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Product Information

GenElute™ FFPE RNA/DNA Purification Plus Kit
Product Number RDP200

TECHNICAL BULLETIN

Product Description

GenElute FFPE RNA/DNA Purification Plus Kit provides a rapid method for the sequential isolation and purification of total RNA (including microRNA) and 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.

GenElute FFPE RNA/DNA Purification Plus Kit provides conditions that allow for the partial reversing of the formalin modifications, resulting in a high quality and yield of nucleic acids. The kit is able to purify all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA), depending on the age of the FFPE tissue as the degree of fragmentation of the RNA will increase over time. The RNA is purified from other cellular components without the use of phenol or chloroform. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays. The purified genomic DNA is also of the highest quality, and can be used in PCR reactions, sequencing, Southern blotting and SNP analysis.

Purification is based on spin column chromatography using the proprietary resin as the separation matrix. The nucleic acids are preferentially purified from other cellular components without the use of phenol or chloroform. The process first involves deparaffinization of the FFPE samples through a series of xylene and ethanol washes. Next, the FFPE samples are digested with the provided Proteinase K and Digestion Buffer A using an incubation time which is specific for the recovery of RNA (please see the flow chart on page 3). The soluble lysate containing the RNA is then collected for RNA purification while the remaining sample is further digested for DNA. Buffer RL and ethanol are then added to the lysate containing RNA or DNA, and the solution is loaded onto an RNA Purification Micro Column or a DNA Purification Micro Column, respectively. The resin binds nucleic acids in a manner that depends on ionic concentrations, thus only the RNA or DNA will bind to the column while the contaminants will be removed in the flow through or retained on the top of the resin. The bound nucleic acid is then washed with the provided Wash Solution in order to remove any impurities, and the purified nucleic acid is eluted with the Elution Solution A or Elution Buffer F.

Components

Component	50 preps
Digestion Buffer A	2 x 25 mL
Buffer RL	40 mL
Enzyme Incubation Buffer A	6 mL
Wash Solution A	2 x 38 mL
Elution Solution A	6 mL
Elution Buffer F	15 mL
Proteinase K	2 x 12 mg
DNase I	1 vial
RNA Purification Micro Columns	50
DNA Purification Micro Columns	50
Collection Tubes	100
Elution Tubes	100

Reagents and Equipment Required But Not Provided

You must have the following in order to use the FFPE RNA/DNA Purification Plus Kit:

- Benchtop microcentrifuge
- 50–55 °C Incubator or Water Bath
- 80 °C Incubator or Water Bath
- 90 °C Incubator or Water Bath
- 96-100% ethanol
- Xylene, histological grade
- Ice Bucket

Precautions and Disclaimer

This kit is designed for research purposes only. It is not intended for human or diagnostic use.
Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Safety Data Sheets (SDSs).

The **Buffer RL** 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

RNases are very stable and robust enzymes that degrade RNA. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes. The RNA area should be located away from microbiological work stations. Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination. There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only. All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water. Clean all surfaces with commercially available RNase decontamination solutions.When working with purified RNA samples, ensure that they remain on ice during downstream applications.

Reagents to be prepared

Before beginning the procedure, prepare the following:

1) Reconstitute each vial of the **Proteinase K** in 600 µL of molecular biology grade water, aliquot into small fractions and store the unused portions at −20 °C until needed.
2) Prepare a working concentration of each bottle of the **Wash Solution A** by adding 90 mL of 96-100% ethanol (provided by the user) to the supplied bottles containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottles has a box that may be checked to indicate that the ethanol has been added. **Wash Solution A** is used for both RNA and DNA Purification.

Storage/Stability

All solutions should be kept tightly sealed and stored at room temperature. The DNase I and Proteinase K should be stored at −20 °C upon receipt. These reagents should remain stable for at least 1 year in their unopened containers.

Procedure

Note:

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.

This kit is provided with 2 separate columns. When columns are removed from the labelled bags they are supplied in they can easily be identified as follows:

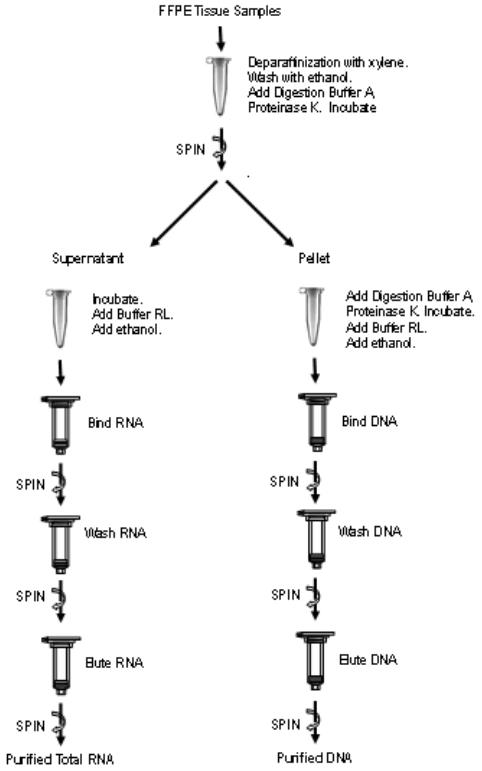
- DNA Purification Micro Columns – Column has predominately white contents with blue o-ring
- RNA Purification Micro Columns – Column has predominately black contents

There are primarily 3 sections to the procedure of RNA/DNA Total extraction using **RNA/DNA Purification kit.**

Section 1: Deparaffinization of FFPE Samples
Section 2: Total RNA Purification
Section 3: Total DNA Purification

Flowchart

Procedure for Purifying Total RNA and DNA using FFPE RNA/DNA Purification Plus Kit



Section 1: *Deparaffinization of FFPE Samples*

1. Deparaffinization

a. Cut four sections up to 20 μm thick from the interior of an FFPE tissue block using a microtome. Trim off any excess paraffin.

Note: Alternatively, from an FFPE block, cut out up to 10 mg of unsectioned core. Trim off any excess paraffin. Grind the sample into fine powder using liquid nitrogen.

b. Transfer the sections or ground block into an RNase-free microcentrifuge tube.
c. Add 1 mL of xylene to the sample. Mix by vortexing.
d. Incubate at 50 °C for 5 minutes.
e. Centrifuge the sample at 14,000 x g (~ 14,000 RPM) for 2 minutes.
f. Carefully remove the xylene without dislodging the pellet.
g. Add 1 mL of 96 - 100 % ethanol. Mix by vortexing.
h. Centrifuge the sample at 14,000 x g (~ 14,000 RPM) for 2 minutes.
i. Carefully remove the ethanol without dislodging the pellet.
j. Repeat Step **1g** to Step **1i** for a second time.
k. Air dry the pellet for about 10 minutes at room temperature.

Note: It is important to remove the ethanol completely.

l. Proceed to **Section 2**. **Total RNA Purification**

Section 2: Total RNA Purification

2. Lysate Preparation

a. Add 300 μL of Digestion Buffer A and 10 μL of the reconstituted Proteinase K to the sample. Mix by vortexing.
b. Incubate at 55 °C for 15 minute. Vortex to mix occasionally.
c. Allow the sample to cool down by placing the tube containing the sample on ice for 3 minutes.
d. Centrifuge the sample at 14,000 x g (~ 14,000 RPM) for 3 minutes.
e. Carefully transfer the RNA-containing supernatant to a new an RNase-free microcentrifuge tube. Retain the microcentrifuge tube containing the pellet for DNA Purification.

Note: The DNA-containing pellet can be stored for 2 hours at room temperature, for up 24 hours at 2–8 °C, or at –20 °C for extensive storage.

f. Incubate the tube of the RNA-containing lysate at 80 °C for 15 minutes. Vortex to mix occasionally.

Note: Do not exceed 15 minutes of incubation at 80 °C as this will increase RNA fragmentation.

g. Add 300 μL of Buffer RL. Vortex to mix.
h. Add 600 μL of 96-100 % ethanol. Vortex to mix.

3. **Binding RNA to Column**

a. Assemble an RNA Purification Micro Column with one of the provided collection tubes
b. Apply up to 600 μL of the lysate with the ethanol (from **Step 2h**) onto the column and centrifuge for 1 minute at **≥ 3,500 x g (~6,000 RPM).**

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at **14,000 x *g* (~14,000 RPM)**.

c. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Repeat Step **3b** and **3c** until all lysate has passed through the column.

Optional Step: The FFPE RNA/DNA Purification Plus Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications. This step should be performed at this point in the protocol using the provided DNase I.

4. RNA Column Wash

a. Apply 500 μL of **Wash Solution A** to the column and centrifuge for 1 minute at 14,000 x g (~ 14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Apply 500 µL of **Wash Solution A** to the column and centrifuge for 1 minute at 14,000 x g (~ 14,000 RPM).
c. Discard the flowthrough and reassemble the spin column with its collection tube
d. Wash column a third time by adding another 500 µL of **Wash Solution A** and centrifuging for 1 minute at 14,000 x g (~ 14,000 RPM).
e. Discard the flowthrough and reassemble the spin column with its collection tube.
f. Spin the column for 2 minutes 14,000 x g (~ 14,000 RPM) in order to thoroughly dry the resin. Discard the collection tube.

5. RNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 20-50 µL of **Elution Solution A** to the column.
c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by a 1 minute spin at **14,000 x *g* (~14,000 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: As little as 15 µL of **Elution Solution A** could be used for higher RNA concentration. However, the RNA yield may be reduced when a smaller elution volume is used. For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 5b** and **5c**).

6. Storage of RNA

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

Section 3: Total DNA Purification

7. Lysate Preparation

a. Add 300 µL of Digestion Buffer A and 10 µL of the reconstituted Proteinase K to the DNA-containing pellet obtained from Step 2e. Mix by vortexing.
b. Incubate at 55 °C for 1 hour. Vortex to mix occasionally.
c. Incubate at 90 °C for 2 hour. Vortex gently occasionally to mix.
d. Allow the sample to cool down by placing the tube containing the sample on ice for 3 minutes.

Note: The FFPE RNA/DNA Purification Plus Kit isolates DNA with minimal amounts of RNA contamination. However, if it is desirable to remove any trace amount of RNA, add 4 µL of RNase A (10 mg/mL) to the cooled lysate and incubate at room temperature for 5 minutes.

e. Add 300 µL of Buffer RL. Vortex to mix.
f. Add 250 µL of 96–100 % ethanol. Vortex to mix.

8. Binding DNA to Column

a. Assemble an DNA Purification Micro Column with one of the provided collection tubes.
b. Apply up to 600 µL of the lysate with the ethanol (from **Step 7f**) onto the column and centrifuge at 14,000 x g (~ 14,000 RPM) for 1 minute.
c. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Repeat Step **8b** and **8c** until all lysate has passed through the column.

9. DNA Column Wash

a. Apply 600 µL of Wash Solution A to the column and centrifuge for 1 minute at 14,000 x g (~ 14,000 RPM).

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flow through and reassemble the spin column with its collection tube. Apply 600 µL of Wash Solution A to the column and centrifuge for 1 minute at 14,000 x g (~ 14,000 RPM).
c. Discard the flow through and reassemble the spin column with its collection tube
d. Wash column a third time by adding another 600 µL of Wash Solution A and centrifuging for 1 minute at 14,000 x g (~ 14,000 RPM).
e. Discard the flow through and reassemble the spin column with its collection tube.
f. Spin the column for 2 minutes 14,000 x g (~ 14,000 RPM) in order to thoroughly dry the resin. Discard the collection tube.

10. DNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 20 -50 µL of Elution Buffer F to the column. Incubate the assembly at room temperature for 1 minute.
c. Centrifuge 1 minute at 14,000 x g (~14,000 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 maximum DNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat Steps 10b and 10c).

11. Storage of DNA

The purified DNA may be stored at 4 °C for a few days. It is recommended that samples be placed at –20 or –70 °C for long term storage.

Appendix A

Protocol for Optional On-Column DNA Removal

Notes Prior to Use

- This optional step is carried out if genomic DNA-free RNA is required.
- Prepare a DNase I mixture by adding 4 µL of the provided RNase-free DNase I to 96 µL of **Enzyme Incubation Buffer A** for each isolation.

a. Apply 500 µL of **Wash Solution A** to the column and centrifuge at 14,000 x g (~14,000 RPM) for 2 minutes. Discard the flowthrough.

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Apply 100 µL **Enzyme Incubation Buffer A** mix containing the RNase-free DNase I to the column and centrifuge at 14,000 x g (~14,000 RPM) for 1 minute.

Note: Ensure that the entire volume of DNase I mix passes through the column. If needed, spin at 14,000 x g for an additional minute.

c. After the centrifugation in Step b, pipette the flowthrough that is present in the collection tube back onto the top of the column.

Note: Ensure that Step c is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

d. Incubate at room temperature for 15 minutes.
e. Proceed to Step **4b** without further centrifugation.

Note: Add the **Wash Solution A** directly to the column containing the **Enzyme Incubation Buffer A** mix containing the RNase-free DNase I.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA or DNA Recovery	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of **Digestion Buffer A** and 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.
	An alternative elution solution was used	It is recommended that the **Elution Solution A** and **Elution Buffer F** supplied with this kit be used for maximum RNA or DNA recovery.
	Ethanol or Buffer RL was not added to the lysate	Ensure that the appropriate amount of ethanol and Buffer RL is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solutions	Ensure that 90 mL of 96-100% ethanol is added to the supplied **Wash Solution A** prior to use.
	Low nucleic acid content in cells or tissues used	Different tissues and cells have different nucleic acid contents, and thus the expected yield of nucleic acid will vary greatly from these different sources. Please check literature to determine the expected nucleic acid content of your starting material.
Clogged Column	Insufficient solubilization of cells or tissues	Ensure that the appropriate amount of **Digestion Buffer A** with Proteinase K added was used. Increase the incubation time.
	Maximum number of sections 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 significant precipitates are 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.

Problem	Possible Cause	Solution and Explanation
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.
	RNase contamination	RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to "*Working with RNA"* at the beginning of this user guide.
	Too long incubation of lysate at high temperature	In order to maintain the integrity of the RNA, it is important that the procedure be performed according to the time indicated. This is especially important for the lysate preparation step when the sample is incubated at 55 and 80°C for 15 minutes each. Incubation beyond the time indicated may lead to fragmentation of RNA.
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at –20 °C for a few days. It is recommended that samples be stored at –70 °C for longer term storage.
	Prolonged incubation at 55 °C	In order to reverse formalin crosslinks, an incubation at 55 °C is required which may lead to degraded RNA.
Nucleic acids does not perform well in downstream applications	Nucleic acids were not washed 3 times with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed 3 times with Wash Solution. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
	Formalin crosslink was not completely reversed	Ensure the sufficient incubation at 80 or 90 °C is performed to remove formalin crosslink.
Genomic DNA contamination	Large amounts of starting material used	Perform RNAse-free DNaseI digestion on the RNA sample after elution to remove genomic DNA contamination.

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