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Product Information

GenElute™ FFPE RNA Purification

Catalog number **RNB400**

Product Description

Sigma's GenElute™ FFPE RNA Purification Kit provides a rapid method for the isolation and purification of total RNA (including microRNA) from formalin-fixed paraffin-embedded (FFPE) tissue samples. Using formalin to fix tissues leads to crosslinking of the RNA and proteins, and the process of embedding the tissue samples can also lead to fragmentation of the RNA over time. The GenElute™ FFPE RNA Purification Kit provides conditions that allow for the partial reversing of the formalin modifications, resulting in a high quality and yield of RNA. 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 preferentially 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 qRT-PCR, reverse transcription PCR, primer extension, expression array assays, and microarray analyses.

Purification is based on spin column chromatography using proprietary resin as the separation matrix. The RNA is 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. Buffer RL and ethanol are then added to the lysate, and the solution is loaded onto a spin-column. The resin binds nucleic acids in a manner that depends on ionic concentrations, thus only the RNA will bind to the column while other contaminants will be removed in the flowthrough or retained on the top of the resin. At this point, any remaining traces of genomic DNA can be digested using an optional protocol, allowing for pure RNA samples to be isolated. The bound RNA is then washed with the provided RNA Wash Solution A in order to remove any impurities, and the purified total RNA is eluted with the Elution Solution A.

Components

Component	50 preps
Digestion Buffer A	25 mL
Buffer RL	30 mL
Enzyme Incubation	6 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Proteinase K	12 mg
DNase I	1 vial
Micro Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Reagents and Equipment Required But Not Provided

You must have the following in order to use the GenElute™ FFPE RNA Purification Kit:

For All Protocols

- Benchtop microcentrifuge
- 96-100% ethanol
- Xylene, histological grade

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).
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

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 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. 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. Care must be taken not to introduce RNAse especially during the final wash and elution.

Reagents to be prepared

Before beginning the procedure, prepare the following:

1) Reconstitute 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 the Wash Solution A by adding 90 mL of 96-100% ethanol to the supplied bottle(s) containing the concentrated Wash Solution A. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

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 arrival. These reagents should remain stable for at least 2 years 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:

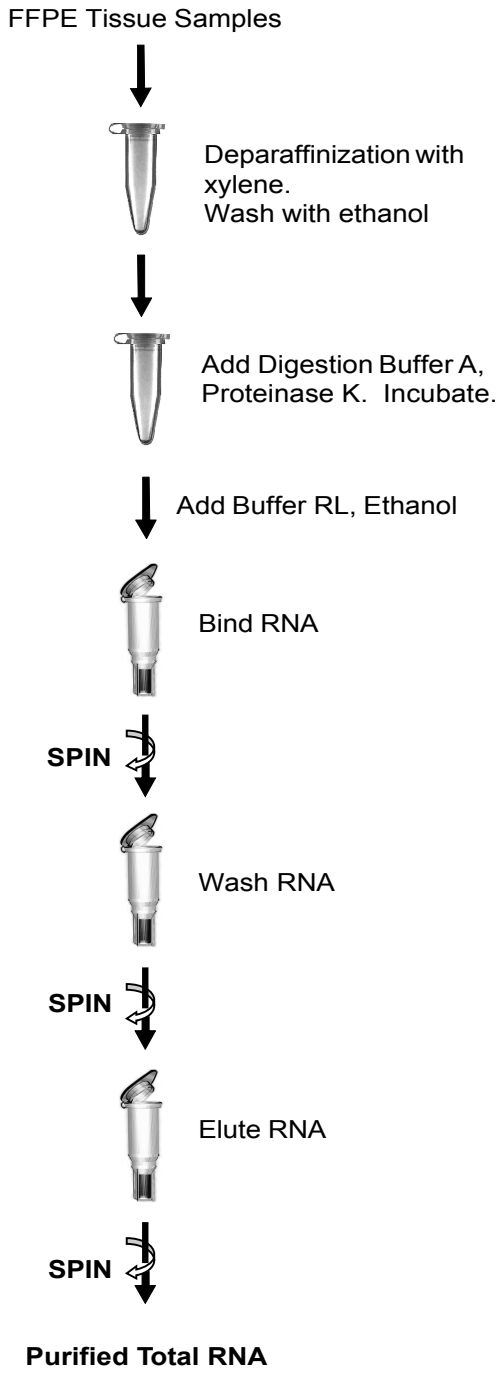
$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

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.

The maximum recommended input is five sections of ≤ 20 µm thick. Alternatively, an unsectioned block of up to 25 mg may be used. It is important to obtain sections from the interior of an FFPE block in order to minimize RNA damage by oxidation. It is important to work quickly during this procedure.

Flowchart

Procedure for purifying Total RNA using GenElute™ FFPE RNA Purification Kit



Section 1: Deparaffinization

Cut 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 25 mg of unsectioned core. Trim off any excess paraffin. Grind the sample into fine powder using liquid nitrogen.

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

Note: It is important to remove the ethanol completely.

Section 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 minutes, followed by 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.

c. Add 300 μL of **Buffer RL**. Vortex to mix.
d. Add 600 μL of 96-100 % ethanol. Vortex to mix.

Section 3. Binding RNA to Column

a. Assemble a column with one of the provided collection tubes
b. Apply up to 600 μL of the lysate with the ethanol (from **Step 2**) 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:

This FFPE RNA Purification 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.

Section 4. Column Wash

a. Apply 400 μL of **Wash Solution A** to the column and centrifuge for 1 minute.

Note: Ensure the entire Wash Solution A 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 flowthrough and reassemble the spin column with its collection tube.
c. Apply 400 μL of **Wash Solution A** to the column and centrifuge for 1 minute.
d. Discard the flowthrough and reassemble the spin column with its collection tube
e. Wash column a third time by adding another 400 μL of **Wash Solution A** and centrifuging for 1 minute.
f. Discard the flowthrough and reassemble the spin column with its collection tube.
g. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

Section 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. Incubate the assembly at room temperature for 1 minute.

Note: Elution Solution A is Nuclease-Free Water

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: For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 5b** and **5c**).

Section 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.

Protocol for Optional On-Column DNA Removal

Notes before 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.

Procedure:

a. Apply 400 µL of **Wash Solution A** to the column and centrifuge for 2 minutes. Discard the flowthrough.

Note: Ensure the entire Wash Solution A 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 of **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 **4c** without further centrifugation.

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 A 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.
	An alternative elution solution was used	It is recommended that the Elution Solution A supplied with this kit be used for maximum RNA recovery.
	Ethanol or Buffer RL was not added to the lysate	Ensure that the appropriate amount of ethanol and Buffer RL are added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that 90 mL of 96 - 100% ethanol is added to the supplied Wash Solution A prior to use.
	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 Digestion Buffer A was used for the amount 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 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.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly. This is especially important for the Cell Lysate Preparation Step in the Animal Tissue protocol, since the RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized.
	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 80°C	In order to reverse formalin crosslinks, an incubation at 80°C is required. Do not exceed 15 minutes of incubation at 80°C as this will increase RNA fragmentation.
RNA does not perform well in downstream applications	RNA was not washed 3 times with the provided Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed 3 times with Wash Solution A. 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°C is performed in Step **2b**. Do not exceed 15 minutes of incubation at 80°C as this will increase RNA fragmentation.

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