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The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-DNA™ FFPE Kit

Catalog Nos. **D3067**

Highlights

- Streamlined purification of high-quality FFPE tissue DNA that is ideal for PCR, Next-Gen library prep, enzymatic manipulations, etc.
- Size selection technology; recover total DNA >50 bp or >500 bp.

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Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

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™ FFPE Kit (Kit Size)	D3067 (50 Preps.)	Storage Temperature
Deparaffinization Solution	20 ml	Room Temp.
Proteinase K & Storage Buffer¹	2 x 5 mg	-20°C (after mixing)
2X Digestion Buffer	5 ml	Room Temp.
Genomic Lysis Buffer²	50 ml	Room Temp.
Genomic DNA Wash 1	25 ml	Room Temp.
Genomic DNA Wash 2³ (concentrate)	12 ml	Room Temp.
DNA Elution Buffer	10 ml	Room Temp.
RNase A⁴	2 mg	4°C
Zymo-Spin™ IIC Columns	50	Room Temp.
Collection Tubes	100	Room Temp.
Instruction Manual	1	-

¹ The Proteinase K is stable as shipped. Add 260 µl **Proteinase K Storage Buffer** to each **Proteinase K** tube prior to use. The final concentration of **Proteinase K** after the addition of **Proteinase K Storage Buffer** is ~20 mg/ml. Store at -20° C.

² *Recommended:* Add beta-mercaptoethanol to 0.5%(v/v) i.e., 250 µl per 50 ml or 500 µl per 100 ml.

³ Before starting, add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **Genomic DNA Wash 2** concentrate.

⁴ Re-suspend lyophilized RNase A in 300 µl of ddH₂O. Store at 4° C.

Specifications

- **Sample Size** – 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.
- **DNA Recovery** – High quality total DNA ($A_{260}/A_{280} > 1.8$) can be eluted into small volumes (i.e., ≥25 µl) allowing for highly concentrated samples. The maximum DNA binding capacity of the provided spin column is ~25 µg.
- **Processing Time** – As little as 4 hours when processing large amounts of tissue. For maximum yields of the highest quality DNA, it is recommended to process samples overnight.
- **Equipment/Reagents** – Microcentrifuge, thermomixer or heat block/bath capable of 55°C and 90°C, isopropanol, beta-mercaptoethanol (optional).

™ 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™ FFPE Kit** provides a simple and reliable method for high yield/quality DNA isolation from formalin-fixed, paraffin embedded (FFPE) tissue samples and sections. The unique chemistries of the product have been optimized for maximum recovery of non-crosslinked, ultra-pure DNA without RNA contamination. Simply digest deparaffinized tissues using the provided **Proteinase K**, heat, and then purify the DNA with the *Fast-Spin* columns in the kit. DNA >50 bp or >500 bp can be *selectively* isolated by altering the lysis buffer conditions as given in the protocol. PCR inhibitors are effectively removed during the isolation procedure, and eluted DNA is ideal for PCR, Next-Gen library prep, enzymatic manipulation, etc. Shown below is a schematic and performance overview of the procedure.

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

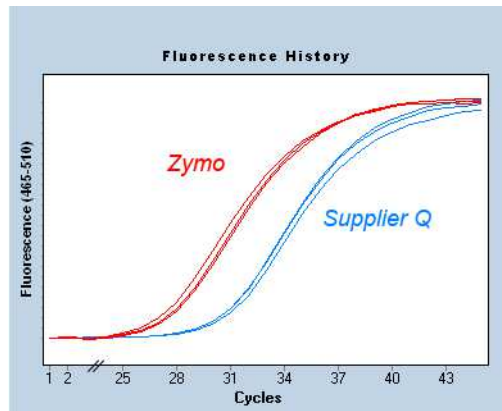
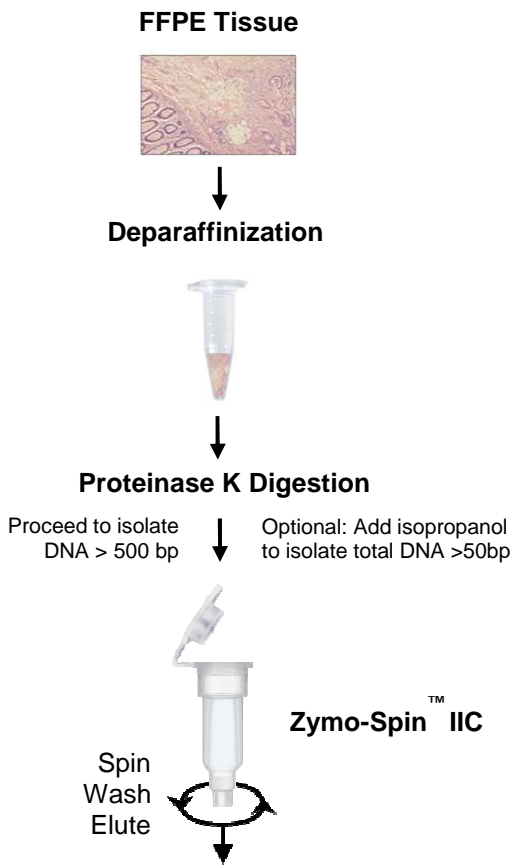


Figure 1. Equivalent amounts of DNA isolated using Zymo and Supplier Q procedures were used for real time PCR analysis. DNA isolated using the **Quick-DNA™ FFPE Kit** consistently yielded lower Ct values as depicted by the amplification curves above.

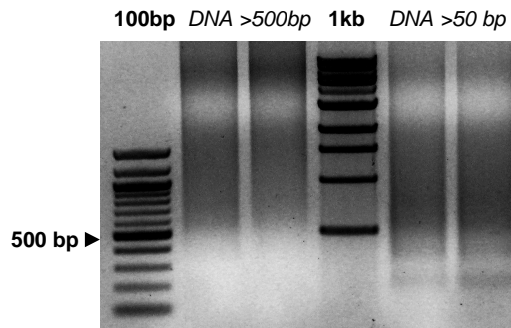


Figure 2. Equivalent amounts of DNA resolved in a 1% agarose/TAE/EtBr gel show binding conditions may be adjusted with the **Quick-DNA™ FFPE Kit** to selectively isolate DNA >50 bp or >500 bp. 100 bp DNA ladder and 1 kb DNA ladder from Zymo Research.

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Note: If using fresh/frozen tissue specimens proceed directly with **Proteinase K Digestion & DNA Isolation** (pg. 4)

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

Buffer Preparation

- ✓ Add 260 µl **Proteinase K Storage Buffer** to reconstitute lyophilized **Proteinase K** at 20 mg/ml. Vortex to dissolve. Store at -20° C.
- ✓ Before starting, add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **Genomic DNA Wash 2** concentrate.
- ✓ Resuspend lyophilized **RNase A** in 300 µl of ddH₂O. Store at 4° C.
- ✓ *Recommended:* Add beta-mercaptoethanol (user supplied) to the **Genomic Lysis Buffer** to a final dilution of 0.5%(v/v) i.e., 250 µl per 50 ml.

Protocol

Deparaffinization

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

Note: Up to 25 mg tissue from a paraffin block or up to four (4) tissue sections (≤20 µm thick) with a total surface area ~20 cm². It is recommended to use start with 1-2 sections.

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) in a microcentrifuge tube, add the following mixture:

H ₂ O	45µl
2X Digestion Buffer	45µl
Proteinase K	10µl

Note: If the tissue sample is too large for the digestion volume, scale up the digestion to 200 µl while keeping the amount of Proteinase K the same. Double the reagent volumes indicated in Step 1 & 2 of the DNA Purification Protocol (Page 4).

2.	<i>Rapid Digestion</i>	<i>Standard Digestion</i>
	Incubate at 55°C for 1-4 hours	Incubate at 55°C overnight (12-16 hrs)

Note: The *Rapid Digestion* is recommended for processing slide tissue sections. The *Standard Digestion* ensures maximum yields of DNA from tough-to-lyse (collagen-rich, fibrous, etc.) or large tissue samples.

3. Transfer the digestion to 94°C and incubate for 20 minutes. Once done, add 5 µl of **RNase A**, mix, and incubate an additional 5 minutes at room temperature.

DNA Purification

1. Add 350 μ l of **Genomic Lysis Buffer** to the tube and mix thoroughly by vortexing.
2. Add 135 μ l of isopropanol¹ (user supplied) to the sample and mix thoroughly. Centrifuge at $\geq 12,000 \times g$ for 1 minute to remove insoluble debris.
3. Transfer the supernatant to a **Zymo-Spin™ IIC Column**² in a **Collection Tube**. Centrifuge at $10,000 \times g$ for 1 minute.
4. Add 400 μ l of **Genomic DNA Wash 1** to the spin column in a new **Collection Tube**. Centrifuge at $10,000 \times g$ for 1 minute. Discard the flow-through.
5. Add 700 μ l of **Genomic DNA Wash 2** to the spin column. Centrifuge at $\geq 12,000 \times g$ for 1 minute. Discard the flow-through.
6. Add 200 μ l of **Genomic DNA Wash 2** to the spin column. Centrifuge at $\geq 12,000 \times g$ for 1 minute.
7. Transfer the **Zymo-Spin™ IIC Column** to a clean microcentrifuge tube. Add $\geq 50 \mu$ l **DNA Elution Buffer**³ or water (add $\geq 100 \mu$ l if sampling 25 mg tissue) to the spin column. Incubate 2-5 minutes at room temperature.
8. Centrifuge at top speed for 30 seconds to elute the DNA.

The eluted DNA can be used immediately for molecular based applications or stored $\leq -20^{\circ}\text{C}$ for future use.

¹ ssDNA will also be purified if present in the sample upon the addition of isopropanol.

This procedure will isolate total DNA > 50 bp. To isolate only DNA > 500 bp, skip *Step 2*.

FFPE DNA may be highly degraded and DNA > 500 bp may not be present in sample.

² The maximum loading volume for the **Zymo-Spin™ Column** is $\sim 700 \mu$ l.

³ Elution of DNA from the column is dependent on pH and temperature. If water is used, ensure the pH is > 6.0 . Also, the total yield may be improved by eluting the DNA with Elution Buffer or water pre-equilibrated to $60-70^{\circ}\text{C}$ or by performing and pooling sequential elutions.

Ordering Information

Product Description	Catalog No.	Kit Size
Quick-DNA™ FFPE Kit	D3067	50 Preps.

For Individual Sale	Catalog No.	Amount
Deparaffinization Solution	D3067-1-20	20 ml
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
Genomic Lysis Buffer	D3004-1-50	50 ml
Genomic DNA Wash 1	D3067-2-25	25 ml
Genomic DNA Wash 2 (concentrate)	D3067-3-12	12 ml
DNA Elution Buffer	D3004-4-4 D3004-4-10 D3004-4-50	4 ml 10 ml 50 ml
Zymo-Spin™ IIC Columns	C1011-50 C1011-250	50 250
RNase A	E1008-2 E1008-8	2 mg 8 mg
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1,000

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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 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).

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