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.

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ZYMO RESEARCH CORP.

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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.

Product Contents

ZR FFPE DNA MiniPrep™ (Kit Size)	D3065 (50 Preps.)	D3066 (200 Preps.)	Storage Temperature
Proteinase K & Storage Buffer[1]	2 x 5 mg	2 x 20 mg	−20°C (after mixing)
2X Digestion Buffer	5 ml	20 ml	Room Temp.
Genomic Lysis Buffer[2]	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 A[3]	2 x 1 mg	8 mg	4°C
Collection Tubes	100	400	Room Temp.
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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.

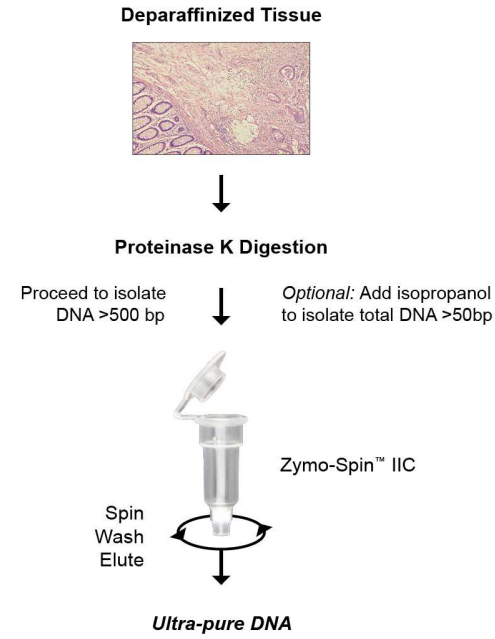
[3] Re-suspend lyophilized RNase A in 150 µl per tube (D3065) or 1,200 µl (D3066) of $ddIH_20$. Store at 4° C.

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 cm^2. 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).

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.



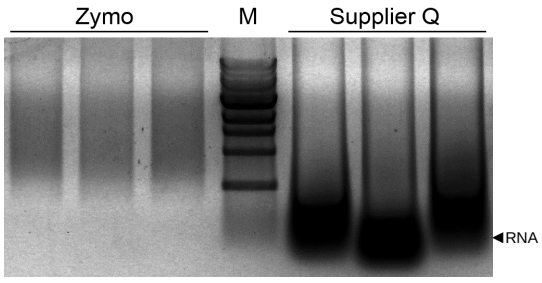


Figure 2. Equivalent volumes of DNA isolated using Zymo and Supplier Q procedures were resolved in a 1% agarose/TAE/EtBr gel (image) and show DNA isolated using the **ZR FFPE DNA MiniPrep™** is high quality and RNA-free. M is a 1 kb DNA ladder

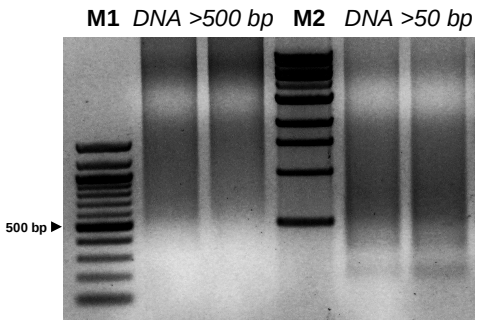


Figure 1. Equivalent amounts of DNA resolved in a 1% agarose/TAE/EtBr gel show binding conditions may be adjusted with the **ZR FFPE DNA MiniPrep™** to selectively isolate DNA >50 bp or >500 bp. M1 is a 100 bp DNA ladder, M2 is a 1 kb DNA ladder (Zymo Research).

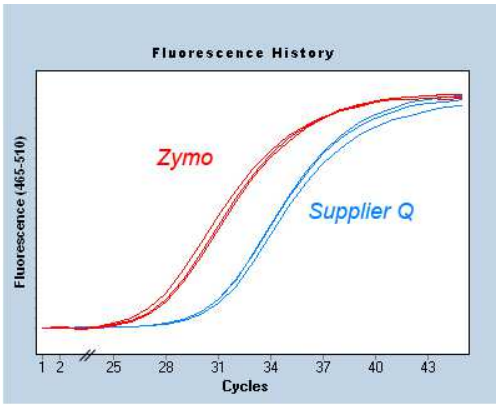


Figure 3. Equivalent amounts of DNA isolated using Zymo and Supplier Q procedures were used for real time PCR analysis. DNA isolated using the **ZR FFPE DNA MiniPrep™** consistently yielded lower *Ct* values as depicted by the amplification curves above.

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

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 150µl per tube (D3065) or 1,200 µl (D3066) of ddIH_20. 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 ddIH_2O 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

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

H_2O	45µl
2X Digestion Buffer	45µl
Proteinase K	10µl

Note: If your 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 3 & 4.*

2.

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.

3. Transfer the digestion to 94°C and incubate for 20 minutes. When done add 5 µl 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).

4. Add 350 µl 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 µl of isopropanol* (user supplied) to the sample, mix thoroughly, and proceed to Step 5	Proceed *directly* 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.

5. Centrifuge at 10,000 *x 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 *x g* for 1 minute.

6. Add 200 µl of **DNA Pre-Wash Buffer** to the spin column in a new **Collection Tube**. Centrifuge at 10,000 *x g* for 1 minute.

7. Add 400 µl of **g-DNA Wash Buffer** to the spin column. Centrifuge at 10,000 *x g* for one 1 minute.

8. Transfer the **Zymo-Spin™ IIC Column** to a clean microcentrifuge tube. Add ≥50 µl **DNA Elution Buffer** or water (e.g., add ≥100 µl 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 ≤-20ºC 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 µl.

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.

Ordering Information

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

For Individual Sale	Catalog No.	Amount
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 D3004-1-100	50 ml 100 ml
DNA Pre-Wash Buffer	D3004-5-15 D3004-5-30 D3004-5-50	15 ml 30 ml 50 ml
g-DNA Wash Buffer	D3004-2-50 D3004-2-100	50 ml 100 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

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