Presto™ FFPE RNA Mini Kit

For research use only

Sample: up to 25 mg sections of FFPE, 4~10 sections of 5~20 µm thick FFPE

Yield: up to 50 µg of total RNA

Format: spin column

Operation Time: within 80 minutes

Elution Volume: 25-100 µl

Storage: dry at room temperature (15-25ºC)

Geneaid

CERTIFICATE NO. QAIC/TW/50077
ISO 9001:2015 QMS

Introduction

The Presto™ FFPE RNA Mini Kit was designed specifically for purifying total RNA from formalin-fixed, paraffin-embedded (FFPE) tissue. Paraffin is initially dissolved with organic solvents such as Xylene followed by Proteinase K digestion. RNA in chaotropic salt is then bound by the glass fiber matrix of the spin column and DNA contamination is removed using an in column DNase treatment. Other contaminants are removed using Wash Buffer and the purified total RNA is eluted in RNase-free Water. High quality total RNA can be purified within 80 minutes without phenol extraction or alcohol precipitation. The purified RNA is ready for use in RT-PCR, primer extension, mRNA selection and cDNA synthesis.

Quality Control

The quality of the Presto™ FFPE RNA Mini Kit is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. Total RNA is isolated from 25 mg sections of FFPE, quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Contents

Component	RF004 RFD004	RF050 RFD050	RF100 RFD100
RT Buffer	1.5 ml	15 ml	30 ml
RB Buffer	2 ml	30 ml	60 ml
Proteinase K[1] (Add RNase-free Water)	1 mg (0.1 ml)	11 mg (1.1 ml)	11 mg x 2 (1.1 ml x 2)
DNase I (2U/µl)[2] (RFD004/050/100 only)	20 µl	275 µl	550 µl
DNase I Reaction Buffer (RFD004/050/100 only)	200 µl	2.5 ml	5 ml
W1 Buffer	2 ml	30 ml	50 ml
Wash Buffer[3] (Add Ethanol)	1.5 ml (6 ml)	25 ml (100 ml)	25 ml + 12.5 ml (100 ml + 50 ml)
RNase-free Water	1 ml	15 ml	15 ml
RB Column	4 pcs	50 pcs	100 pcs
2 ml Collection Tube	8 pcs	100 pcs	200 pcs

IMPORTANT BEFORE USE!

[1]Add RNase-free Water to Proteinase K (see the bottle label for volume) then vortex to ensure it is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. For extended periods, the RNase-free Water and Proteinase K mixture should be stored at 4ºC.

[2]DNase I is shipped at room temperature and should be stored at -20ºC for extended periods.

[3]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation. The additional Wash Buffer x 12.5 ml is only included in RFD100.

Caution

RB Buffer and W1 Buffer contain chaotropic salt. During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

Steps to prevent RNase contamination

Disposable and nondisposable plasticware and automatic pipettes should be sterile and used only for RNA procedures.

Additional Requirements

Xylene, Limonene or CitriSolv, Absolute Ethanol, ß-mercaptoethanol, 60ºC and 80ºC dry bath incubators

Troubleshooting

Clogged column

Reduce the amount of starting material or separate it into multiple tubes. Centrifugation temperature must be between 20ºC to 25ºC. Lower centrifuge temperature can cause formation of precipitates that can clog the RB Column.

Low RNA yield

Samples that were stored for very long periods and mounted on microscope slides may contain lower amounts of RNA. At the RNA elution step, add RNase-free Water into the CENTER of the column matrix and let stand for 5 minutes to ensure the RNase-free Water is absorbed. Repeat the elution step by adding the eluate into the RB Column and centrifuge again, the additional elution step can raise the RNA concentration.

DNA contamination

Reduce sample amount. Perform In Column DNase I Digestion or DNA Digestion In Solution.

RNA does not perform well in downstream applications

Residual ethanol contamination. Following the wash step, dry the RB Column with additional centrifugation at 14-16,000 x g for 5 minutes. At the sample lysis step, 80ºC incubation is crucial for optimal RNA performance in downstream applications.

FFPE RNA Extraction Protocol

Step 1 Deparaffinization

- Cut up to **25 mg sections of FFPE** or **4~10 sections of 5~20 µm thick FFPE sections**.
- Transfer sections to a 1.5 ml microcentrifuge tube.
- Add **1 ml of Xylene**, **Limonene** or **CitriSolv** then mix by shaking vigorously.
- Incubate at room temperature for approximately 10 minutes.
- Centrifuge at 14-16,000 x g for 3 minutes then remove the supernatant.
- Add **1 ml ethanol (96–100%)** to the pellet, vortex to mix then centrifuge at 16,000 x g for 2 minutes.
- Carefully remove the supernatant by pipetting, being careful **NOT** to disturb the pellet.
- Open the tube and incubate at room temperature for 10 minutes to evaporate residual ethanol.

Step 2 Sample Lysis

- Add **200 µl of RT Buffer** and **20 µl of Proteinase K (10 mg/ml)** to the pellet then vortex to mix.
- Incubate at 60ºC for 15-30 minutes. During incubation, invert the tube every 5 minutes.
- Incubate at 80ºC for 15 minutes.

NOTE: If using only one dry bath incubator, leave the sample at room temperature after the 60ºC incubation until the incubator has reached 80ºC. The 15 minute incubation at 80ºC is critical for reversal of crosslinks and optimal RNA performance in downstream applications such as real-time RT-PCR.

- Cool the sample on ice for 3 minutes then centrifuge at 14-16,000 x g for 15 minutes.
- Transfer the supernatant to a new 1.5 ml tube, being careful **NOT** to disturb the pellet.

Step 3 RNA Binding

- Add **400 µl of RB Buffer** and **4 µl of β-mercaptoethanol** then mix well by vortex. Incubate at room temperature for 5 minutes.
- Add **800 µl of absolute ethanol (96-100%)** and mix well by vortex.
- Place a **RB Column in a 2 ml Collection Tube**. Transfer **700 µl** of sample to the RB Column.
- Centrifuge at 14-16,000 x g for 1 minute then discard the flow-through.
- Transfer the remaining mixture to the same RB Column. Centrifuge at 14-16,000 x g for 1 minute.
- Discard the flow-through then place the RB Column in a new 2 ml Collection Tube.

Optional Step 1: In Column DNase I Digestion

The amount of DNA contamination is significantly reduced following In Column DNase I Digestion. However, traces of residual DNA may be detected in very sensitive applications. In this situation, please perform Optional Step 2: DNA Digestion In Solution instead to efficiently remove trace amounts of DNA. Standard DNase buffers are incompatible with In Column DNase I Digestion and may affect RNA integrity and reduce yield.

1. Add 400 µl of Wash Buffer (make sure ethanol was added) to the RB Column then centrifuge at 14-16,000 x g for 30 seconds.
2. Discard the flow-through and place the RB Column back in the 2 ml Collection Tube.
3. Prepare DNase I solution in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

DNase I	5 µl (2 U/µl)
DNase I Reaction Buffer	45 µl
Total Volume	50 µl

4. Gently pipette DNase I solution (DO NOT vortex) then add DNase I solution (50 µl) into the CENTER of the RB column matrix.
5. Incubate the column for 15 minutes at room temperature (20-30ºC) then proceed with Step 4 RNA Wash.

Step 4 RNA Wash

- Add **400 µl of W1 Buffer** into the RB Column then centrifuge at 14-16,000 x g for 30 seconds.
- Discard the flow-through then place the RB Column back in the 2 ml Collection Tube.
- Add **600 µl of Wash Buffer (make sure ethanol was added)** into the RB Column.
- Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through.
- Place the RB Column back in the 2 ml Collection Tube.
- Add **600 µl of Wash Buffer (make sure ethanol was added)** into the RB Column.
- Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through.
- Place the RB Column back in the 2 ml Collection Tube.
- Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.

Step 5 RNA Elution

- Place the dried **RB Column** in a clean 1.5 ml microcentrifuge tube.
- Add **50 µl of RNase-free Water** to the CENTER of the column matrix.
- Let stand for at least 1 minute to ensure the **RNase-free Water** is absorbed by the matrix.
- Centrifuge at 14-16,000 x g for 1 minute to elute the purified RNA.

Optional Step 2: DNA Digestion In Solution

1. Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

RNA in RNase-free Water	1-40 µl
DNase I	0.5 µl/µg RNA
DNase I Reaction Buffer	5 µl
RNase-free Water	Add to final volume = 50 µl
Total Volume	50 µl

2. Gently pipette the DNase I reaction solution (DO NOT vortex) then incubate the tube at 37ºC for 15-30 minutes.
3. Stop the reaction by adding 1µl of 0.5 M EGTA (pH=8.0) then incubate the Tube at 75ºC for 10 minutes.

NOTE: DNase I Reaction Buffer may cause aberrant migration or smearing of RNA on gels. If analyzing RNA by gel electrophoresis, repurify the RNA sample by using the Geneaid™ RNA Cleanup Kit instead of stopping the reaction with EGTA and heat inactivation.