GeneDia

FFPE RNA Extraction Kit MANUAL

GENEDIA™ **life Science Co**.

Product # EK0350R

 # EK03100R

 # EK03200R

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Introduction

Formalin-fixed and paraffin-embedded (FFPE) tissue is commonly used in histopathological analysis. Recently, there is more and more interest in also investigating RNA modifications, RNA expression or miRNA profiles of old, archived FFPE samples. However, fixation, embedding and storage lead to crosslinking and fragmentation of RNA. Especially crosslinks cannot be detected by standard quality control assays, such as gel electrophoresis, spectrophotometry or microfluidic analysis, but the efficiency of enzymatic reactions is significantly reduced, for example in RT-PCR. Standard RNA purification procedures do not remove these chemical modifications and therefore result in low RNA yield or poor downstream application performance. The **GENEDIA™ FFPE RNA Extraction Kit** implements buffers and procedural steps to efficiently de-crosslink nucleic acids and yield high quality RNA for the most demanding applications.

Kit Specifications

The **GENEDIA™ FFPE RNA Extraction Kit** uses a simple, reliable, phenol-free protocol to isolate RNA from FFPE tissue sections. First, tissue sections are deparaffinized with Paraffin Dissolver and then washed in ethanol-based solutions to remove the deparaffinization Reagent. Next, the sections are treated with Proteinase K to solubilize the fixed tissue and release the nucleic acids into solution. Finally, total RNA is isolated using the **GENEDIA™ FFPE RNA Extraction Kit**. The RNA isolation protocol begins with suspending the sample in Absolute Ethanol, containing a strong protein denaturant (the chaotropic salt, guanidine salt), which prevents ribonuclease (RNase) degradation of the RNA. The sample is filtered using a micro-spin containing a silica-based fibre matrix. The nucleic acids in the sample bind to the fibre matrix and then the RNA component is efficiently removed from the matrix-bound sample. The immobilized RNA is washed to remove contaminants, and total RNA is recovered in a final volume of 30 µl. The isolated pure RNA is ready for gene expression analysis by qRT-PCR.

Kit Components

Components	Product # EK0350R (50 preps)	Product # EK03100R (100 preps)	Product # EK03200R (200 preps)
Paraffin Dissolver	20 ml	40 ml	40 ml*2
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	4 ml	8 ml
Proteinase K	1 ml	2 ml	2 ml*2
Spin Columns	50	100	200
Collection Tubes	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 RNA 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
- 70% ethanol
- Absolute ethanol
- Carrier RNA (for quantitative precipitation/purification of RNA. It improves recovery of short fragments (< 200 bp) or low amounts of nucleic acids.)

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 # EK0350R**
- **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 # EK03100R**
- **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 # EK03200R**

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 # EK0350R**
- **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 # EK03100R**
- **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 # EK03200R**

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

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™ RNA 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: *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

(Optional): Add 10 µl Carrier RNA can be improving recovery of short fragments (< 200 bp) or low amounts of nucleic acids.)

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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 number 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 RNA 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 minutes (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 RNA Spin column.
19) Centrifuge at **11,000 x g** (~ 10,000 RPM) for **1 minutes**.
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 minutes** 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 RNA

24) Place the column into a fresh Elution tube provided with the kit.
25) Add **40 µl** of **GeneEB** (preheated at 60°C) to the column. Incubate the assembly at room temperature for 2 minute.
26) Centrifuge for **1 minute 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 RNA

The purified RNA 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 RNA Recovery	**Incomplete lysis of cells or tissue**	Ensure that the appropriate amount of Digestion Buffer GeneLB with Proteinase K added 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 RNA content in cells or tissues used	Different tissues and cells have different RNA contents, and thus the expected yield of RNA will vary greatly from these different sources. Please check literature to determine the expected RNA 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 number 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 precipitates to form that can cause the columns to clog.
RNA is Degraded	**FFPE sample is old**	The quality of RNA purified may drastically decrease in old samples. For best performance, freshly prepared samples are highly recommended

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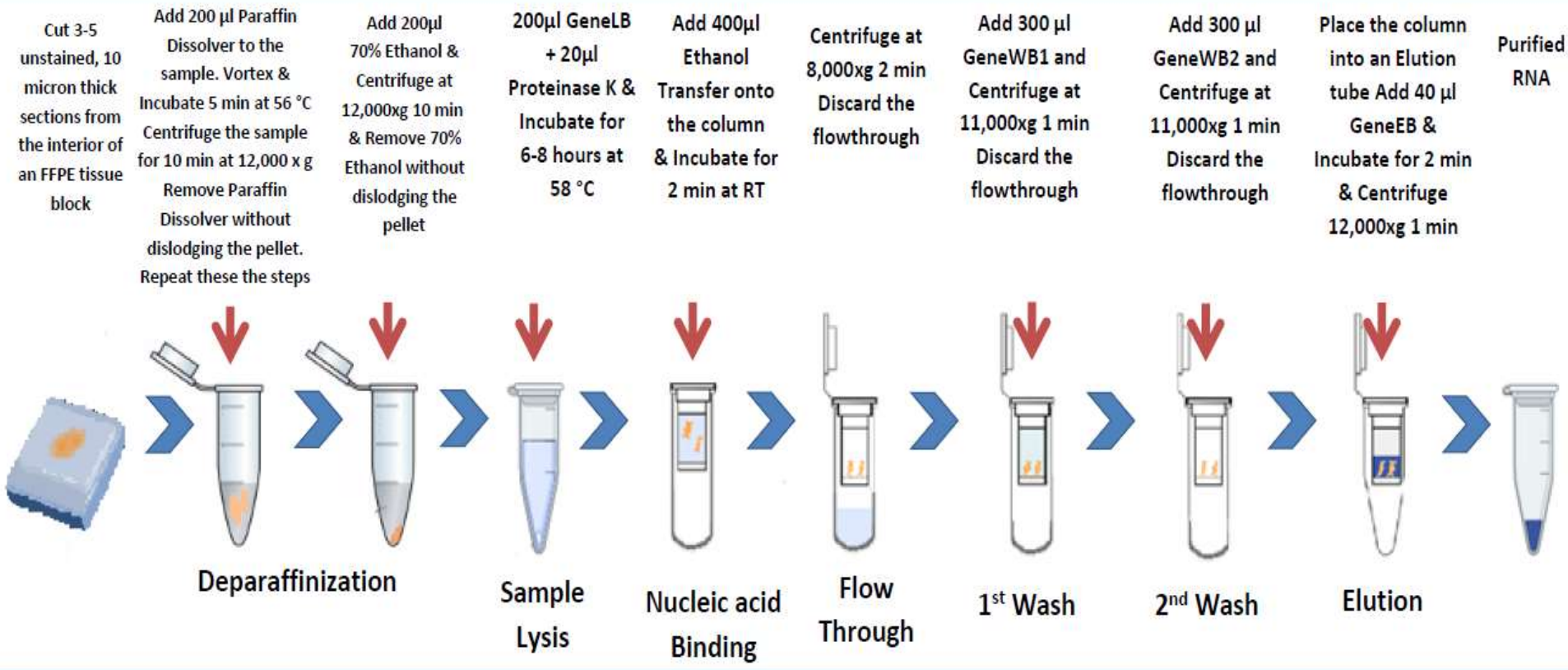
Isolation Method of RNA from FFPE tissue samples

Workflow



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