

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 60 minutes

Elution Volume: 25-100 µl

Storage: dry at room temperature (15-25°C)

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 60 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!

¹Add 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 4°C.

²DNase I is shipped at room temperature and should be stored at -20°C for extended periods.

³Add 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, 60°C and 80°C dry bath incubators

Troubleshooting

Clogged column

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

DNA contamination

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

Low RNA integrity

Typical RIN (RNA Integrity Number) of RNA purified with Presto™ FFPE RNA Mini Kit are in range of 2–6. However, the RIN values from degraded FFPE samples are not a sensitive measure of RNA quality. The DV₂₀₀ (percentage of RNA fragments that are > 200 nucleotides) developing by Illumina is used to assess FFPE RNA quality instead. In general, the DV₂₀₀ of purified RNA are in range of 30%-90% using Geneaid FFPE RNA kit. The RNA is qualified for NGS and RT-qPCR.

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, 80°C incubation is crucial for optimal RNA performance in downstream applications.

FFPE RNA Extraction Protocol

<p>Step 1 Deparaffinization</p>	<ul style="list-style-type: none"> • 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 vortex vigorously for 10 seconds. • Centrifuge at 14-16,000 x g for 2 minutes then remove the supernatant without disturbing the pellet. • 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. • Repeat the ethanol wash steps for a second time. • Open the tube and incubate at room temperature for 10 minutes to evaporate residual ethanol. 										
<p>Step 2 Sample Lysis</p>	<ul style="list-style-type: none"> • Add 200 µl of RT Buffer and 20 µl of Proteinase K (10 mg/ml) to the pellet then vortex to mix. • Incubate at 55°C for 15 minutes. During incubation, invert the tube every 5 minutes. • Incubate at 80°C for 15 minutes. <p>NOTE: If using only one dry bath incubator, leave the sample at room temperature after the 55°C incubation until the incubator has reached 80°C. The 15 minutes incubation at 80°C is critical for reversal of crosslinks and optimal RNA performance in downstream applications such as real-time RT-PCR. Do not exceed 15 minutes of incubation at 80°C as this will increase RNA fragmentation.</p> <ul style="list-style-type: none"> • 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 2 ml tube, being careful NOT to disturb the pellet. 										
<p>Step 3 RNA Binding</p>	<ul style="list-style-type: none"> • Add 400 µl of RB Buffer then mix well by vortex. • 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. <p>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.</p> <ol style="list-style-type: none"> 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: <table border="1" data-bbox="320 1198 772 1276"> <tr> <td>DNase I</td> <td>5 µl (2 U/µl)</td> </tr> <tr> <td>DNase I Reaction Buffer</td> <td>45 µl</td> </tr> <tr> <td>Total Volume</td> <td>50 µl</td> </tr> </table> <ol style="list-style-type: none"> 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 (20-30°C) then proceed with Step 4 RNA Wash. 	DNase I	5 µl (2 U/µl)	DNase I Reaction Buffer	45 µl	Total Volume	50 µl				
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<p>Step 4 RNA Wash</p>	<ul style="list-style-type: none"> • 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. 										
<p>Step 5 RNA Elution</p>	<ul style="list-style-type: none"> • 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. <p>Optional Step 2: DNA Digestion In Solution</p> <ol style="list-style-type: none"> 1. Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows: <table border="1" data-bbox="320 1863 871 1995"> <tr> <td>RNA in RNase-free Water</td> <td>1-40 µl</td> </tr> <tr> <td>DNase I</td> <td>0.5 µl/µg RNA</td> </tr> <tr> <td>DNase I Reaction Buffer</td> <td>5 µl</td> </tr> <tr> <td>RNase-free Water</td> <td>Add to final volume = 50 µl</td> </tr> <tr> <td>Total Volume</td> <td>50 µl</td> </tr> </table> <ol style="list-style-type: none"> 2. Gently pipette the DNase I reaction solution (DO NOT vortex) then incubate the tube at 37°C for 15-30 minutes. 3. Stop the reaction by adding 1µl of 0.5 M EGTA (pH=8.0) then incubate the Tube at 75°C for 10 minutes. <p>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.</p>	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
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