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The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-DNA/RNA™ FFPE Kit

Catalog No. **R1009**

Highlights

- High performance sample prep technology for total nucleic acid (DNA & RNA) recovery from the same FFPE tissue sample/section.
- High quality DNA and *DNA-free* RNA is ready for use in any downstream application. *DNase I included.*

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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-DNA/RNA™ FFPE Kit (Kit Size)	R1009 (50 Preps.)
Deparaffinization Solution	20 ml
Proteinase K¹ & Storage Buffer	2x 5 mg
2X Digestion Buffer²	5 ml
DNA/RNA Lysis Buffer	50 ml
DNA/RNA Prep Buffer	50 ml
DNA/RNA Wash Buffer³ (concentrate)	24 ml
DNase/RNase-Free Water	30 ml
DNase I⁴ (lyophilized)	1
DNA Digestion Buffer	4 ml
Zymo-Spin™ IIC Columns	100
Collection Tubes	150
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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 **DNA/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 cm². It is recommended to use 1-2 sections if performing the protocol for the first time. Compatible with fresh/frozen tissue specimens.
- **Size Limits** – Capable of recovering DNA and total RNA ≥ 17 nucleotides.
- **Purity** – High quality DNA and RNA ($A_{260}/A_{280} > 1.8$, $A_{260}/A_{230} > 1.8$) are recovered.
- **Recovery** – The DNA/RNA binding capacity of the **Zymo-Spin™ IIC Column** is ~ 50 µg.
- **Storage** – DNA and RNA eluted with **DNase/RNase-Free Water** can be stored at $\leq 70^\circ\text{C}$. The addition of RNase inhibitors is highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge, vortex, and heat block.

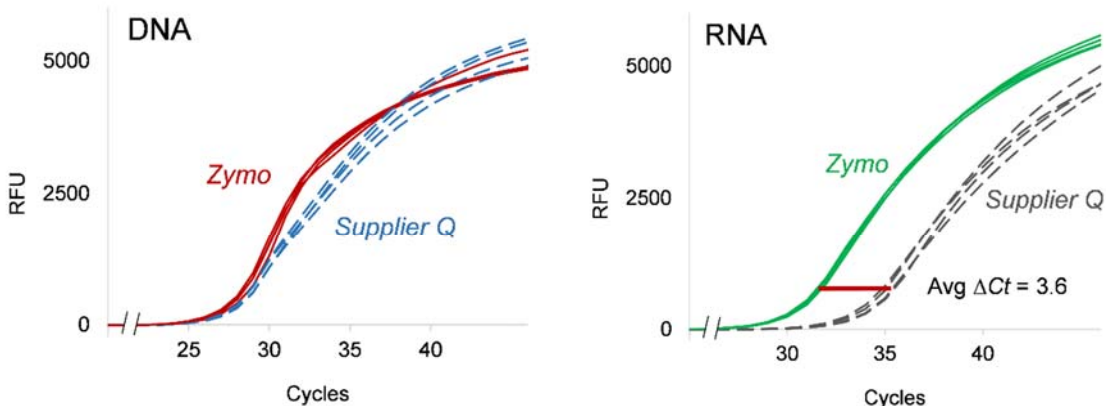
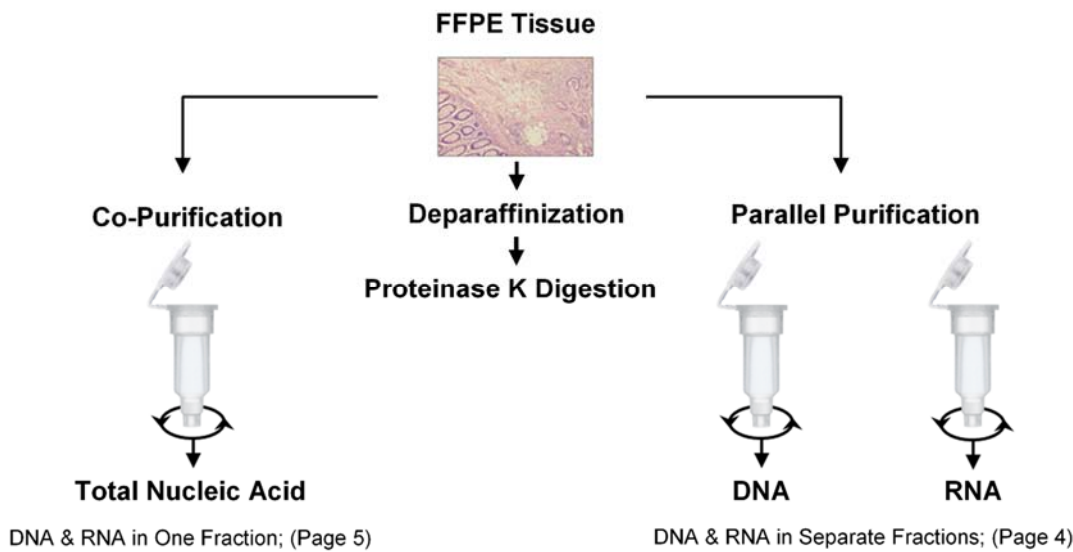
™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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Product Description

The **Quick-DNA/RNA™ FFPE Kit** provides a simple and reliable method for total nucleic acid isolation (DNA & RNA) from the same formalin-fixed, paraffin embedded (FFPE) tissue sample. The unique chemistries of the product have been optimized for maximum recovery of DNA and 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 DNA & 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.



DNA & RNA isolated using the **Quick-DNA/RNA™ FFPE Kit** are high quality and consistently outperforms RNA isolated using Supplier Q procedures (Avg ΔC_t = 3.6) as depicted by the RT-PCR amplification curves (n=4).

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Notes:

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

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

Note: Xylene may also be used for deparaffinization. See the Appendix on page 6 for instructions.

Buffer Preparation

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

Protocol

The isolation consists of three steps: (I) Deparaffinization, (II) Tissue Digestion, and (III) Parallel Purification or Co-Purification.

Deparaffinization

1. Remove (trim) excess paraffin wax from sample and transfer the sample to a new microcentrifuge tube (not provided).

Note: Up to 25 mg tissue from paraffin block or up to four (4) tissue section ($\leq 20 \mu$ m thick) with a total surface area $\sim 20 \text{ cm}^2$. It is recommend to use 1-2 sections if performing the protocol for the first time.

2. Add 400 μ l 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 ($\leq 25 \text{ mg}$), add the following mixture:

DNase/RNase-Free Water	95 μ l
2X Digestion Buffer	95 μ l
Proteinase K	10 μ l

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 94°C for 20 minutes to de-crosslink the sample.
4. Proceed to DNA & RNA Parallel Purification (Page 4) for isolation into two separate fractions or DNA & RNA Co-Purification (Page 5) for isolation into one fraction.

DNA & RNA Parallel Purification

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 (20-30°C) unless specified.

1. Add 600 µl of **DNA/RNA Lysis Buffer** to the deparaffinized and digested tissue and mix thoroughly. Centrifuge at max speed for 1 minute to remove insoluble debris.
2. Transfer the supernatant into a **Zymo-Spin™ IIC Column** in a **Collection Tube** and centrifuge. **Save the flow-through.**

DNA Purification

(DNA is bound to the column)

3. Transfer the **Zymo-Spin™ IIC Column** into a new **Collection Tube**.

RNA Purification

(RNA is in the flow-through)

3. Add 1 volume¹ ethanol (95-100%) to the flow-through in the **Collection Tube** from Step 2 and mix well by pipetting. Then transfer the sample into a new **Zymo-Spin™ IIC Column**² in a **Collection Tube** and centrifuge. Discard the flow-through.³

4. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
5. Add 700 µl **DNA/RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
6. Add 200 µl **DNA/RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into a new microcentrifuge tube.
7. Add 50 µl **DNase/RNase-Free Water** directly to the column matrix and let stand for 1 minute, then centrifuge to elute DNA & RNA respectively.

Alternatively, for highly concentrated DNA & RNA use ≥25 elution.

The eluted DNA & RNA can be used immediately or stored at ≤-70°C.

In-Column DNase I Treatment⁴

1. Wash the column with 400 µl **DNA/RNA Wash Buffer** and centrifuge. Discard the flow-through.
2. In a microcentrifuge 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.
3. Incubate the column at room temperature (20-30°C) for 15 minutes. Proceed to step 4 above.

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 At this point, RNA samples can be in-column DNase I treated (below).

⁴ 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.*

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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.

DNA & RNA Co-Purification

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 (20-30°C) unless specified.

1. Add 600 µl of **DNA/RNA Lysis Buffer** to the deparaffinized and digested tissue and mix thoroughly. Centrifuge at max speed for 1 minute to remove insoluble debris and then transfer the supernatant to a new microcentrifuge tube (not provided).
2. Add 1 volume¹ 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.
4. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
5. Add 700 µl **DNA/RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
6. Add 200 µl **DNA/RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into a new microcentrifuge tube (not provided).
7. Add 50 µl **DNase/RNase-Free Water** directly to the column matrix, let stand for 1 minute and centrifuge to elute DNA & RNA into one fraction.

Alternatively, for highly concentrated DNA/RNA use ≥25 elution.

The eluted DNA/RNA can be used immediately or stored at ≤-70°C.

Appendices

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 ddH₂O 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-DNA/RNA™ FFPE Kit	R1009	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
DNA/RNA Lysis Buffer	D7001-1-50	50 ml
DNA/RNA Prep Buffer	D7010-2-10 D7010-2-25 D7010-2-50	10 ml 25 ml 50 ml
DNA/RNA Wash Buffer (concentrate)	D7010-3-6 D7010-3-12 D7010-3-24	6 ml 12 ml 24 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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