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siehe unsere Liefer- und Versandbedingungen

Zuschläge

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- Trockeneiszuschlag
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ZYMO RESEARCH

DNA Purification Made Simple™

Quick-DNA™ FFPE Kit

Isolation of ultra-pure DNA from FFPE tissue.

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.

Catalog Numbers:
D3067

Scan with your smart-phone camera to view the online protocol/video.

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

Table of Contents

Product Contents

Quick-DNA™ FFPE Kit	D3067-E (50 Preps.)	Storage Temperature
Deparaffinization Solution	20 ml	Room Temp.
Proteinase K & Storage Buffer[1]	2 x 5 mg	−20°C (after mixing)
2X Digestion Buffer	5 ml	Room Temp.
Genomic Lysis Buffer[2]	50 ml	Room Temp.
Genomic DNA Wash 1	25 ml	Room Temp.
Genomic DNA Wash 2[3] (concentrate)	12 ml	Room Temp.
DNA Elution Buffer	10 ml	Room Temp.
RNase A[4]	2 mg	4°C
Zymo-Spin™ IICR Columns	50	Room Temp.
Collection Tubes	100	Room Temp.
Instruction Manual	1	-

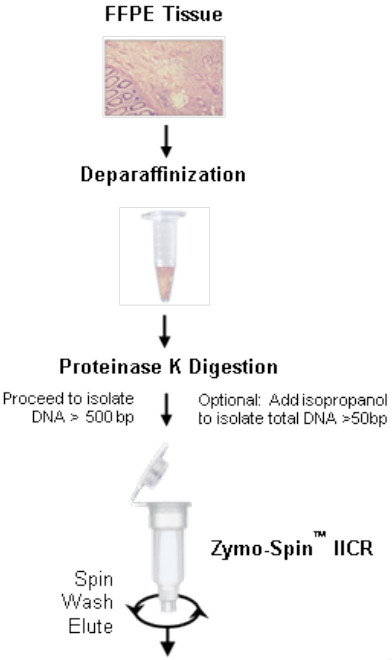
[1] The Proteinase K is stable as shipped. Add 260 µl **Proteinase K Storage Buffer** to each **Proteinase K** tube prior to use. The final concentration of **Proteinase K** after the addition of **Proteinase K Storage Buffer** is ~20 mg/ml. Store at -20° C.
[2] *Recommended*: Add beta-mercaptoethanol 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 ddH_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 mm^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, isopropanol (optional), beta-mercaptoethanol (optional).

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.



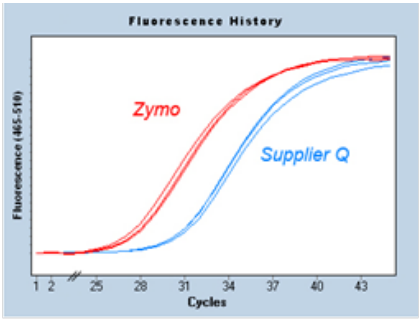


Figure 1. Equivalent amounts of DNA isolated using Zymo and Supplier Q procedures were used for rea time PCR analysis. DNA isolated using the ***Quick*-DNA™ FFPE Kit** consistently yielded lower *Ct* values as depicted by the amplification curves above.

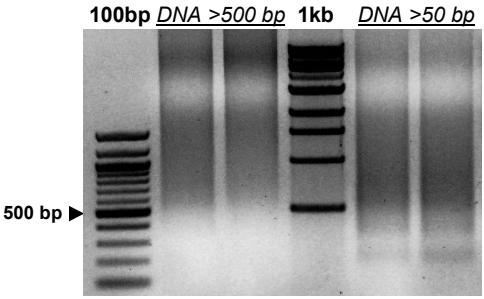


Figure 2. Equivalent amounts of DNA resolved in a 1% agarose/TAE/EtBr gel show binding conditions may be adjusted with the ***Quick*-DNA™ FFPE Kit** to selectively isolate DNA >50 bp or >500 bp. 100 bp DNA ladder and 1 kb DNA ladder from Zymo Research.

Protocol

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 ddH_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.

Deparaffinization[1]

1. Remove (trim) excess paraffin wax from sample and transfer sample to a 1.5 m microcentrifuge tube.
2. Add 400 µl of **Deparaffinization Solution**[2] to the sample. Incubate at 55°C for 1 minute. Vortex briefly.
3. Remove **Deparaffinization Solution** from the sample and proceed to next section.

   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 mm^2 can be used per purification. It is recommended to start with just 1-2 sections.

Tissue Digestion

1. To the deparaffinized tissue sample (≤ 25 mg) in a microcentrifuge tube, add the following mixture[3]:

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

Continued on next page

[1] If using fresh/frozen tissue specimens proceed directly with Proteinase K Digestion & DNA Isolation.
[2] Xylene may also be used for deparaffinization. See the Appendix on page 6 for instruction.
[3] 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 5).

2. Incubate the sample at 55°C according to the table below.

Rapid Digestion	*Standard Digestion*
Incubate at 55°C for 1-4 hours	Incubate at 55°C overnight (12-16 hrs)

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 µl of **Genomic Lysis Buffer** to the tube and mix thoroughly by vortexing.

2. *Optional:* Add 135 µl of isopropanol[1] (user supplied) to the sample and mix thoroughly[2]. Centrifuge at ≥ 12,000 *x g* for 1 minute to remove insoluble debris.

3. Transfer the supernatant to a **Zymo-Spin™ IICR Column**[3] in a **Collection Tube**. Centrifuge at 10,000 *x g* for 1 minute.

4. Add 400 µl of **Genomic DNA Wash 1** to the spin column in a **new Collection Tube**. Centrifuge at 10,000 *x g* for 1 minute. Discard the flow-through.

5. Add 700 µl of **Genomic DNA Wash 2** to the spin column. Centrifuge at ≥ 12,000 *x g* for 1 minute. Discard the flow-through.

6. Add 200 µl of Genomic DNA Wash 2 to the spin column. Centrifuge at ≥ 12,000 *x g* for 1 minute.

Continued on next page

[1] ssDNA will also be purified if present in the sample upon the addition of isopropanol.
[2] 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.
[3] The maximum loading volume for the **Zymo-Spin™ Column** is ~700 µl.

7. Transfer the Zymo-Spin™ IICR Column to a clean microcentrifuge tube. Add ≥ 50 µl **DNA Elution Buffer**[1,2] or water (add ≥100 µl 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 ≤-20ºC for future use.

[1] 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 60-70ºC or by performing and pooling sequential elutions.

[2] The **DNA Elution Buffer** contains 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.

Appendix: Xylene Deparaffinization

Rapid Deparaffinization (Slide Tissue Sections Only)

1. Remove (trim) excess paraffin wax from sample and transfer sample to a 1.5 m 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 4).

Standard Deparaffinization (Tissue Samples and Slide Sections)

1. Remove (trim) excess paraffin wax from sample and transfer sample to a 1.5 m 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.

   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 ddIH2O 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 4).

Ordering Information

Product Description	Catalog No.	Kit Size
***Quick*-DNA™ FFPE Kit**	D3067-E	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™ IICR Columns	C1078-50 C1078-250	50 250
RNase A	E1008-8	8 mg
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1,000

Complete Your DNA Methylation Workflow

✓ **Rapid Method for Complete Bisulfite Conversion of DNA**

EZ DNA Methylation Kits	Size	Catalog No.
EZ DNA Methylation-Lightning Kit	50 Rxns. 200 Rxns.	D5030 D5031
EZ-96 DNA Methylation-Lightning Kit	2x96 Rxns. (Deep-Well) 2x96 Rxns. (Shallow-Well)	D5032 D5033
EZ DNA Methylation-Lightning Automation Kit	96 Rxns.	D5049
EZ-96 DNA Methylation Lightning MagPrep	4 X 96 Rxns. 8 X 96 Rxns.	D5046 D5047

✓ **Innovative Solutions for Next Generation Sequencing**

Library Prep Kits	Size	Catalog No.
Zymo-Seq WGBS Library Kit	24 Preps.	D5465
Pico Methyl-Seq Library Prep Kit	10 Preps. 25 Preps.	D5455 D5456
Zymo-Seq RRBS Library Kit	24 Preps. 48 Preps.	D5460 D5461

✓ **Optimal Amplification of Bisulfite-Treated DNA**

ZymoTaq Polymerase	Size	Catalog No.
ZymoTaq Premix	50 Rxns. 200 Rxns.	E2003 E2004
ZymoTaq DNA Polymerase	50 Rxns. 200 Rxns.	E2001 E2002
ZymoTaq qPCR Premix	50 Rxns. 200 Rxns.	E2054 E2055

✓ **Industry Leading Tools for Assessing Your DNA Methylation Workflow**

DNA Methylation Standards	Size	Catalog No.
Human Methylated & Non-methylated DNA Set	5 µg/20 µl	D5014
Universal Methylated DNA Standard	Human Mouse	D5011 D5012
Bisulfite-Converted Universal Methylated Human DNA Standard	1 µg/50 µl	D5015
Human Methylated & Non-Methyated (WGA) DNA Set	5 µg/20 µl	D5013

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