ZYMO RESEARCH

RNA Purification Made Simple™

# *Quick*-RNA™ FFPE Kit

RNA from any sample

## Highlights

- Spin-column purification of total RNA (including small/microRNAs) from FFPE (formalin-fixed paraffin embedded) tissue sections.
- Proteinase K and DNase I enzymes are included for efficient lysis and DNA-free RNA.
- DNA & RNA can be eluted in one eluate or in two separate fractions, ready for Next-Gen Sequencing, RT/qPCR, etc.

Catalog Numbers:
R1008

Scan with your smart-phone camera to view the online protocol/video.

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# Table of Contents

# Product Contents

| *Quick*-RNA™ FFPE Kit | R1008 (50 prep) |
|---|---|
| Deparaffinization Solution | 20 ml |
| Proteinase K[1] (lyophilized) & Storage Buffer | 5 mg (x2) |
| 2X Digestion Buffer | 5 ml |
| RNA Lysis Buffer | 50 ml |
| RNA Prep Buffer | 25 ml |
| RNA Wash Buffer[2] (concentrate) | 24 ml |
| DNase/RNase-Free Water | 10 ml |
| DNase I[3] (lyophilized) | 250 U |
| DNA Digestion Buffer | 4 ml |
| Zymo-Spin™ IICR Columns | 50 |
| Collection Tubes | 50 |
| Instruction Manual | 1 |

**Storage Temperature** - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add **Proteinase K Storage Buffer** to the lyophilized **Proteinase K**, 5 mg, see Buffer Preparation, page 4. Store frozen aliquots.
2 Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate
3 Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:
**#E1009-A (250 U)**, add 275 µl **water**

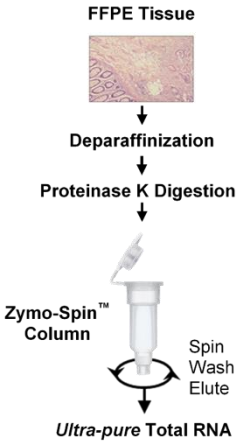
# Specifications

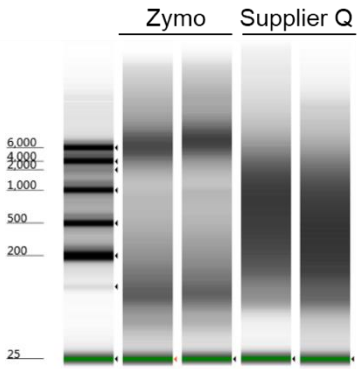
- **Sample Sources –** Up to 25 mg tissue from paraffin block or up to 4 tissue sections (≤ 20 µm thick) with a total surface area of ~ 20 $mm^2$. Recommended: Use 1-2 sections if performing the protocol for the first time.
- **Compatibility –** Fresh or frozen tissue specimens can also be processed.
- **Size –** Total RNA (≥ 17 nt).
- **Purity –** $A_{260}/A_{280}$ & $A_{260}/A_{230}$ > 1.8. RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.
- **Binding Capacity – Zymo-Spin™ IICR Column** yield up to 50 µg RNA.
- **Elution Volume –** ≥ 25 µl **DNase/RNase-Free Water.**
- **Equipment Needed** (user provided) **–** Microcentrifuge, vortex, heat block, water bath or incubator.

# Product Description

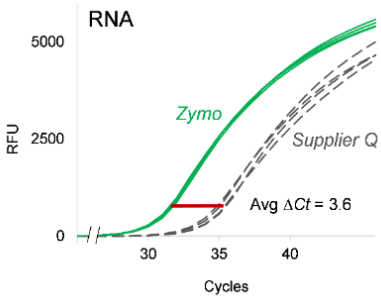
The **Quick-RNA™ FFPE Kit** provides a simple and reliable method for total RNA isolation from formalin-fixed paraffin embedded (FFPE) tissue samples. The unique chemistries of the product have been optimized for maximum recovery of total RNA including small/micro RNA species.

Simply deparaffinize tissues using **Deparaffinization Solution**, digest using **Proteinase K**, heat to reverse chemical crosslinks, and then purify using **Zymo-Spin™ Column** technology. The result is high-quality RNA (> 17 nt) that is DNA-free and is ready for RT/qPCR, hybridization, sequencing, etc.




RNA isolated with the ***Quick*-RNA™ FFPE Kit** is higher quality (left); compared to Supplier Q procedures (right). Quality assessed by Agilent 2200 TapeStation.




RNA isolated using the ***Quick*-RNA™ FFPE Kit** is high quality and consistently outperforms RNA isolated using Supplier Q procedures (Avg Δ Ct = 3.6) as depicted by the RT/qPCR amplification curves (n=4).

# Protocol

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation and (III) Total RNA Purification

## (I) Buffer Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.
- ✓ Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:
  **#E1009-A (250 U)**, add 275 µl **water**
- ✓ Add 260 µl **Proteinase K Storage Buffer** to the lyophilized **Proteinase K**, 5 mg. Vortex to dissolve and store frozen aliquots.

## (II) Sample Preparation

- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

### Deparaffinization[1,2]

1. Remove (trim) excess paraffin wax from sample[3] and transfer into a nuclease-free tube (not provided).

2. Add 400 µl of **Deparaffinization Solution** to the sample. Incubate at 55°C for 1 minute. Vortex briefly.

3. Remove **Deparaffinization Solution** from the sample and proceed to Tissue Digestion, below.

### Tissue Digestion

1. To the deparaffinized tissue sample (≤ 25 mg), add the following mixture:

| | |
|---|---|
| **DNase/RNase-Free Water** | 95 µl |
| **2X Digestion Buffer** | 95 µl |
| **Proteinase K** | 10 µl |

2. Incubate at 55°C for 1 hour (microdissection) or up to 4 hours (tissue block).

3. After digestion, transfer the tube (e.g., heat-block) and incubate at 65°C for 15 minutes to de-crosslink the sample.

4. Proceed to Total RNA Purification, page 6.

1 If using fresh or frozen tissue specimens, proceed directly to Tissue Digestion.
2 Alternatively, xylene may also be used for deparaffinization. See page 7.
3 Up to 25 mg tissue from paraffin block or up to 4 tissue sections (≤ 20 µm thick) with a total surface area of ~20 $mm^2$. Recommended: Use 1-2 sections if performing the protocol for the first time.

## (III) Total RNA Purification

✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Add 600 µl **RNA Lysis Buffer** to the tissue and mix thoroughly. Centrifuge at max speed for 1 minute to remove insoluble debris and then transfer the supernatant into a nuclease-free tube (not provided).

2. Add 1 volume ethanol (95-100%) to the supernatant (1:1) and mix well.

   Example: Add 600 µl ethanol to 600 µl supernatant.

3. Then transfer the mixture into a **Zymo-Spin™ IICR Column**[1] in a **Collection Tube** and centrifuge[2]. Discard the flow-through.

   Recommended: At this point, DNase I Treatment can be performed, see page 8.

4. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.

5. Add 700 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.

6. Add 400 µl **RNA Wash Buffer** and centrifuge the column for 1 minute to ensure complete removal of the wash buffer. Then carefully, transfer the column into a nuclease-free tube (not provided).

7. Add 50 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

   Alternatively, for highly concentrated RNA use ≥ 25 µl elution.

   The eluted RNA can be used immediately or stored frozen.

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1 To process samples > 700 µl, columns may be reloaded.
2 At this point, proteins can be purified from the flow-through. See page 8.

# Appendices

## Xylene Deparaffinization

✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

### Slide Tissue Sections Only (rapid deparaffinization)

1. Remove (trim) excess paraffin wax from sample and transfer the sample to a nuclease-free tube (not provided).
2. Add 1 ml xylene (not provided) to the sample. Vortex vigorously for 30 seconds and then centrifuge for 1 minute. Remove and discard the xylene.
3. Wash sample with 1 ml ethanol (95-100%). Vortex vigorously for 30 seconds then centrifuge. Remove and discard ethanol. Repeat this step.
4. Dry the sample using vacuum centrifugation (e.g., SpeedVac) or by heating uncapped tubes at 37° C for up to 40 minutes.
5. Continue to Tissue Digestion, page 5.

### Tissue Samples and Slide Tissue Sections (standard deparaffinization)

1. Remove (trim) excess paraffin wax from sample and transfer the sample to a nuclease-free tube (not provided).
2. Add 1 ml xylene (not provided) to the sample. Vortex and incubate at room temperature for 1 hour with gentle rocking. Centrifuge, discard supernatant. Repeat this step.
3. Wash with 1 ml ethanol (100%) for 5 minutes with gentle rocking. Centrifuge and discard supernatant. Repeat this step.
4. Wash with 1 ml ethanol (95%) for 5 minutes with gentle rocking. Centrifuge and discard supernatant. Repeat this step.
5. Wash with 1 ml ethanol (75%) for 5 minutes with gentle rocking. Centrifuge and discard supernatant. Repeat this step.
6. Wash with 1 ml dd$H_2O$ for 5 minutes with gentle rocking. Remove the water from the sample as much as possible.
7. Continue to Tissue Digestion, page 5.

# Appendices

## DNase I Treatment (in-column)

✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Following RNA binding step (page 6, step 3), add 400 µl **RNA Wash Buffer** to the column, centrifuge and discard the flow-through.
2. For each sample to be treated, prepare **DNase I Reaction Mix** (see table below) in an RNase-free tube (not provided) and mix by gentle inversion. Then add 80 µl directly into column matrix and incubate at room temperature (20-30°C) for 15 minutes. Proceed with the purification (page 6, step 4).

| **DNase I Reaction Mix** | |
|---|---|
| **DNase I** (reconstituted; 1 U/ul)[1] | 5 µl |
| **DNA Digestion Buffer** | 75 µl |

## Protein Purification: Acetone Precipitation of Proteins

✓ After the RNA binding to the column (page 6, step 3), the protein content in the flow-through can be purified:

1. Add 4 volumes of cold acetone (-20°C) to the flow-through and mix.
2. Incubate the samples for 30 minutes on ice.
3. Centrifuge at max speed for 10 minutes. Discard the supernatant. Keep the pellet.
4. Add 400 µl ethanol (95-100%) to the protein pellet. Centrifuge at max speed for 1 minute. Discard the supernatant.
5. Air-dry the protein pellet for 10 minutes at room temperature.
6. Resuspend and vortex the pellet in a buffer appropriate for downstream application (e.g., SDS-PAGE sample loading buffer).

1 Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 $A_{260}$ units/ml of reaction mixture at 25°C.

# Ordering Information

| Product Description | Catalog No. | Size |
|---|---|---|
| **Quick-RNA™ FFPE Kit** | R1008 | 50 preps. |

| Individual Kit Components | Catalog No. | Amount |
|---|---|---|
| RNA Lysis Buffer | R1060-1-50<br>R1060-1-100 | 50 ml<br>100 ml |
| RNA Prep Buffer | R1060-2-25<br>R1060-2-100 | 25 ml<br>100 ml |
| RNA Wash Buffer (concentrate) | R1003-3-24<br>R1003-3-48 | 24 ml<br>48 ml |
| DNase/RNase-Free Water | W1001-6<br>W1001-30 | 6 ml<br>30 ml |
| DNase I Set (lyophilized)<br>(250 U supplied with DNA Digestion Buffer, 4 ml) | E1010 | 1 set |
| Deparaffinization Solution | D3067-1-20 | 20 ml |
| Proteinase K (lyophilized) & Storage Buffer | D3001-2-5<br>D3001-2-20 | 5 mg<br>20 mg |
| 2X Digestion Buffer | D3050-1-5<br>D3050-1-20 | 5 ml<br>20 ml |
| Zymo-Spin™ IICR Columns | C1078-50 | 50 |
| Collection Tubes | C1001-50 | 50 |

# Complete Your Workflow

✓ For tough-to-lyse samples, use ZR BashingBead Lysis Tubes:

| **ZR BashingBead Lysis Tubes** | |
|---|---|
| 2.0 mm beads #S6003 | Plant/animal tissue |
| 0.1 + 0.5 mm beads #S6012 | Microbes |
| 0.1 + 2.0 mm beads #S6014 | Microbes in tissue/insects |

✓ For isolation of DNA/RNA from any sample:

| **Quick-RNA Plus kits** | |
|---|---|
| Miniprep Plus #R1057 | For cells, tissue, biological liquids |
| MagBeads #R2130 | Automatable (Tecan, Hamilton, Kingfisher, etc.) |

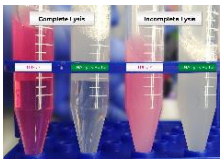
✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

| **RNA Clean & Concentrator kits** | |
|---|---|
| Microprep #R1013-R1014 | DNase I Set included |
| MagBeads #R1082 | Automatable (Tecan, Hamilton, Kingfisher, etc.) |

✓ For NGS:

| **Zymo-Seq RiboFree Total RNA Library Prep kit** | |
|---|---|
| #R3000 | 12 preps |
| #R3003 | 96 preps |

# Troubleshooting Guide

| Problem | Possible Causes and Suggested Solutions |
|---|---|
| **Precipitation, viscous lysate** | Incomplete lysis and/or high-mass input:<br>- If precipitation occurs (upon adding ethanol to the lysate) or if the lysate is extremely viscous, increase the volume of RNA Lysis Buffer to ensure complete lysis and homogenization until lysate is transparent (see image).<br>  |
| **Low purity** ($A_{260}/A_{230}$ nm, $A_{260}/A_{280}$ nm) | Sample handling:<br>- Ethanol and/or salt contamination. After centrifugation steps, carefully remove the column from the collection tube to prevent buffer carryover. Alternatively, blot emptied collection tube with a tissue or towel.<br>- Make sure lysate and wash buffers have passed completely through the matrix of the column. This may require centrifuging at a higher speed and/or longer time.<br>Incomplete lysis and/or cellular debris:<br>- Increase the volume of RNA Lysis Buffer (proportionally) to ensure complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate. |
| **Low yield** | Sample input:<br>- Too much input or incomplete lysis/homogenization can cause cellular debris to clog or overload the column and result in compromised nucleic acid recovery. Use less input material and/or increase the volume of RNA Lysis Buffer.<br>High-protein content:<br>- Perform Proteinase K treatment to the sample prior to purification. See appropriate sample preparation protocol. |
| **DNA contamination** | To remove DNA:<br>- Perform in-column DNase I treatment or perform DNase I treatment post-purification, then re-purify the treated sample.<br>- For future preps, increase the volume of RNA Lysis Buffer to ensure complete lysis and homogenization of the sample. |
| **RNA degradation** | To prevent RNA degradation:<br>- Immediately collect and lyse fresh sample RNA Lysis Buffer ensure stability. Homogenized samples can be stored frozen for later processing. |
| **2X Digestion Buffer** | If there is visible precipitation, incubate reagent at 37°C until solubilized. |

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

# Notes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