GeneDia

FFPE DNA Extraction Kit MANUAL

GENEDIA™ **life Science Co**.

Product # EK0450R

 # EK04100R

 # EK04200R

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Introduction

The **GENEDIA™ FFPE DNA Extraction Kit** provides a rapid method for the isolation and purification of genomic DNA from formalin-fixed paraffin-embedded (FFPE) tissue samples. Using formalin to fix tissues leads to crosslinking of the nucleic acids and proteins, and the process of embedding the tissue samples can also lead to fragmentation of the nucleic acids over time. The **GENEDIA™ FFPE DNA Extraction Kit** provides conditions that allow for the partial reversing of the formalin modifications, resulting in a high quality and yield of nucleic acids. The DNA is purified from other cellular components without the use of phenol or chloroform. The core kits contain the core items like binding column and buffers and accessories like enzymes. The purified genomic DNA is of the highest integrity, and can be used in a number of downstream applications including qPCR, mutation screening, microarray analyses, sequencing, Southern blotting and SNP analysis.

Kit Specifications

Purification is based on spin column chromatography using **GENEDIA**'s proprietary resin as the separation matrix. The DNA is preferentially purified from other cellular components without the use of phenol or chloroform. First, Paraffin Dissolver enables an efficient deparaffinization of the sample. Next, the FFPE samples are digested with the provided Proteinase K and Digestion Buffer –GeneLB - using an incubation time which is specific for the recovery of DNA. Absolute Ethanol is then added to the lysate, and the solution is loaded onto a spin-column. GENEDIA's column binds nucleic acids in a manner that depends on ionic concentrations, thus only the DNA will bind to the column while the contaminants will be removed in the flowthrough or retained on the top of the resin. The bound DNA is then washed with the provided Wash Solution in order to remove any impurities, and the purified DNA is eluted with GeneEB.

Kit Components

Components	Product # EK0450R (50 preps)	Product # EK04100R (100 preps)	Product # EK04200R (200 preps)
Paraffin Dissolver	20 ml	40 ml	40 ml*4
GeneLB (Lysis)	10 ml	20 ml	40 ml
GeneWB1 (Wash 1)	12 ml	22 ml	43 ml
GeneWB2 (Wash 2)	7 ml	13 ml	25 ml
GeneEB (Elution)	2 ml	5 ml	10 ml
Proteinase K	1 ml	2 ml	2 ml*2
Spin Columns	50	100	200
Elution tubes	50	100	200
Product Insert	1	1	1

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Storage Conditions

All components of the **GENEDIA™ FFPE DNA Extraction Kit** should be stored at room temperature (20-25 °C) and are stable for 1 year. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

Recommended Equipment and Reagents

- 56-65 °C incubator
- Benchtop microcentrifuge
- Sampler in 100 to 1000 Microliter size
- Sampler tips
- 2 ml microcentrifuge tubes
- Vortex
- Absolute Ethanol
- 70% ethanol

Precautions and Disclaimers

- Prior to using the protocol, Genetic ID recommends that care be taken in homogenizing the sample in a manner that minimizes the risk of inadvertently contaminating the sample. Contamination can occur using improperly cleaned equipment or using poor laboratory practices during homogenization, weighing and labeling of the subsample.
- The Buffer GeneLB contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.
- All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. 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.

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.

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Notes Prior to Use

• Prepare a working concentration of the **GeneWB1** by adding:

- **5 ml** of absolute Ethanol (not provided) to each of the bottles containing **12 ml** of concentrated **GeneWB1**. This will give a final volume of 17 ml for **Product # EK0450R**
- **9 ml** of absolute Ethanol (not provided) to the supplied bottle containing **22 ml** concentrated **GeneWB1**. This will give a final volume of 31 ml for **Product # EK04100R**
- **18 ml** of absolute Ethanol (not provided) to the supplied bottle containing **43 ml** concentrated **GeneWB1**. This will give a final volume of 61 ml for **Product # EK04200R**

The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

• Prepare a working concentration of the **GeneWB2** by adding:

- **10 ml** of absolute Ethanol (not provided) to each of the bottles containing **7 ml** of concentrated **GeneWB2**. This will give a final volume of 17 ml for **Product # EK0450R**
- **19 ml** of absolute Ethanol (not provided) to the supplied bottle containing **13 ml** concentrated **GeneWB2**. This will give a final volume of 32 ml for **Product # EK04100R**
- **37 ml** of absolute Ethanol (not provided) to the supplied bottle containing **25 ml** concentrated **GeneWB2**. This will give a final volume of 62 ml for **Product # EK04200R**

The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

Sampling and Extraction Procedure

A. Sample preparation

Cut **3-5 unstained, 10-micron** thick sections from the interior of an FFPE tissue block using a microtome. Trim off any excess paraffin.

Insert FFPE section(s) in a microcentrifuge tube (not provided).

The amount of embedded tissue can be up to 50 mg for GENEDIA™ DNA Extraction Kit.

B. Deparaffinization of the sample

1) Add **200 µl Paraffin Dissolver** to the sample.
2) Vortex the sample.
3) Incubate **5 minutes** at **56 °C** (to melt the paraffin).

Note: *Make sure that paraffin completely melts during the heat incubation step and is mixed well after melting in order for paraffin to be completely dissolved.*

4) Centrifuge the sample for **10 min** at **12,000 x g** (~ 12,000 RPM).
5) Remove Paraffin Dissolver without dislodging the pellet.
6) Repeat the steps 1 to 5.
7) Add **200 µl 70% ethanol**. Mix by vortexing.
8) Centrifuge sample for **10 minutes** at **12,000 x g** (~ 12,000 RPM).
9) Remove **70% ethanol** without dislodging the pellet.

Note: *It is important to remove the ethanol completely.*

10) Proceed to Step C. Lysate Preparation

C. Lysate Preparation

11) Add **200 µl GeneLB** and **20 µl Proteinase K**. Mix by vortexing

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12) Incubate for **6-8 hours** at **58 °C**, and after the lysing process wait for the sample to reach room temperature.

Note: *Most tissue samples will be digested or clarified within the time indicated. If significant amount of visible debris remains, centrifuge the samples at 12,000 x g (~ 12,000 RPM) for 2 minutes and transfer the supernatant to a new microcentrifuge tube (not provided).*

13) After the lysing process wait for the sample to reach room temperature.
14) Add **400 µl absolute Ethanol** (not provided) and mix by vortexing (2 x 5 s).

D. Binding DNA to Column

14) Assemble a Spin column with collection tube.
15) Transfer lysate onto the column and incubate for 2 minutes at room temperature.
16) Centrifuge at **8,000 x g** (~ 6,000 RPM) for **2 minutes**.
17) Discard the flowthrough. Reassemble the spin column with its collection tube.

Typically, samples will pass through the columns within ≤ 1 minute (in less than 1 minute). If the entire volume has not passed, spin for an additional minute.

E. Column Wash

1st wash

18) Add **300 µl Buffer GeneWB1** to the GENEDIA DNA Spin column.
19) Centrifuge at **11,000 x g** (~ 10,000 RPM) for **1 minute**.
20) Discard the flowthrough. Reassemble the spin column with its collection tube.

2nd wash

21) Add **300 µl Buffer GeneWB2** to the column and centrifuge for **1 minute** at **11,000 x g** (~10,000 RPM).
22) Discard the flowthrough and reassemble the spin column with its collection tube.
23) Spin the column for **2 minutes** in order to thoroughly dry the resin. Discard the collection tube.

F. Elute DNA

24) Place the column into a fresh Elution tube provided with the kit.
25) Add **40 µl** of **GeneEB** to the column. Incubate the assembly at room temperature for 2 minutes.
26) Centrifuge for **1 minute** at **12,000 x g** (~12,000 RPM). Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

Note: *For an improved yield, elute the sample twice and use after concentration process*

G. Storage of DNA

The purified DNA may be stored at –20°C for a few weeks. It is recommended that samples be placed at –70°C for long term storage.

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Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	**Incomplete lysis of cells or tissue**	Ensure that the appropriate amount of Digestion Buffer GeneLB with added Proteinase K was used. Increase the incubation time
	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also “Clogged Column” below.
	Low DNA content in cells or tissues used	Different tissues and cells have different DNA contents, and thus the expected yield of DNA will vary greatly from these different sources. Please check literature to determine the expected DNA content of your starting material.
Clogged Column	**Insufficient solubilization of cells or tissues**	Ensure that the appropriate amount of lysis buffer was used for the number of cells or tissue.
	Maximum number of cells or amount of tissue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications
	Clarified lysate was not used for the binding step	Ensure that after the lysis step the sample is centrifuged if a significant amount of debris is present, and that only the clarified lysate is used in subsequent steps.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitate formation that can clog the columns.
DNA is Degraded	**FFPE sample is old**	The quality of DNA purified may drastically decrease in old samples. For best performance, freshly prepared samples are highly recommended

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Isolation Method of DNA from FFPE tissue samples

Workflow

Cut 3-5 unstained, 10 micron thick sections from the interior of an FFPE tissue block

Add 200 µl Paraffin Dissolver to the sample. Vortex & Incubate 5 min at 56 °C Centrifuge the sample for 10 min at 12,000 xg Remove Paraffin Dissolver without dislodging the pellet. Repeat these the steps

200µl 70% ethanol & Centrifuge at 12,000xg 10 min & Remove 70% ethanol without dislodging the pellet

200µl GeneLB + 20µl Proteinase K & Incubate for 6-8 h min at 58 °C

Add 400µl Ethanol Transfer onto the column & Incubate for 2 min at RT

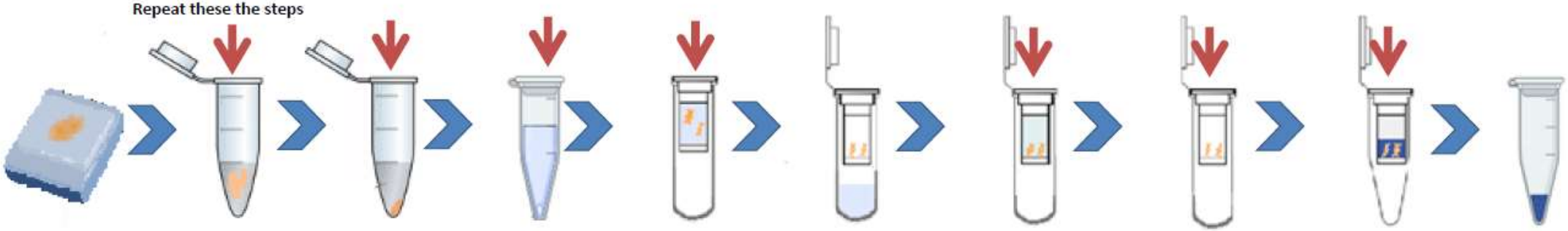
Centrifuge at 8,000xg 2 min Discard the flowthrough

Add 300 µl GeneWB1 and Centrifuge at 11,000xg 1 min Discard the flowthrough

Add 300 µl GeneWB2 and Centrifuge at 11,000xg 1 min Discard the flowthrough

Place the column into an Elution tube Add 40 µl GeneEB & Incubate for 2 min & Centrifuge 12,000xg 1 min

Purified DNA



Deparaffinization | Sample lysis | Nucleic acid Binding | Flow Through | 1st Wash | 2nd Wash | Elution

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