Quick-DNA™ FFPE Kit Protocol

Zymo Research

Abstract

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.

Citation: Zymo Research Quick-DNA™ FFPE Kit Protocol. **protocols.io**
dx.doi.org/10.17504/protocols.io.giybufw
Published: 21 Dec 2016

Guidelines

Product Contents

Quick-DNA™ FFPE Kit (Kit Size)	D3067 (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™ 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 ddH_2O. 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.

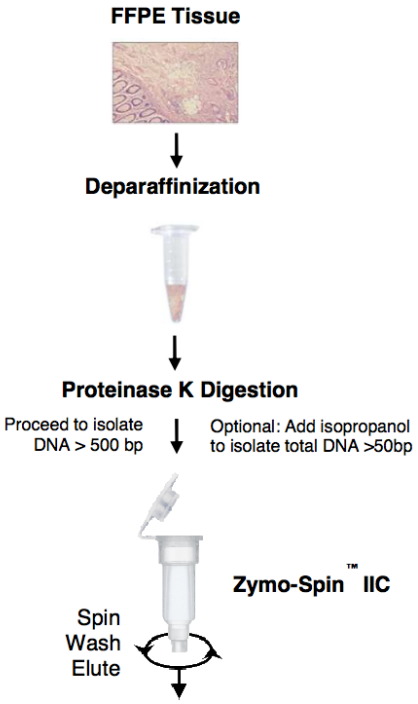
- **DNA Recovery** High quality total DNA (A_{260}/A_{280} >1.8) can be eluted into small volumes (i.e., ≥25 µl) allowing for highly concentrated samples. The maximum DNA binding capacity of the provided spin column is ~25 µg.

- **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, isopropanol, 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.



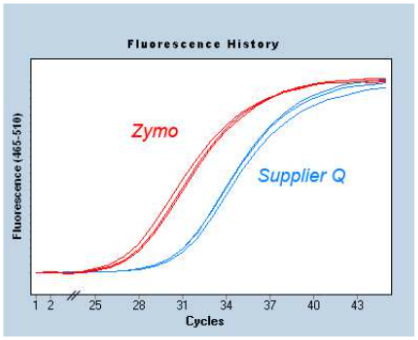


Figure 1. Equivalent amounts of DNA isolated using Zymo and Supplier Q procedures were used for real time PCR analysis. DNA isolated using the ***Quick*-DNA™ FFPE Kit** consistently yielded lower *Ct* values values as depicted by the amplification curves above.

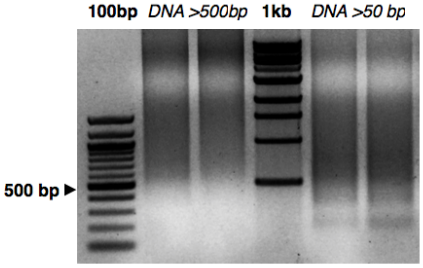


Figure 2. Equivalent amounts of DNA resolved in a 1% agarose/TAE/EtBr gel show binding conditions may be adjusted with the ***Quick*-DNA™ FFPE Kit** to selectively isolate DNA >50 bp or >500 bp. 100 bp DNA ladder and 1 kb DNA ladder from Zymo Research.

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.

Before start

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.

Materials

Quick-DNA™ FFPE Kit D3067 by Zymo Research

Protocol

Deparaffinization

Step 1.

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

NOTES

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

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Note: If using fresh/frozen tissue specimens proceed directly with Proteinase K Digestion & DNA Isolation.

Deparaffinization

Step 2.
Add 400 µl of **Deparaffinization Solution** to the sample. Incubate at 55°C for 1 minute. Vortex briefly.

DURATION
00:01:00

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Note: Xylene may also be used for deparaffinization. See the Appendix for more instructions.

Deparaffinization

Step 3.
Remove **Deparaffinization Solution** from the sample and proceed to next section.

Tissue Digestion

Step 4.

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

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

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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 8 & 9 of the DNA Purification section.

Tissue Digestion

Step 5.

Digest 1-4 hours or overnight at 55 °C.

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

NOTES

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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.

Tissue Digestion

Step 6.

Transfer the digestion to 94°C and incubate for 20 minutes.

DURATION

00:20:00

Tissue Digestion

Step 7.

Once done, add 5 µl of **RNase A**, mix, and incubate an additional 5 minutes at room temperature.

DURATION

00:05:00

DNA Purification

Step 8.

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

DNA Purification

Step 9.

Add 135 µl of isopropanol[1] (user supplied) to the sample and mix thoroughly.

NOTES

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[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 this step.

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

DNA Purification

Step 10.

Centrifuge at ≥ 12,000 x g for 1 minute to remove insoluble debris.

DURATION

00:01:00

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This procedure will isolate total DNA > 50 bp. To isolate only DNA > 500 bp, skip this step.

DNA Purification
Step 11.

Transfer the supernatant to a **Zymo-Spin™ IIC Column**[2] in a **Collection Tube**.

NOTES
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The maximum loading volume for the Zymo-Spin™ Column is ~700 µl.

DNA Purification
Step 12.

Centrifuge at 10,000 x g for 1 minute.

DURATION
00:01:00

DNA Purification
Step 13.

Add 400 µl of **Genomic DNA Wash 1** to the spin column in a new **Collection Tube**.

DNA Purification
Step 14.
Centrifuge at 10,000 x g for 1 minute. Discard the flow-through.

DURATION
00:01:00

DNA Purification
Step 15.
Add 700 µl of **Genomic DNA Wash 2** to the spin column.

DNA Purification
Step 16.

Centrifuge at ≥ 12,000 x g for 1 minute. Discard the flow-through.

DURATION
00:01:00

DNA Purification
Step 17.
Add 200 µl of **Genomic DNA Wash 2** to the spin column.

DNA Purification
Step 18.

Centrifuge at ≥ 12,000 x g for 1 minute.

DURATION
00:01:00

DNA Purification

Step 19.

Transfer the **Zymo-Spin™ IIC Column** to a clean microcentrifuge tube. Add ≥ 50 µl **DNA Elution Buffer**[3] or water (add ≥100 µl if sampling 25 mg tissue) to the spin column.

NOTES
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[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° C or by performing and pooling sequential elutions.

DNA Purification

Step 20.

Incubate 2-5 minutes at room temperature.

DURATION
00:05:00

DNA Purification

Step 21.

Centrifuge at top speed for 30 seconds to elute the DNA.

DURATION
00:00:30

DNA Purification

Step 22.

The eluted DNA can be used immediately for molecular based applications or stored ≤-20°C for future use.