at 13,000-15,000 x g
RNA Yield:	Varies with source material

Experimental Overview

Introduction

The flow chart illustrates the steps for isolating total RNA from FFPE tissues.

Sample Sectioning



General Information

General Handling of RNA

Observe the following guidelines to prevent RNase contamination and maximize the RNA yield:

- Use disposable, individually wrapped, sterile plastic ware
 - Use sterile, new pipette tips and microcentrifuge tubes
 - Wear latex or nitrile gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin
 - Use proper microbiological aseptic techniques when working with RNA
 - Use RNase AWAY® Reagent (page viii) to remove RNase contamination from surfaces
-

Elution Parameters

Elution Buffer

The RNA is eluted from the Spin Cartridge using RNase-Free water (provided). Alternatively, Tris Buffer (10 mM Tris-HCl), pH 7.5 in RNase-free water can be used.

Elution Buffer Volume

The protocol recommends eluting the RNA in 50 µl RNase-free water. You may vary the volume of RNase-free water to obtain total RNA in the desired final concentration. For best results, always use at least 30 µl of RNase-free water.

Note: Reducing the elution volume may increase the RNA concentration but may result in a reduced total yield of RNA.

Number of Elutions

Performing a second elution step increases the RNA yield by up to 30%.

Elution Buffer Temperature

Pre-warming the elution buffer to 65°C increases the RNA yield by up to 15-20%.

Safety Information

Follow the safety guidelines below when using the Kit.

- Treat all reagents supplied in the kit as potential irritants.
 - Wear a lab coat, disposable gloves, and protective eyewear.
 - If a buffer spill occurs, clean with a suitable laboratory detergent and water.
 - Dispose of any tissue in designated biohazard containers.
 - If a spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with 1% (v/v) sodium hypochlorite or a suitable laboratory disinfectant.
-

Methods

Preparing Lysates

Introduction

This section provides instructions for preparing lysates from FFPE tissues.

To obtain high-quality total RNA, follow the guidelines on page 4 for handling RNA.

Materials Needed

- RNase-free tubes, 1.5 ml
- RNase-free pipette tips
- Optional: RNase-free orifice pipette tips
- Two heat blocks set to 60°C and 72°C
- Microcentrifuge capable of centrifuging at 13,000-15,000 × g
- Recommended: microtome suitable for sectioning paraffin-embedded tissue that is capable of producing 10 μm sections

Components provided with the kit:

- Melting Buffer
 - Proteinase K
-

Sample Amount

For each RNA purification, use 3-8 pieces of 10 μm thick sections of FFPE tissue with a tissue surface area between 0.5-1 cm².

Continued on next page

Preparing Lysates, Continued

Guidelines for Sectioning Paraffin Blocks

To use this kit, you need 10 μm sections of the tissue in paraffin block. You may use any method for sectioning the paraffin blocks. General guidelines for sectioning paraffin blocks are outlined below:

- Avoid nuclease contamination by using a clean, sharp microtome blade and tweezers
- When multiple samples are processed, clean the microtome blade and tweezers with RNase-inactivating agents such as RNase AWAY[®] to avoid cross-contamination of nucleic acid and RNases. UV irradiation for 10 minutes is recommended after cleaning.
- Always wear latex or nitrile gloves
- Cut 10 μm thick sections from trimmed paraffin blocks with a tissue surface area of about 0.5-1 cm^2 .

Continued on next page

Preparing Lysates, Continued

De-paraffinization and Lysis

Perform all centrifugation steps at room temperature.

1. Place 3-8 pieces of 10 μm sections of FFPE tissue samples into a sterile, RNase-free 1.5-ml microcentrifuge tube.
2. Add 300 μl Melting Buffer to the tube.
3. Centrifuge for 10-20 seconds at maximum speed to submerge the sections in Melting Buffer.
4. Incubate for 10 minutes at 72°C with intermittent gentle mixing every 2-3 minutes by tapping the tube (make sure the tissue stays submerged) to melt the paraffin.
5. Collect any liquid remaining in the tube cap by tapping the tube.
6. Add 20 μl Proteinase K to the tube containing the tissue and mix well by pipetting up and down and ensure the tissue is well suspended.
7. Incubate at 60°C for 10-60 minutes with occasional mixing. At the end of the incubation, there should be no visible tissue debris in the solution. If you notice any tissue debris, extend the incubation time by an additional 30-60 minutes and up to 3 hours.
8. **Immediately** centrifuge at maximum speed for 1 minute to separate the tissue lysate from paraffin and allow the paraffin to form a thin layer at the top.
9. Using a clean, RNase-free pipette tip (1 ml), penetrate the paraffin layer and aspirate the solution containing the tissue lysate and transfer to a clean RNase-free tube. If the paraffin plugs the tip, change to a new pipette tip and insert the new tip in the same location to collect the tissue lysate.

Note: You may use a large orifice pipette tip, which reduces the likelihood to plug the tip with paraffin and allows for easier transfer of the lysate solution to the new tube. Alternatively, cut off the tip of a pipette tip using a clean RNase-free razor blade to enlarge the tip opening.

Proceed to **Isolating Total RNA**, next page.



Note

After preparing the lysate, we recommend isolating RNA immediately. Do not store the lysate in Melting Buffer, as it may adversely affect the quality of your RNA.

Isolating Total RNA

Introduction

This section provides instructions for isolating total RNA from the prepared tissue lysate.



The Binding Buffer (L3) contains guanidine isothiocyanate. Always wear a laboratory coat, disposable gloves, and eye protection when handling solutions containing this chemical.

Do not add bleach or acidic solutions directly to solutions containing guanidine isothiocyanate or sample preparation waste. Guanidine isothiocyanate forms reactive compounds and toxic gases when mixed with bleach or acids.

Materials Needed

- RNase-free pipette tips
- 100% ethanol
- Microcentrifuge capable of centrifuging 13,000-15,000 x g

Components provided with the kit:

- Binding Buffer (L3)
 - Wash Buffer (W5)
 - RNase-free Water
 - Spin Cartridge with Collection Tube
 - Wash Tubes
 - Recovery Tubes
-

Before Starting

- Add 80 ml 100% ethanol to 20 ml Wash Buffer (W5). Mix well and mark the bottle to indicate that ethanol has been added. Store Wash Buffer with ethanol at room temperature.
 - Preheat an aliquot of the RNase-free water supplied with the kit to 65°C. This is required for the elution step.
-

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Isolating Total RNA, Continued

Binding RNA

Perform all centrifugation steps at room temperature.

1. Add 400 μl Binding Buffer (L3) and 800 μl 100% ethanol to the sample from Step 9, page 7. Mix well by vortexing.
2. Transfer 700 μl of the sample from Step 1 to the Spin Cartridge in a Collection Tube.
3. Centrifuge the cartridge at 800 x g for 1 minute. Discard the flow through and reinsert the cartridge into the Collection Tube.
4. Transfer the remaining sample (~700 μl) from Step 1 to the Spin Cartridge in the Collection Tube.
5. Centrifuge the cartridge at 800 x g for 1 minute.
Note: If you notice any sample still remaining in the cartridge, centrifuge the cartridge at maximum speed for an additional minute.
6. Discard the flow through and replace the used Collection Tube with a clean Wash Tube supplied in the kit.

Proceed to **Washing Step**, below.

Washing Step

1. To the cartridge from Step 6, above, add 500 μl Wash Buffer (W5) with ethanol (see **Before Starting**, previous page) to the cartridge.
2. Centrifuge the cartridge at maximum speed for 1 minute. Discard the flow through and reinsert the cartridge into the Wash Tube.
3. Repeat Step 2 twice for a total of 3 wash steps using 500 μl Wash Buffer (W5) with ethanol for each wash.
4. Centrifuge the cartridge at maximum speed for 1 minute to remove any residual Wash Buffer with ethanol. Discard the Wash Tube.

Proceed to **Eluting Step**, next page.

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Isolating Total RNA, Continued

Eluting Step

1. Place the Spin Cartridge from **Washing Step, Step 4** (previous page) into a clean 1.5 ml RNA Recovery Tube supplied with the kit.
2. Add 50 μ l RNase-free water heated to 65°C (see page 8) into the center of the cartridge.
3. Incubate the cartridge for 1 minute.
4. Centrifuge the cartridge at maximum speed for 1 minute.

The Recovery Tube contains total RNA.

Note: The recovery volume may be 5-10% less than 50 μ l.

Optional: You may increase the total yield of total RNA by up to 25% by performing a second elution step (repeat Steps 2-4). However, the second elution reduces the concentration of the recovered total RNA due to a larger total RNA sample volume.

5. Remove and discard the cartridge.
 6. Place the tube on ice before proceeding to downstream applications, or store the RNA at -80 °C until further use.
-

The Next Steps

You may use the purified total RNA for the following procedures:

- If highly pure RNA without genomic DNA contamination is required perform optional DNase I treatment (see page 11 for protocol).
 - If desired, determine the quality and quantity of the purified RNA (page 13).
 - Immediately use total RNA for the desired downstream applications (page 2).
 - Store the total RNA at -80°C until further use.
-

Optional DNase I Digestion

Introduction

If your downstream application requires DNA-free total RNA perform the optional DNase I digestion after elution of RNA, following the instructions in this section.

The DNase I, Amplification Grade is included in the kit and is also available separately from Invitrogen (see page viii).

Materials Needed

- RNase-free microcentrifuge tubes, 1.5 ml
- RNase-free pipette tips
- Heating block set at 65°C
- Microcentrifuge capable of centrifuging 13,000-15,000 x g
- Ice

Components provided with the kit:

- 10X DNase I Buffer
 - DNase I, Amplification Grade
 - 25 mM EDTA
-

Continued on next page

Optional DNase I Digestion, Continued

DNase I Digestion

This protocol is designed for use with 5-8 μ l RNA sample.

If you perform your digestion for more than 5-8 μ l RNA sample, scale up the amount of reagents accordingly.

You may perform DNase I digestion with an aliquot of the purified RNA sample or with the entire amount of purified RNA sample.

1. On ice, add the following components to a sterile tube:

<u>Component</u>	<u>Amount per sample</u>
Purified RNA Sample:	5-8 μ l
DNase I, Amplification Grade (1 U/ μ l):	1 μ l
10X DNase I Buffer:	1 μ l
<u>DEPC-treated water:</u>	<u>to 10 μl</u>
Total Volume:	10 μ l

2. Mix by gently pipetting up and down or by vortexing briefly, and spin briefly to collect the tube contents.
 3. Incubate the tube at room temperature for 5-15 minutes.
 4. Centrifuge briefly and add 1 μ l of 25 mM EDTA to each tube on ice.
 5. Mix by gently pipetting up and down, and centrifuge briefly to collect the tube contents.
 6. Incubate at 65°C for 10 minutes.
 7. Centrifuge briefly and place the tube on ice before proceeding to reverse transcription or store the RNA at -80°C.
-

Determining RNA Quality and Quantity

Introduction

Once you have isolated the total RNA, determine the quantity and quality of the purified RNA as described in this section.

RNA Yield

Analyze the yield of purified total RNA by checking the UV absorbance at 260 nm or by using the Quant-iT™ RNA Assay Kits.

UV Absorbance

1. Dilute an aliquot of the total RNA sample in 10 mM Tris-HCl, pH 7.0. Mix well.

Note: The RNA must be in a neutral pH buffer to accurately measure the UV absorbance.

2. Determine the OD₂₆₀ of the sample using a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.0.
3. Calculate the amount of total RNA using the formula:

$$\text{Total RNA } (\mu\text{g}) = \text{OD}_{260} \times 40 \mu\text{g} / (1 \text{ OD}_{260} \times 1 \text{ ml}) \times \text{dilution factor} \times \text{total sample volume (ml)}$$

Quant-iT™ RNA Assay Kits

The Quant-iT™ RNA Assay Kit (see page viii) provides a rapid, sensitive, and specific method for RNA quantitation with minimal interference from DNA, protein, or other common contaminants that affect UV absorbance readings.

The kit contains a state-of-the-art quantitation reagent, pre-diluted standards for standard curve, and a ready-to-use buffer. The assay is performed in a microtiter plate format and is designed for reading in standard fluorescent microplate readers.

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Determining RNA Quality and Quantity,

Continued

Analyzing RNA Quality

Typically, RNA isolated using the PureLink™ FFPE Total RNA Isolation Kit has an $OD_{260/280}$ of 1.5-2.0 when samples are diluted in Tris-HCl (pH 7.0). An $OD_{260/280}$ ratio of 1.5-2.0 indicates that RNA is reasonably clean of proteins and other contaminants for downstream applications (see page 2).

Agarose gel electrophoresis of RNA isolated using this kit **does NOT** usually show the typical 18S rRNA and 28S rRNA bands. This is a result of random cross-linking during sample fixation. The extracted RNA is suitable for downstream applications (see page 2). Gel analysis reveals if any contaminating DNA is present. Contaminating DNA is visible as a band at the well or as background smearing. Contaminating DNA is easily removed by treating the RNA samples with DNase I after eluting the RNA (page 11).

For quality assessment of the total RNA from FFPE tissue, we recommend performing RT-PCR followed by gel electrophoresis of the PCR products (see example on next page).

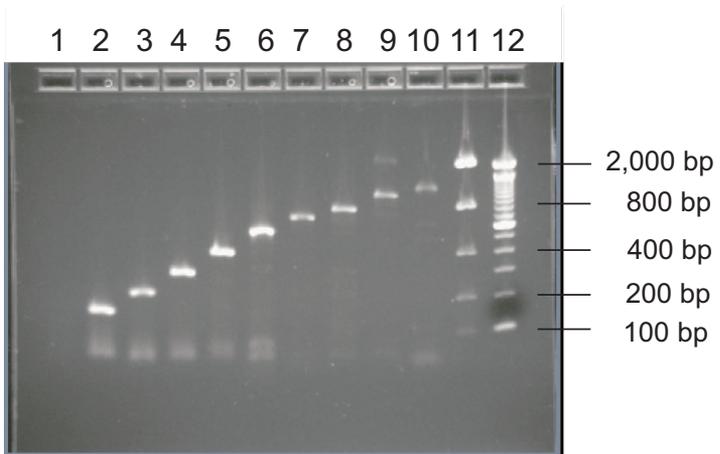
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Determining RNA Quality and Quantity, Continued

Example Results

The quality of total RNA obtained from FFPE mouse brain tissue using the PureLink™ FFPE Total RNA Isolation Kit was tested by RT-PCR using primers specific for size-defined PCR products to determine the amplifiable size of the purified RNA. Eight microliters of PCR product were loaded into each well of a 2% E-Gel® (see page viii for catalog number). The results are shown below.

Note: The actual length of RT-PCR products amplified from RNA purified using the PureLink™ FFPE Total RNA Isolation Kit varies depending on the fixation protocol used and age of the FFPE tissue.



Lane 1: No Reverse Transcriptase Negative Control

Lane 2: 169 bp PCR product

Lane 3: 231 bp PCR product

Lane 4: 311 bp PCR product

Lane 5: 416 bp PCR product

Lane 6: 563 bp PCR product

Lane 7: 683 bp PCR product

Lane 8: 782 bp PCR product

Lane 9: 986 bp PCR product

Lane 10: 1105 bp PCR product

Lane 11: E-Gel® Low Range Quantitative DNA Ladder

Lane 12: 100 bp DNA Ladder

Troubleshooting

Introduction

Review the information below to troubleshoot your experiments.

Problem	Cause	Solution
Low RNA yield	RNA contaminated with RNase	Follow the guidelines on page 4 to prevent RNase contamination.
	Less than the recommended amount of FFPE tissue used	Use the recommended amount of 3-8 pieces of 10 μm sections and make sure the sections contain tissue.
No RNA yield	No tissue in sections used for RNA extraction	Make sure that the sections used for RNA extraction contain tissue.
	Tissue was not submerged properly	Ensure the tissue section is submerged in Melting Buffer during deparaffinization and lysis.
No paraffin layer after melting and centrifugation	Heating block was not set at 72°C	Make sure to set your heating block at 72°C for melting the paraffin.
	Solution not centrifuged immediately	Make sure to centrifuge the sample IMMEDIATELY after melting.
Tissue pieces remain in solution after degradation step	No Proteinase K was added	Be sure to add Proteinase K for degradation at 60°C. Increase the time for Proteinase K digestion to up to 3 hours, if you notice tissue pieces.
No 18S and 28S rRNA bands after gel electrophoresis of RNA	RNA from purified FFPE samples usually does not show the 18S and 28S rRNA bands in the gel due to random cross-linking during fixation	If desired, use other procedures to evaluate the quality of your RNA such as RT-PCR followed by gel electrophoresis of the PCR products (see previous page) and proceed with downstream applications.
Genomic DNA contamination	--	Use optional DNase I digestion step after eluting RNA to remove genomic DNA contamination.
Low volume of eluted RNA	Centrifugation speed was too low	Make sure the centrifugation speed is 13,000 – 15,000 \times g.

Technical Support

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our web page (www.invitrogen.com).

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MSDS Requests

MSDSs (Material Safety Data Sheets) are available on our website at www.invitrogen.com/msds.

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**Limited Use
Label License
No: 283 FFPE
Technology**

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