



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR FFPE DNA MiniPrep™

Catalog Nos. **D3065 & D3066**

Highlights

- High performance sample prep technology for high quality DNA (*up to ~25 µg/prep*) from FFPE tissue samples and sections.
- *Selectable* size cut-off technology; recover total DNA >50 bp *or* >500 bp.
- Eluted DNA is RNA-free and ideal for PCR, Next-Gen library prep, enzymatic manipulation, etc.

Contents

Product Contents	1
Specifications.....	1
Product Description.....	2
Buffer Preparation.....	3
Protocol.....	3, 4
Ordering Information	5
Related Products	6

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

Product Contents

ZR FFPE DNA MiniPrep™ (Kit Size)	D3065 (50 Preps.)	D3066 (200 Preps.)	Storage Temperature
Proteinase K & Storage Buffer¹	2 x 5 mg	2 x 20 mg	-20°C (after mixing)
2X Digestion Buffer	5 ml	20 ml	Room Temp.
Genomic Lysis Buffer²	50 ml	2 x 100 ml	Room Temp.
DNA Pre-Wash Buffer	15 ml	50 ml	Room Temp.
g-DNA Wash Buffer	50 ml	100 ml	Room Temp.
DNA Elution Buffer	10 ml	50 ml	Room Temp.
Zymo-Spin™ IIC Columns	50	200	Room Temp.
RNase A³	2 mg	8 mg	4°C
Collection Tubes	100	400	Room Temp.
Instruction Manual	1	1	-

Note - 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.

¹ The Proteinase K is stable as shipped. Add 260 µl (D3065) or 1,040 µl (R3066) **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 Re-suspend lyophilized RNase A in 300 µl (D3065) or 1,200 µl (D3066) 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, xylene, ethanol, 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 **ZR FFPE DNA MiniPrep™** 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.

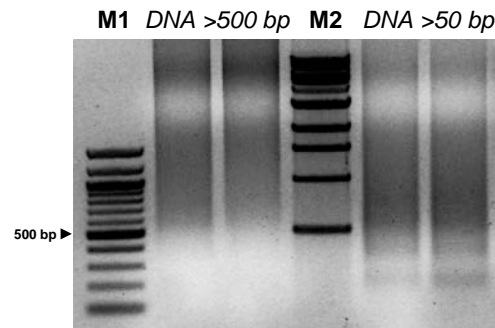
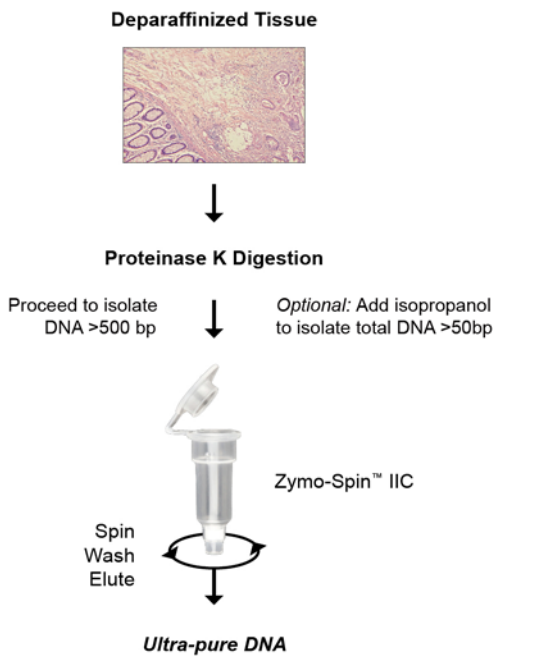


Figure 1. Equivalent amounts of DNA resolved in a 1% agarose/TAE/EtBr gel show binding conditions may be adjusted with the **ZR FFPE DNA MiniPrep™** to selectively isolate DNA >50 bp or >500 bp. M1 is a 100 bp DNA ladder, M2 is a 1 kb DNA ladder (Zymo Research).

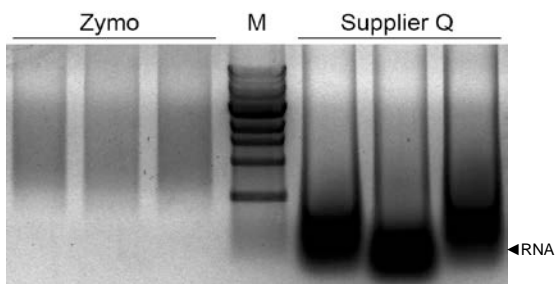


Figure 2. Equivalent volumes of DNA isolated using Zymo and Supplier Q procedures were resolved in a 1% agarose/TAE/EtBr gel (image) and show DNA isolated using the **ZR FFPE DNA MiniPrep™** is high quality and RNA-free. M is a 1 kb DNA ladder

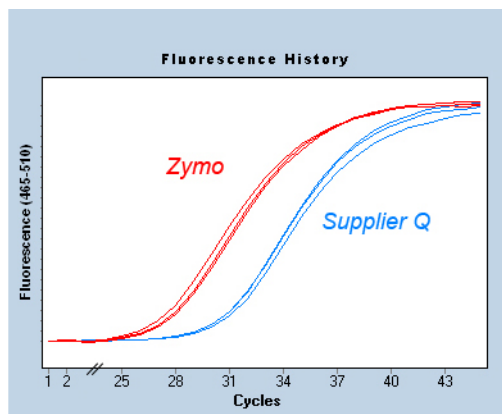


Figure 3. Equivalent amounts of DNA isolated using Zymo and Supplier Q procedures were used for real time PCR analysis. DNA isolated using the **ZR FFPE DNA MiniPrep™** consistently yielded lower Ct values as depicted by the amplification curves above.

Buffer Preparation

- ✓ Add 260 µl (D3065) or 1,040 µl (D3066) **Proteinase K Storage Buffer** to each **Proteinase K** tube prior to use and store at -20° C. The final concentration of **Proteinase K** after the addition of **Proteinase K Storage Buffer** is ~20 mg/ml.
- ✓ Resuspend lyophilized **RNase A** in 300 µl (D3065) or 1,200 µl (D3066) 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 or 500 µl per 100 ml.

Protocol

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

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 **Proteinase K Digestion & DNA Isolation** (see page 4).

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 **Proteinase K Digestion and DNA Isolation** (see page 4).

Proteinase K Digestion & DNA Isolation

- To a deparaffinized tissue sample (≤ 25 mg) in a microcentrifuge tube, add the following:

H ₂ O	45 μ l
2X Digestion Buffer	45 μ l
Proteinase K	10 μ l

Note: If your tissue sample is too large for the digestion volume, scale up the digestion to 200 μ l while keeping the amount of Proteinase K the same. *Double the reagent volumes indicated in Step 3 & 4.*

Rapid Digestion	Standard Digestion
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 but should also be sufficient for most tissue samples. However, the *Standard Digestion* ensures maximum yields of DNA from even tough-to-lyse (collagen-rich, fibrous, etc.) tissue samples.

- Transfer the digestion to 94°C and incubate for 20 minutes. When done add 5 μ l of **RNase A**, mix, and then incubate an additional 5 minutes at room temperature.

Note: It is recommended to skip the heat treatment in *Step 3* and the addition of isopropanol in *Step 4* if only double stranded DNA is required (e.g. *OneStep qMethyl™* Array, Cat. No. D5312).

- Add 350 μ l of **Genomic Lysis Buffer** to the tube and mix thoroughly by vortexing.

To Isolate Total DNA >50 bp	To Isolate DNA >500 bp
Add 135 μ l of isopropanol* (user supplied) to the sample, mix thoroughly, and proceed to Step 5	Proceed <i>directly</i> to Step 5

Note: When working with a new sample, it is recommended to isolate total DNA as a precaution. FFPE DNA may be highly degraded and DNA >500 bp may not be present in sample.

- Centrifuge at 10,000 $\times g$ for 1 minute to remove insoluble debris and then transfer the supernatant to a **Zymo-Spin™ IIC Column** in a **Collection Tube**. Centrifuge at 10,000 $\times g$ for 1 minute.
- Add 200 μ l of **DNA Pre-Wash Buffer** to the spin column in a new **Collection Tube**. Centrifuge at 10,000 $\times g$ for 1 minute.
- Add 400 μ l of **g-DNA Wash Buffer** to the spin column. Centrifuge at 10,000 $\times g$ for one 1 minute.
- Transfer the **Zymo-Spin™ IIC Column** to a clean microcentrifuge tube. Add ≥ 50 μ l **DNA Elution Buffer** or water (e.g., add ≥ 100 μ l if sampling 25 mg tissue) to the spin column. Incubate 2-5 minutes at room temperature, then 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.

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

The maximum loading volume for the **Zymo-Spin™ Column** is ~ 700 μ l.

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°C or by performing and pooling sequential elutions.

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Ordering Information

Product Description	Catalog No.	Kit Size
ZR FFPE DNA MiniPrep™	D3065	50 Preps.
	D3066	200 Preps.

For Individual Sale	Catalog No.	Amount
Proteinase K & Storage Buffer	D3001-2-5	5 mg set
	D3001-2-20	20 mg set
2X Digestion Buffer	D3050-1-5	5 ml
	D3050-1-20	20 ml
Genomic Lysis Buffer	D3004-1-50	50 ml
	D3004-1-100	100 ml
DNA Pre-Wash Buffer	D3004-5-15	15 ml
	D3004-5-30	30 ml
	D3004-5-50	50 ml
g-DNA Wash Buffer	D3004-2-50	50 ml
	D3004-2-100	100 ml
DNA Elution Buffer	D3004-4-4	4 ml
	D3004-4-10	10 ml
	D3004-4-50	50 ml
Zymo-Spin™ IIC Columns	C1011-50	50
	C1011-250	250
RNase A	E1008-2	2 mg
	E1008-8	8 mg
Collection Tubes	C1001-50	50
	C1001-500	500
	C1001-1000	1,000

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Popular DNA Purification Products from Zymo Research

Product	Format	Kit Size	Cat No.
Fragment DNA Clean-up, Concentration & Recovery			
DNA Clean & Concentrator™-5	Spin Column Format (up to 5 µg/prep.)	50 preps. 200 preps.	D4003*, D4013 D4004*, D4014
DNA Clean & Concentrator™-25	Spin Column Format (up to 25 µg/prep.)	50 preps. 200 preps.	D4005*, D4033 D4006*, D4034
DNA Clean & Concentrator™-100	Spin Column Format (up to 100 µg/prep.)	25 preps. 50 preps.	D4029 D4030
DNA Clean & Concentrator™-500	Spin Column Format (up to 500 µg/prep.)	10 preps. 20 preps.	D4031 D4032
ZR-96 DNA Clean & Concentrator™-5	96-Well Format (up to 5 µg/well; deep well)	2x96 preps. 4x96 preps.	D4023 D4024
Genomic DNA Clean & Concentrator™	Spin Column Format (up to 10 µg/prep.)	25 preps. 100 preps.	D4010 D4011
ZR-96 DNA Clean-up Kit™	96-Well Format (up to 5 µg/well; shallow well)	2x96 preps. 4x96 preps.	D4017 D4018
ZR DNA Sequencing Clean-up Kit™	Spin Column Format (up to 5 µg/prep.)	50 preps. 200 preps.	D4050 D4051
ZR-96 DNA Sequencing Clean-up Kit™	96-Well Format (up to 5 µg/well)	2x96 preps. 4x96 preps.	D4052 D4053
OneStep™ PCR Inhibitor Removal Kit	Spin Column Format (up to 25 µg/prep.)	50 preps.	D6030
OneStep-96™ PCR Inhibitor Removal Kit	96-Well Format (up to 5 µg/well)	2x96 preps.	D6035
Zymoclean™ Gel DNA Recovery Kit	Spin Column Format (up to 5 µg/prep.)	50 preps. 200 preps.	D4001 D4002
ZR-96 Zymoclean™ Gel DNA Recovery Kit	96-Well Format (up to 5 µg/well)	2x96 preps. 4x96 preps.	D4021 D4022
Zymoclean™ Large Fragment DNA Recovery Kit	Spin Column Format (up to 10 µg/prep.)	25 preps. 100 preps.	D4045 D4046
Plasmid DNA Isolation			
Zyppy™ Plasmid Miniprep Kit	Pellet Free, Spin Column Format	50 preps. 100 preps. 400 preps. 800 preps.	D4036 D4019 D4020 D4037
Zyppy™ Plasmid Midiprep Kit	Pellet Free, Spin Column Format	25 preps. 50 preps.	D4025 D4026
Zyppy™ Plasmid Maxiprep Kit	Spin/Vacuum Column Format	10 preps. 20 preps.	D4027 D4028
ZR Plasmid Miniprep™-Classic	Spin Column Format	100 preps. 400 preps. 800 preps.	D4015 D4016 D4054
ZR BAC DNA Miniprep Kit	BAC/PAC plasmid DNA Isolation. Spin Column Format	25 preps. 100 preps.	D4048 D4049
Environmental DNA Isolation			
ZR Soil Microbe DNA MicroPrep™ ZR Soil Microbe DNA MiniPrep™ ZR Soil Microbe DNA MidiPrep™ ZR-96 Soil Microbe DNA Kit™	Bead Bashing, Spin Column Format (up to 5 µg/prep.) Bead Bashing, Spin Column Format (up to 25 µg/prep.) Bead Bashing, Spin Column Format (up to 125 µg/prep.) Bead Bashing, 96-Well Format (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	D6003 D6001 D6101 D6002
ZR Fungal/Bacterial DNA MicroPrep™ ZR Fungal/Bacterial DNA MiniPrep™ ZR Fungal/Bacterial DNA MidiPrep™ ZR-96 Fungal/Bacterial DNA Kit™	Bead Bashing, Spin Column Format (up to 5 µg/prep.) Bead Bashing, Spin Column Format (up to 25 µg/prep.) Bead Bashing, Spin Column Format (up to 125 µg/prep.) Bead Bashing, 96-Well Format (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	D6007 D6005 D6105 D6006
ZR Fecal DNA MicroPrep™ ZR Fecal DNA MiniPrep™ ZR Fecal DNA MidiPrep™ ZR-96 Fecal DNA Kit™	Bead Bashing, Spin Column Format (up to 5 µg/prep.) Bead Bashing, Spin Column Format (up to 25 µg/prep.) Bead Bashing, Spin Column Format (up to 125 µg/prep.) Bead Bashing, 96-Well Format (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	D6012 D6010 D6110 D6011
ZR Tissue & Insect DNA MicroPrep™ ZR Tissue & Insect DNA MiniPrep™ ZR Tissue & Insect DNA MidiPrep™ ZR-96 Tissue & Insect DNA Kit™	Bead Bashing, Spin Column Format (up to 5 µg/prep.) Bead Bashing, Spin Column Format (up to 25 µg/prep.) Bead Bashing, Spin Column Format (up to 125 µg/prep.) Bead Bashing, 96-Well Format (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	D6015 D6016 D6115 D6017
ZR Plant/Seed DNA MicroPrep™ ZR Plant/Seed DNA MiniPrep™ ZR Plant/Seed DNA MidiPrep™ ZR-96 Plant/Seed DNA Kit™	Bead Bashing, Spin Column Format (up to 5 µg/prep.) Bead Bashing, Spin Column Format (up to 25 µg/prep.) Bead Bashing, Spin Column Format (up to 125 µg/prep.) Bead Bashing, 96-Well Format (up to 5 µg/well)	50 preps. 50 preps. 25 preps. 2x96 preps.	D6022 D6020 D6120 D6021

* Uncapped Spin Column Format

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