ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-RNA™ FFPE Kit

Catalog Nos. **R1008**

Highlights

- High performance sample prep technology for high quality total RNA (including small/micro RNAs) from FFPE tissue samples and sections.
- *DNA-free* RNA is ready for use in any downstream application. *DNase I included.*

Contents

ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please contact us.

Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

For assistance, contact us at tech@zymoresearch.com.

Product Contents

***Quick-RNA*™ FFPE Kit** (Kit Size)	**R1008** (50 Preps.)
Deparaffinization Solution	20 ml
Proteinase K[1] & Storage Buffer	2 x 5 mg
2X Digestion Buffer[2]	5 ml
RNA Lysis Buffer	50 ml
RNA Prep Buffer	25 ml
RNA Wash Buffer[3] (concentrate)	24 ml
DNase/RNase-Free Water	10 ml
DNase I[4] (lyophilized)	1
DNA Digestion Buffer	4 ml
Zymo-Spin™ IIC Columns	50
Collection Tubes	50
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Storage Temperature - Store all kit components (*i.e.,* buffers, columns) at room temperature.

[1] Prior to use, reconstitute each lyophilized **Proteinase K** with 260 µl **Proteinase K Storage Buffer**. Vortex to dissolve. Store at -20°C.

[2] The **2X Digestion Buffer** may have formed a precipitate. If this is the case, incubate at 37°C to solubilize.

[3] Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.

[4] Prior to use, reconstitute the lyophilized **DNase I** with 275 µl **DNase/RNase-Free Water**. Mix by gentle inversion. Store aliquots at -20°C.

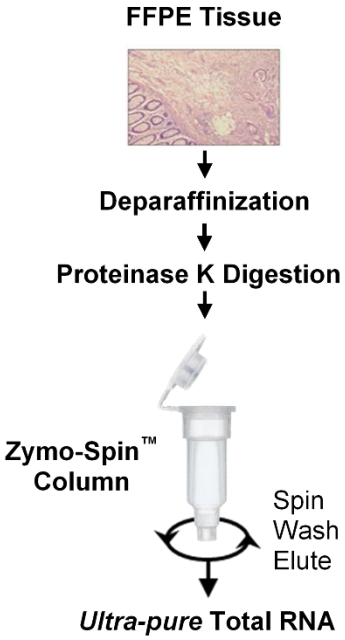
Specifications

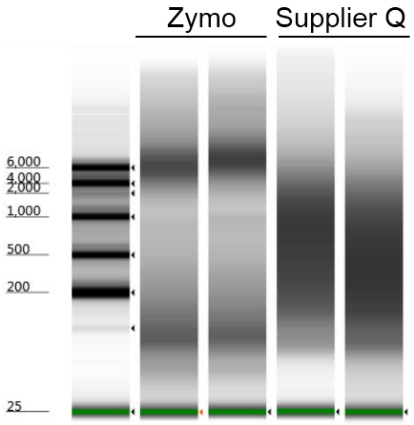
- **Sample Sources** – Up to 25 mg tissue from paraffin block or up to four (4) tissue sections (≤20 µm thick) with a total surface area ~20 cm^2. It is recommended to use 1-2 sections if performing the protocol for the first time. Compatible with fresh/frozen tissue specimens.
- **RNA Size** – RNAs ≥17 nucleotides.
- **RNA Purity** - A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8. DNase I provided for complete removal of DNA.
- **RNA Recovery** – The RNA binding capacity of the **Zymo-Spin™ IIC Column** is ~50 µg.
- **RNA Storage** - RNA eluted with **DNase/RNase-Free Water** (provided) can be stored at ≤70ºC. The addition of RNase inhibitors in highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge, vortex, and heat block.

Product Description

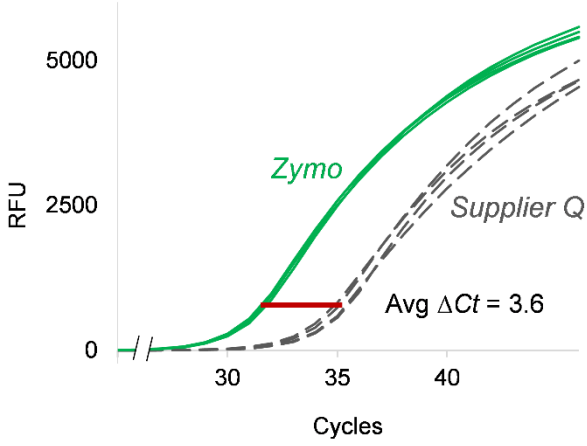
The ***Quick*-RNA™ FFPE Kit** provides a simple and reliable method for RNA isolation from formalin-fixed, paraffin embedded (FFPE) tissue samples. The unique chemistries of the product have been optimized for maximum recovery of both large and small RNA species. Simply deparaffinize tissues using **Deparaffinization Solution**, digest using **Proteinase K**, heat to reverse chemical crosslinks, and then purify using *Zymo-Spin*® column technology. The result is high-quality total RNA (*including small RNAs 17-200 nt*) that is *DNA-free* and is ready for RT-PCR, hybridization, sequencing, *etc*.

For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.





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 ΔCt = 3.6) as depicted by the RT-PCR amplification curves (n=4).

Notes:

Ensure the RNA isolation procedure is performed in an RNase-free environment.

The lyophilized **Proteinase K** and **DNase I** are stable as shipped.

Buffer Preparation

- ✓ Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.
- ✓ Add 275 µl **DNase/RNase-Free Water** per vial to reconstitute the lyophilized **DNase I** at 1 U/µl. Mix by gentle inversion. Store frozen aliquots at -20°C.
- ✓ Prior to use, reconstitute each lyophilized **Proteinase K** with 260 µl **Proteinase K Storage Buffer**. Vortex to dissolve. Store at -20°C.

Protocol

Deparaffinization

Note: If using fresh/frozen tissue specimens proceed directly with **Tissue Digestion** below.

1. Remove (trim) excess paraffin wax from sample and transfer the sample to an RNase-free tube (not provided).

   Note: *Up to 25 mg tissue from paraffin block or up to four (4) tissue section (≤20 µm thick) with a total surface area ~20 cm². It is recommend to use 1-2 sections if performing the protocol for the first time.*

Note: Xylene may also be used for deparaffinization. See the Appendix on page 5 for instructions.

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 next section.

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.

RNA Isolation

All centrifugation steps should be performed at 10,000 – 16,000 *x g* for 30 seconds unless specified.
All steps should be performed at room temperature (20-30ºC) unless specified.

1. Add 600 µl of **RNA Lysis Buffer** to the tube and mix thoroughly. Centrifuge at max speed for 1 minute to remove insoluble debris and then transfer the supernatant to an RNase-free tube (not provided).
2. Add 1 volume[1] ethanol (95-100%) to the sample and mix well.
3. Transfer the mixture into a **Zymo-Spin™ IIC Column**[2] in a **Collection Tube** and centrifuge. Discard the flow-through.

   Recommended: **DNase I** treatment (in-column)[3]:

   (D1) Wash the column with 400 µl **RNA Wash Buffer** and centrifuge. Discard the flow-through.
   (D2) In an RNase-free tube, add 5 µl **DNase I** (1 U/µl)*, 75 µl **DNA Digestion Buffer** and mix by inversion. Add the mix directly to the column matrix.
   (D3) Incubate the column at room temperature (20-30ºC) for 15 minutes. Proceed to step 4.

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 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not provided).
7. Add 50 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge to elute RNA.

   Alternatively, for high concentrated RNA use ≥25 elution.

   The eluted RNA can be used immediately or stored at ≤-70ºC.

Notes:

[1] For small RNA recovery, use 2 volumes of ethanol (95-100%)

[2] To process samples > 700 µl, **Zymo-Spin™** columns may be reloaded.

[3] Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial. Store frozen aliquots.

** Unit definition - one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A260 units/min/ml of reaction mixture at 25°C.*

Appendix: Xylene Deparaffinization

Rapid Deparaffinization (Slide Tissue Sections Only)

1. Remove (trim) excess paraffin wax from sample and transfer the sample to a 1.5 ml microcentrifuge tube.
2. Add 1 ml xylene (*not provided*) to the sample. Vortex vigorously for 30 seconds and then centrifuge sample at 10,000 x *g* (~10,000 rpm) for 1 minute. Remove and discard the xylene.
3. Wash sample with 1 ml ethanol (95-100%). Vortex vigorously for 30 seconds then centrifuge samples at 10,000 x *g* for 1 minute. Remove and discard ethanol. Repeat this step.
4. Dry the sample using vacuum centrifugation (e.g., SpeedVac or similar) or by heating uncapped tubes at ~37° C for up to 40 minutes.
5. The sample is now ready for **Tissue Digestion** (see page 3).

Standard Deparaffinization (Tissue Samples and Slide Tissue Sections)

1. Remove (trim) excess paraffin wax from sample and transfer the sample to a 1.5 ml microcentrifuge tube.
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, and repeat this step.

   Note: *Centrifuge at 10,000 x g for 1 minute and remove/discard supernatant after washing for the following steps.*
3. Wash twice with 1 ml ethanol (100%) for 5 minutes with gentle rocking.
4. Wash twice with 1 ml ethanol (95%) for 5 minutes with gentle rocking.
5. Wash twice with 1 ml ethanol (75%) for 5 minutes with gentle rocking.
6. Wash once with 1 ml ddIH_2O for 5 minutes with gentle rocking. Remove as much water from the sample as possible.
7. The sample is now ready for **Tissue Digestion** (see page 3).

Ordering Information

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

For Individual Sale	Catalog No.	Amount
Deparaffinization Solution	D3067-1-20	20 ml
Proteinase K (lyophilized) (supplied with Proteinase K Storage Buffer)	D3001-2-5 D3001-2-20	5 mg set 20 mg set
2X Digestion Buffer	D3050-1-5 D3050-1-20	5 ml 20 ml
RNA Lysis Buffer	R1060-1-50 R1060-1-100	50 ml 100 ml
RNA Prep Buffer	R1060-2-10 R1060-2-25 R1060-2-100	10 ml 25 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48	6 ml 12 ml 24 ml 48 ml
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10 W1001-30	1 ml 4 ml 6 ml 10 ml 30 ml
DNase I Set (lyophilized) DNase I (250 U) & DNA Digestion Buffer (4 ml)	E1010	1 set
Zymo-Spin™ IIC Columns	C1011-50 C1011-250	50 250
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1,000

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