FormaPure RNA: 
Extended Protocol for RNA Isolation from FFPE Sample

Refer to www.beckmancoulter.com/ifu for updated protocols. For questions regarding this protocol, call Technical Support at Beckman Coulter at 1-800-369-0333.

Contents
- Introduction
- Kit Specifications
- Warnings and Precautions
- Materials Supplied
- Materials Required but not Supplied
- Process Overview
- Protocol for RNA Isolation

Introduction

The FormaPure RNA extraction and purification kit uses the patented Beckman Coulter SPRI paramagnetic bead-based technology to isolate RNA from formalin-fixed, paraffin-embedded (FFPE) tissue without the use of xylene. This kit has been optimized for use with downstream sequencing and PCR-based assays. Specifically, RNA isolated using the FormaPure RNA kit is compatible with the following downstream applications:

- RNA-seq
- Endpoint or qRT-PCR

FormaPure RNA isolates RNA from tissue sections totaling a thickness of up to $3 \times 10$ µm. The protocol can be performed in both 96-well plates (manually and automated) and in 1.5 mL tubes (manually only). Nucleic acid extraction begins with the solubilization of the paraffin from the tissue slices in tubes. An enzymatic lysis step digests the tissue and releases the nucleic acids, as well as gently decrosslinks RNA. The remaining protocol can be carried out in plates or tubes. A binding solution is added to immobilize the nucleic acids to the surface of the SPRI beads. Contaminants are rinsed away using a simple washing procedure, DNA is removed from the sample and RNA is again immobilized on the surface of the SPRI beads before elution with water.
Kit Specifications

<table>
<thead>
<tr>
<th>Kit Type</th>
<th>Part Number</th>
<th>Number of Preps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>C19158</td>
<td>96</td>
</tr>
<tr>
<td>Small</td>
<td>C19157</td>
<td>50</td>
</tr>
</tbody>
</table>

Warnings and Precautions

Read and observe the following safety information.

**IMPORTANT** The ![danger](image) symbol indicates a potential safety risk involving the material, action, or equipment required for executing a procedural action; when you see the ![danger](image) symbol, return to this section to review relevant safety information.

![danger](image) **DANGER**

<table>
<thead>
<tr>
<th>H315</th>
<th>Causes skin irritation.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H319</td>
<td>Causes serious eye irritation.</td>
</tr>
<tr>
<td>H334</td>
<td>May cause allergy or asthma symptoms or breathing difficulties if inhaled.</td>
</tr>
<tr>
<td>H335</td>
<td>May cause respiratory irritation.</td>
</tr>
<tr>
<td>P261</td>
<td>Avoid breathing vapors.</td>
</tr>
<tr>
<td>P280</td>
<td>Wear protective gloves, protective clothing and eye/face protection.</td>
</tr>
<tr>
<td>P284</td>
<td>In case of inadequate ventilation, wear respiratory protection.</td>
</tr>
<tr>
<td>P304+P340</td>
<td>IF INHALED: Remove person to fresh air and keep at rest in a position comfortable for breathing.</td>
</tr>
<tr>
<td>P342+P311</td>
<td>If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.</td>
</tr>
</tbody>
</table>

**CAUTION**

Risk of chemical injury from Proteinase K. To avoid contact with Proteinase K, wear appropriate personal protective equipment, including protective eyewear, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details about chemical exposure before using the chemical.
CAUTION

Risk of burning from hot liquid splattering into your eyes or onto your skin. Wear appropriate personal protective equipment while incubating the samples. Place tube cap locks on the tubes to prevent the tops of the tubes from opening during incubation.

Materials Supplied

The following reagents are supplied in the FormaPure RNA kit. The reagent icon, which is located on the top of the corresponding bottle, is included in the instructions as a visual aid to ensure the correct reagent is used.

NOTE Refer to the product labels for expiration dates.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Icon</th>
<th>Storage Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral Oil</td>
<td>MO</td>
<td>15 to 30 °C</td>
</tr>
<tr>
<td>Lysis</td>
<td>LBA</td>
<td>15 to 30 °C</td>
</tr>
<tr>
<td>Bind</td>
<td>BBA</td>
<td>15 to 30 °C</td>
</tr>
<tr>
<td>Re-Bind</td>
<td>RBA</td>
<td>15 to 30 °C</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>-</td>
<td>15 to 30 °C</td>
</tr>
</tbody>
</table>

Materials Required but not Supplied

FormaPure samples can be processed in a 96-well plate or tube format. Refer to the tables below for the items required for this procedure:

- Hardware & Accessories
- Consumables
- Reagents
# Hardware & Accessories

<table>
<thead>
<tr>
<th>Item</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjustable Heat Source</td>
<td>• Thermomixer with 1.5 mL tubes and plate adaptor and heated lid</td>
</tr>
<tr>
<td></td>
<td><strong>Or</strong></td>
</tr>
<tr>
<td></td>
<td>• Hybex with 1.5 mL tubes and plate adaptor</td>
</tr>
<tr>
<td></td>
<td><strong>Two heat sources of any type are recommended for the protocol.</strong></td>
</tr>
<tr>
<td>Vortexer</td>
<td><strong>Not specified.</strong></td>
</tr>
<tr>
<td>Microcentrifuge</td>
<td>Beckman Coulter Microcentrifuge 16</td>
</tr>
<tr>
<td></td>
<td><strong>Or equivalent.</strong></td>
</tr>
<tr>
<td>Bead Separation Magnet</td>
<td>• Agencourt SPRIStand Magnetic 6-Tube Stand (for 1.5, 1.7, or 2.0 mL tubes) (Beckman Coulter), PN A29182</td>
</tr>
<tr>
<td></td>
<td><strong>Or</strong></td>
</tr>
<tr>
<td></td>
<td>• V&amp;P Scientific 7 Bar Magnet, PN VP 771MWZM-1ALT (for 96-Well Plate)</td>
</tr>
</tbody>
</table>

# Consumables

<table>
<thead>
<tr>
<th>Item</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcentrifuge Tubes</td>
<td>1.5 mL</td>
</tr>
<tr>
<td>Cap Locks</td>
<td>for Microcentrifuge Tubes</td>
</tr>
<tr>
<td>96-Well Plate</td>
<td>1.2 mL, ThermoFisher Scientific, PN AB1127</td>
</tr>
<tr>
<td></td>
<td><strong>Or equivalent.</strong></td>
</tr>
<tr>
<td>96-Well Storage Plate</td>
<td>200 µL</td>
</tr>
<tr>
<td>PCR Adhesive Seals</td>
<td>for 96-Well Plate</td>
</tr>
</tbody>
</table>

# Reagents

<table>
<thead>
<tr>
<th>Item</th>
<th>Supplier</th>
<th>Catalog Name</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>100% Ethanol</strong> (Molecular Grade)³</td>
<td>AmericanBio</td>
<td>Ethanol, Absolute Alcohol, 200 Proof, Anhydrous</td>
<td>AB00138</td>
</tr>
<tr>
<td><strong>DNase I</strong></td>
<td>ThermoFisher Scientific</td>
<td>Ambion DNase I (RNase-free)</td>
<td>AM2222 or AM2224</td>
</tr>
<tr>
<td><strong>Nuclease-Free Water</strong>³</td>
<td>ThermoFisher Scientific</td>
<td>Nuclease-Free Water (not DEPC-Treated)</td>
<td>AM9932</td>
</tr>
</tbody>
</table>

³ The recommended **Supplier**, **Catalog Name**, and **Catalog Number** for this item is provided; if necessary, an equivalent product may be substituted for the listed product.
Process Overview

Before You Begin

• Preheat adjustable heat sources to 80°C and 60°C.
• Prepare 80% Ethanol from 100% stock using Nuclease-Free Water.

  IMPORTANT Do not use a previously-prepared solution, as it may have a lower ethanol percentage, causing yield loss.

  IMPORTANT This protocol uses ethanol in multiple steps. Dispose of supernatant containing ethanol waste in accordance with local regulations and acceptable laboratory practices.

• Wear appropriate personal protective equipment (PPE) when handling samples and reagents.

Procedure

1. Sample Preparation:
   For each sample, transfer one to three 10 µm FFPE tissue sections into a 1.5 mL tube.

2. Deparaffinization:
   a. Add 450 µL of Mineral Oil to each sample and immerse the sections completely with a pipette tip.

   NOTE Make sure that the sample is completely immersed and does not float due to attached bubbles.
b. **Incubate at 80°C for 5 minutes.**

c. After incubation, vortex the tubes two times, for five seconds each time, to solubilize the paraffin and disperse the tissue.

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3 **Tissue Digestion:**

a. Add 200 µL of Lysis to each sample.

b. Centrifuge the tubes at 10,000 x g for 15 seconds. The mineral oil forms a separate upper phase.

**NOTE** Incubate the tubes for 3 more minutes at 80°C if the mineral oil layer appears cloudy and the tissue is stuck at the interface of mineral oil and lysis buffer. After the incubation, make sure to cool the tubes for 2 minutes before adding Proteinase K.

c. Add 20 µL of Proteinase K to the aqueous, lower phase and mix by pipetting up and down 10 times without disrupting the upper phase.

d. Incubate the tubes at 60°C for 120 minutes.

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4 **Lysate Transfer:**

a. Take the tubes out of the heat source and centrifuge the tubes at 10,000 x g for 5 minutes.

b. Transfer all of the clear lysate (lower phase) to a 96-well plate, or to 1.5 mL tubes, without disrupting the upper phase (mineral oil) or the pellet.

**NOTE** If the tissue is clogging the pipette tip, you may centrifuge the tubes for additional time.

**NOTE** Minimize the amount of Mineral Oil that is transferred along with the lysate. However, a small amount of Mineral Oil carryover does not affect downstream applications.

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5 **First Bind:**

a. Fully re-suspend the Bind by shaking or vortexing.

b. Add 300 µL of Bind to each sample and mix by pipetting up and down 10 times with a P1000 pipette set at 350 µL. Mix gently to minimize the generation of bubbles.

c. Incubate at room temperature for 5 minutes.
d. Place the samples on the magnet for 10 minutes; if the solution is not fully clear after 10 minutes, incubate until clear. If using:
   - A 96-well plate, place the samples on the bar magnet plate.
   - Tubes, place the tubes in an Agencourt SPRIStand Magnetic 6-Tube Stand.

e. With the samples on the magnet, aspirate the supernatant without disrupting the beads. Discard the supernatant.

6 Ethanol Wash:

   a. Remove the samples from the magnet.
   b. Add 750 µL of freshly prepared 80% Ethanol to each sample.
   c. Using a P1000 pipette set at 600 µL, mix by pipetting up and down 20 times, or until the beads are fully re-suspended.
   d. Place the samples on the magnet for 3 minutes; if the solution is not fully clear after 3 minutes, incubate until clear.
   e. With the samples on the magnet, aspirate the supernatant without disrupting the beads. Discard the supernatant.
   f. Air dry the samples on the magnet for 10 minutes.

7 DNase I Treatment:

   a. Remove the samples from the magnet.
   b. Add 80 µL of Nuclease-Free Water to each sample.
   c. Add 10 µL of 10× DNase I buffer and 10 µL of DNase I to each sample.
   d. Mix by pipetting up and down 5 times with a P200 pipette set at 80 µL to thoroughly distribute the buffer and enzyme. Mix gently to minimize the generation of bubbles.
   e. Cover the plate with an adhesive seal, or close the tubes, and incubate at 37°C for 20 minutes.

8 Re-Bind:

   a. Add 150 µL of Re-Bind to each sample and mix by pipetting up and down 10 times with a P200 pipette set at 150 µL. Mix gently to minimize the generation of bubbles.
   b. Incubate at room temperature for 5 minutes.
   c. Place the samples on the magnet for 10 minutes; if the solution is not fully clear after 10 minutes, incubate until clear.
   d. With the samples on the magnet, aspirate the supernatant without disrupting the beads. Discard the supernatant.
9 Ethanol Wash:
   a. Remove the samples from the magnet.
   b. Add 750 µL of freshly prepared 80% Ethanol to each sample.
   c. Using a P1000 pipette set at 600 µL, mix by pipetting up and down 20 times, or until the beads are fully re-suspended.
   d. Place the samples on the magnet for 3 minutes; if the solution is not fully clear after 3 minutes, incubate until clear.
   e. With the samples on the magnet, aspirate the supernatant without disrupting the beads. Discard the supernatant.
   f. Air dry the samples on the magnet for 10 minutes.

10 Elution:
   a. Remove the samples from the magnet.
   b. Add a minimum of 40 µL of Nuclease-Free Water to each sample and mix by pipetting up and down 10 times, or until beads are fully re-suspended, with a P200 pipette set at 30 µL.
   c. Cap tubes or cover the plate with a PCR adhesive seal and incubate at 60°C for one minute.
   d. Place the samples on the magnet for 1 minute; if the solution is not fully clear after 1 minute, incubate until clear.
   e. With the samples on the magnet, transfer as much of the supernatant as possible to a 96-well storage plate, or to a new tube, without disturbing the magnetic beads.
   f. Store at -20°C, or -80°C for long-term storage.
Product Availability

FormaPure RNA
- C19157 — FormaPure RNA, 50 Prep Kit
- C19158 — FormaPure RNA, 96 Prep Kit

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Glossary of Symbols is available at techdocs.beckmancoulter.com (PN C05838).