ROJETECHNOLOGIES

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DNJia FFPE Tissue Kit

DNA isolation based on silica technology

- MiniPrep

For DNA Isolation from

Formalin-fixed, paraffin-embedded tissues

By ROJE

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ROJETechnologies has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality, affordable Molecular and diagnostic Kits and reagents comparable to the world leaders to research centers, laboratories, clinics, hospitals, and diagnostic centers all over the world.

Contents

Kit Content

Component	50 preps	100 preps
TLB	13ml	26ml
GLB	14ml	28ml
BWB1 (concentrate)	16ml	2 x 16ml
BWB2 (concentrate)	16ml	2 x 16ml
RRB	5ml	10ml
RJ-Protease	2 X 1.25ml	4 X 1.25ml
HiPure DR Column	50	100
Collection Tube	50	100

Storage

Shipment condition is checked by ROJETechnologies. After arrival, all reagents should be kept dry, at room temperature. We suggest storing RJ-Protease at 2-8°C, and for routine use, it is recommended that you aliquot it to 100µl volumes and storage at 2-8°C. When storage condition is as directed, all reagents are stable until expiration date, as indicated on the kit box.

Intended Use

DNJia FFPE Tissue Kit provides the components and procedures necessary for purifying genomic DNA from formalin-fixed, paraffin-embedded tissues. Notice that DNJia FFPE Tissue Kit is intended for molecular biology applications, not for diagnostic use. We recommend all users study DNA experiment guidelines before starting their work.

Guarantee & Warranty

ROJETechnologies guarantee the efficiency of all manufactured kits and reagents. For more information on choosing proper kits based on your needs, please contact our technical support team. If any product does not satisfy you for reasons other than misuse, please contact our technical support team. If the problem is due to the manufacturing process, the ROJE team will replace the Kit for you.

Notice to Purchaser

This product is only for experiments and not for commercial use—no right to resell this Kit or any components. For information about our licensing or distributors, contact the ROJE business team.

Warning and Precautions

Due to chemical material usage that may be hazardous, always make sure to wear a suitable lab coat, disposable gloves, and protective eyewear. Material Safety Data Sheet (MSDS) for all products and reagents are provided. They are accessible online at www.rojetechnologies.com.

Quality Control

DNJia FFPE Tissue Kit is tested against predetermined experiments on a lot-to-lot basis according to ROJETechnologies ISO-certified quality management system to ensure consistent product quality. For your information, the results of all experiments are accessible by addressing REF and Lot number on the web at www.rojetechnoloes.com.

Description

DNJia FFPE Tissue Kit provides a fast, reliable, meticulous method for genomic DNA isolation from formalin-fixed, paraffin-embedded tissues. This Kit is based on spin-column technology for isolation of concentrated, highly purified, intact genomic DNA, which is suitable for various downstream processes such as PCR, Southern blot, genotyping, etc.

Procedure

DNJia FFPE Tissue Kit is designed for isolating DNA from up to 5 sections (5-10 μm thick).

Lysis is achieved by incubation the sample in an RJ-Protease enzyme solution and TLB. Appropriate conditions for DNA binding to the silica membrane are achieved by adding chaotropic salts and Ethanol to the lysate. Then, DNA is selectively bound to the membrane. Contaminants are removed by two specific washing buffers. Pure genomic DNA is finally eluted in a rehydration buffer. Isolated DNA is ready to use in downstream applications. It has A 260/ A 280 ratios of 1.7–2 and an asymmetric peak at 260 nm by spectrophotometer, confirming high purity.

Equipment & Reagents to Be Supplied by User

- Xylene
- Ethanol (96-100%)
- Pipets and pipet tips
- 1.5ml Microtube
- Vortex
- Centrifuge
- Micro centrifuge
- Dry Heat Block/ Water Bath

Applications

The isolated DNA can be used in many downstream applications:

- Different kinds of PCRs, including Long-range PCR
- Sequencing
- Restriction digestion
- Southern blotting
- Cloning

Features

Specific features of DNJia FFPE Tissue Kit are listed here in Table 1.

Table 1. DNJia FFPE Tissue Kit features and specifications

Features	Specifications
Elution volume	50-100µl
Technology	Silica technology
Main sample type	Formalin-fixed, paraffin-embedded tissues
Processing	Manual
Sample amount	• Up to 5 sections (5-10 µm thick)
Operation time per reaction	Less than 3 h
Average purity	A260/A280= 1.7-2.0
Size of DNA purified	≈ 50 Kb
Enzyme	**RJ-Protease**

Recommended Starting Material

Recommended using freshly cut sections of FFPE tissue, each with a thickness of 5 to 10 µm. For each reaction, use up to 5 sections, each with a thickness of 5 to 10 µm and a surface area of up to 250 mm2. If you are not sure about section properties, use up to 3 sections for each reaction.

Before Start

- If GLB or TLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage conditions and will not influence the efficiency of the buffer.
- Not forget to add the appropriate amount of Ethanol (96–100%) to BWB1 and BWB2 as indicated on the bottle; before using for the first time, refer to washing buffer preparation.

Washing Buffer Preparation

Before the first use, add the appropriate amount of Ethanol (96-100%) to each washing buffer tube, then mix thoroughly to prepare washing buffer, refer to Table 2. Do not forget to tick the check box on the bottle label to indicate that ethanol has been added. Before each use, mix reconstituted buffer by shaking and storing at room temperature.

Table 2: Washing buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
BWB1	16ml	24ml	40ml
BWB1	16ml	24ml	40ml

Protocols

Protocol: Isolation of Genomic DNA (Formalin-fixed, paraffin-embedded tissues)

Sample type: Formalin-fixed, paraffin-embedded tissues

Some tips to know

• All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.

• If RNase treatment is desired, Prime-RNase A can be ordered separately from ROJETechnologies, Cat No. EB983013.

• If TLB or GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage conditions and won't influence the efficiency of the buffer.

• Do not forget to add the appropriate amount of Ethanol (96–100%) to BWB1 and BWB2 as indicated on the bottle; before using for the first time, refer to the washing buffer preparation part.

Process

Trim excess paraffin off the sample block by using a scalpel. Cut the samples into sections 5-10 µm thick. Transfer up to 5 sections in a 1.5ml microcentrifuge tube.

- Add 1ml xylene to the tube. Vortex vigorously for 30-60 s until paraffin becomes wholly separated from the tissue and soluble in xylene. Centrifuge at 14000 rpm for 3 min.

- Remove the supernatant by pipetting. Do not remove any of the pellets.

Attention! Do not disturb the pellet at the bottom of the tube.

- Add 1ml ethanol (96-100%) to the pellet and mix by vortexing. Centrifuge at 14000 rpm for 3 min.
- Remove the supernatant by pipetting. Attention not to disturb the pellet.
- Repeat steps 4-5.
- Open the tube and incubate at room temperate for 10-15 min until the Ethanol has evaporated thoroughly.
- Add 236 µl TLB. Mix by pulse vortexing for 30 s and then incubate at 56˚C for 10 min.
- Add 50 µl RJ-Protease to the sample. Mix thoroughly by pulse vortexing for 30 s and then incubate at 56˚C for 1-2 h. Pulse vortex every 10 min during incubations to intersperse the sample, or place it in a thermomixer or shaking water bath.

Note: you might observe white precipitates in the lysate during lysis, these white precipitates will not disappear, and you must apply them to the column.

Optional: This protocol is specialized for DNA isolation. For typical and routine applications, RNase treatment isn't needed. If RNA-free genomic DNA is required, add 10µl PrimeRNase A (Cat No. EB983013), mix by vortexing, and incubate for 5 min at room temperature before step 3.

- Add 250 µl GLB to the mixture, mix by pulse vortexing for 10 s and then incubate at 70°C for 15 min.
- Add 250 µl cold absolute ethanol, then mix thoroughly by pulse vortexing for 15 s.
- Pipette the mixture from step 11 to a HiPure DR Column in a 2ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 1 min. Discard flow-through and place the spin column in the collection tube again.

Note: Apply the white precipitate onto the column. If the lysate did not pass the column, centrifuge at full speed until it passes through the column.

- Add 700 µl BWB1 to the HiPure DR Column. Centrifuge at 13000 rpm for 1 min; just discard the flow-through. Place the HiPure DR Column in the previous collection tube and go to the next step.
- Add 600 µl BWB2 to the HiPure DR Column. Centrifuge at 14000 rpm for 3 min. Discard both the flow-through and the collection tube. Place the HiPure DR Column in a new clean 1.5ml microcentrifuge tube (not provided).

Note: To avoid Ethanol carry-over, be careful that the column does not come into contact with the flow-through, if it happens, discard the flowthrough, place the column back in a collection tube, and centrifuge for another 1 min at 14000 rpm.

- Pipette 50-100 µl RRB directly onto HiPure DR Column. Incubate at room temperature for 5 min. Then Centrifuge it at 13000 rpm for 2 min.

Troubleshooting

Here we try to cover as many problems as you may see in using this product, however scientists in ROJE technical support team are eager to answer all your questions. Do not hesitate to contact us for more information.

Symptoms	Problem	Suggestion
Low yield	Incomplete sample lysis	• Dehydrate thoroughly samples before embedding. Residual formalin can inhibit the RJ-Protease function.
	Low-percentage ethanol	• Make sure using 96–100% molecular biology grade ethanol.
	Incorrect BWB1 and BWB2	• Prepare BWB1 and BWB2 with correct volume of 96–100% molecular biology grade ethanol, as mentioned in Table 2 and repeat the process with new sample.
Not perform well in downstream application	Formaldehyde modification results in fragmented DNA	• Do the incubation at stage 8 at 90°C, which removes most of the formaldehyde modifications. However, do not expect DNA purified from FFPE sections do as well as DNA purified from fresh sample in downstream application.
	Ethanol carryover	• Preform another centrifugation before rehydration step to ensure no remaining of Ethanol on column.
	Washing buffers do not work well	• Always mix BWB1 and BWB2 before each isolation process.
Clogged Column	Maximum amount of sample exceeded kit specifications	• Refer to specifications to determine if amount of starting material falls within kit specifications.
	Incomplete lysis	• Increase the lysis time to lysate the sample completely.

Appendix 1: Yield and Purity of DNA

The absorbance of DNA can be measured by any spectrophotometer. The ratio of absorbance at 260 nm and 280 nm is used to evaluate the purity of DNA. Pure DNA has an A260/A280 ratio of 1.7–2.0 and a symmetric absorbance peak at 260 nm. If the ratio is lower in either case, it may indicate the presence of contamination. Proteins have absorbance at 280 nm. EDTA, carbohydrate, and phenol all have absorbance near 230 nm.

Appendix 2: Convert RPM to RCF (centrifuge)

All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})(r)}}$$

Where **RCF** = required gravitational acceleration (relative centrifugal force in units of g); **r** = radius of the rotor in cm; and **RPM** = the number of revolutions per minute required to achieve the necessary g-force.

Ordering Information

Category	Product name	Cat NO.	Size
DNA Technologies	DNJia FFPE Tissue Kit	DN983057	50 preps
	DNJia FFPE Tissue Kit	DN983058	100 preps
Related Products	DNJia Plus Blood and Cell Kit	DN983047	50 preps
	DNJia Plus Blood and Cell Kit	DN983046	100 preps
	DNall Plus Kit	DN983048	50 preps
	DNall Plus Kit	DN983049	100 preps
	DNall Kit	DN983037	50 preps
	DNall Kit	DN983038	100 preps
	DNSol, MiniPrep	DN983002	50 preps
	DNSol, MiniPrep	DN983003	100 preps
	DNSol, MiniPrep	DN983004	200 preps
	DNSol, MidiPrep	DN983014	50 preps
	DNSol, MaxiPrep	DN983018	50 preps
	DNSol Clotted Blood Kit	DN983032	50 preps
	DNJia AmnioPure Kit	DN983044	50 preps
	DNJia AmnioPure Kit	DN983045	100 preps
	DNJia Plus Tissue and Bacteria Kit	DN983050	50 preps
	DNJia Plus Tissue and Bacteria Kit	DN983051	100 preps
	Hashin	LD983003	2ml
	Sor	LD983005	2ml
	RJ-Protease, Recombinant (20mg/ml)	EB983121	1ml

Technical Assistance

ROJETechnologies guarantee your complete satisfaction. ROJE technical support team comprises highly trained, experienced scientists who can troubleshoot most problems you face. Our technical support team can offer expert advice to help you select a suitable product.

Contact our technical support at any time by selecting one of these ways:

• Through our telephone and fax number available at the ROJETechnologies website.

• You can submit your question directly to ROJE technical support Team from our website (www.rojetechnologies.com)

• Or send your questions to this email address, ROJETechnologies guarantee your complete satisfaction. ROJE technical support team comprises highly trained, experienced scientists who can troubleshoot most problems you face. Our technical support team can offer expert advice to help you select a suitable product.

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• You can submit your question directly to ROJE technical support Team from our website (www.rojetechnologies.com)

• Or send your questions to this email address, Technicalsupport@Rojetechnologies.com

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