Instructions For Use

FormaPure XL RNA:

Extended Protocol for RNA Isolation from FFPE Sample

PN C40292AB
April 2019

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BECKMAN COULTER

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Contact Us

- For questions regarding this protocol, call Technical Support at Beckman Coulter at 1-800-369-0333.
- For additional information, or if damaged product is received, call Beckman Coulter Customer Service at 800-742-2345 (USA or Canada) or contact your local Beckman Coulter Representative.
- Refer to *www.beckman.com/techdocs* for updated protocols.

Glossary of Symbols is available at www.beckman.com/techdocs (PN C05838).

Product Availability

REF C36000 — FormaPure XL RNA, 50 Prep Kit

REF C36001 — FormaPure XL RNA, 96 Prep Kit

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Protocol for RNA Isolation

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Introduction

The FormaPure XL 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 XL RNA kit is compatible with the following downstream applications:

- RNA-seq
- Endpoint or qRT-PCR

FormaPure XL RNA isolates RNA from tissue sections totaling a thickness of up to 7 × 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 SPRIbeads before elution with water.

Kit Specifications

Kit Type	Part Number	Number of Preps
Medium	C36001	96
Small	C36000	50

Warnings and Precautions

Read and observe the following safety information.

IMPORTANT The ⚠ symbol indicates a potential safety risk involving the material, action, or equipment required for executing a procedural action; when you see the ⚠ symbol, return to this section to review relevant safety information.

	**DANGER**
Proteinase K	
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
P261	Avoid breathing vapors.
P280	Wear protective gloves, protective clothing and eye/face protection.
P284	In case of inadequate ventilation, wear respiratory protection.
P304+P340	IF INHALED: Remove person to fresh air and keep at rest in a position comfortable for breathing.
P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
SDS	Safety Data Sheet is available at www.beckman.com/techdocs.

⚠ 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 XL RNA kit. The reagent icon 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.

Reagent	Icon	Storage Conditions
Mineral Oil		15 to 30 °C
Lysis		15 to 30 °C
Bind		15 to 30 °C
Re-Bind		15 to 30 °C
Proteinase K	-	15 to 30 °C

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

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

Consumables

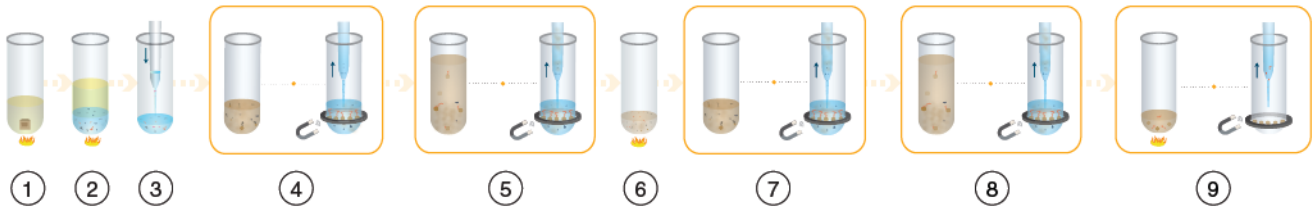
Item	Type
Microcentrifuge Tubes	1.5 mL
Cap Locks	for Microcentrifuge Tubes
96-Well Plate	1.2 mL, ThermoFisher Scientific, **PN AB1127** ***Or equivalent.***
96-Well Storage Plate	200 µL
PCR Adhesive Seals	for 96-Well Plate

Reagents

Item	Supplier	Catalog Name	Catalog Number
100% Ethanol (Molecular Grade)[a]	AmericanBio	Ethanol, Absolute Alcohol, 200 Proof, Anhydrous	AB00138
DNase I	ThermoFisher Scientific	Ambion DNase I (RNase-free)	AM2222 or AM2224
Nuclease-Free Water[a]	ThermoFisher Scientific	Nuclease-Free Water (not DEPC-Treated)	AM9932

a. 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



1. Deparaffinization
2. Tissue digestion.
3. Transfer
4. First Bind
5. Ethanol wash
6. DNase I treatment
7. Re-Bind
8. Ethanol wash.
9. Elution

Protocol for RNA Isolation

Before You Begin

- Preheat adjustable heat sources to 80°C and 60°C.
- Prepare fresh 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 seven **10 µm** FFPE tissue sections into a 1.5 mL tube.

2 Deparaffinization:

a. Add **450 µL** of **Mineral Oil** (MO) 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.

3 Tissue Digestion:

a. Add **200 µL** of **Lysis** (LBD) to each sample.

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

c. ⚠ Incubate at **80°C** for an additional **5 minutes.**

d. Allow samples to cool for **2 minutes** at room temperature.

NOTE If tissue is stuck at the interface of the mineral oil and Lysis buffer, spin tubes at **10,000 × *g*** for **15 seconds**.

e. ⚠ Add **30 µL** of Proteinase K to the aqueous, lower phase and mix by pipetting up and down 10 times without disrupting the upper phase.

NOTE If paraffin solidifies before lysis, keep the sample tubes at 60°C while adding Proteinase K.

f. Incubate the tubes at **60°C** for 120 minutes.

4 Lysate Transfer:

a. Take the tubes out of the heat source and centrifuge the tubes at **10,000 × *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.

5 First Bind:

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

b. Add **300 µL** of **Bind** BBA 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. Prepare DNase solution mix (100 µL per sample):

1) Add the following components per sample.

- **80 µL** of **Nuclease-Free Water**
- **10 µL** of **10× DNase I buffer**
- **10 µL** of **DNase I**

2) Mix the solution by pipetting up and down 5 times.

c. Add **100 µL** of DNase solution 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** RBA 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.

Troubleshooting Guide

This troubleshooting guide may be helpful to maximize nucleic acid yield, integrity, and purity from FFPE tissues, or to solve any issues that may arise. The scientists at Beckman Coulter are available to answer any questions you may have about the information in this troubleshooting guide and the protocols in this manual (refer to Contact Us on page 2 for contact information).

NOTE Visit *www.Formapure.com* for instructional videos and updated information.

This section includes the following tables:

Table 1 Troubleshooting Low Yield

Problem	Possible Solution(s) and Comment(s)
Poor Starting Sample Quality	The processes of formalin fixation, paraffin embedding and/or storage of FFPE tissues cause damage to the nucleic acids. While the FormaPure chemistry is designed to maximize yield and integrity for challenging FFPE samples, this chemistry cannot repair damaged nucleic acids.
Low Tissue Input or Tissue Type	• Some FFPE samples may contain very low amounts of tissue or cells, depending on the tissue and disease types; therefore, the amount of nucleic acids may be inherently low prior to extraction. If possible, increase the amount of FFPE samples to obtain the desired yield. • Certain tissue types are more difficult to digest than others. An extended **Tissue Digestion** incubation can be performed (with DNA isolations only) to free up more of the nucleic acids.
Bead/Sample Loss	• Disruption of the bead pellet during supernatant removal may cause decreased yields. The pipette tip should not contact the bead pellet during aspirations. If a brown color is seen in the pipette tip during aspiration, beads are present and the solution should be dispensed back into the tube or well. Place samples back on magnet until solution is fully cleared and let the beads settle towards the magnet before aspirating again. • Insufficient bead clearing during magnetic separation may lead to decreased yields. Ensure that the beads are completely settled to the magnet and the supernatant is clear before removing the supernatant. • Undigested tissue can trap the beads and prevent efficient nucleic acid binding or lead to bead and sample loss. Tissue should be thoroughly digested in the **Tissue Digestion** step before bead addition. If undigested tissue remains after the **Tissue Digestion** step, avoid transferring the undigested tissue to another tube or well before proceeding to the **Bind** step. For additional information, see *Incomplete Tissue Digestion* below.

Table 1 Troubleshooting Low Yield

Problem	Possible Solution(s) and Comment(s)
Incomplete Tissue Digestion	If the tissue is not completely digested after 3 hours, longer **Tissue Digestion** incubations may be performed (for DNA isolations only). If an extended **Tissue Digestion** time is not desirable, or when performing RNA isolations, avoid transferring any undigested tissue. Samples may be centrifuged for **5 minutes** at **10,000 × *g***, and only the supernatant should be transferred for the ensuing steps. The samples can be centrifuged again for **5 minutes** at **10,000 × *g*** if tissues are still not pelleted toward the bottom of the tube or well. If small tissue pieces are unavoidable during transfer, these will be washed away with other contaminants in the **Wash** steps of the protocol.
Inaccurate Incubation Temperatures	• Higher than recommended temperatures during the **Tissue Digestion** and **Decrosslinking** steps can result in the degradation of nucleic acids, particularly of RNA. Ensure that the temperature of the heat source is accurate and not fluctuating significantly. • Precise incubation temperatures throughout this method are important for optimal chemistry performance. Verify that the heat sources are calibrated and functioning properly, and adjust settings on heat sources to maintain specified in-well/ tube temperatures. • Although **5 minutes** at **80°C** should remove all of the paraffin during the **Deparaffinization** step, depending on the age, embedding process, and the type of paraffin used, longer incubations may be required. We recommend incubating the samples in **80°C** for an additional **3 minutes**, even if you have already added the Lysis buffer, but before the addition of Proteinase K.
Inaccurate or Insufficient Incubation Times	Incubation times provided have been optimized to balance highest possible yield and quality of the extracted sample. Unless otherwise indicated within the troubleshooter, it is not recommended to deviate from the incubation times provided.
Cloudy Eluents	*Depending on the cause of the cloudy eluent, there may or may not be an impact on downstream functionality of the extracted nucleic acids.* **Causes that should be inert:** • Too much mineral oil carryover during the lysate transfer and **Wash** steps may make the eluents appear cloudy. Minimize the amount of mineral oil that is carried over during these steps. However, if some mineral oil is transferred, it can be removed during the subsequent **Wash** steps. Since mineral oil will always remain on the top of the wash solutions, aspirating from the top of the supernatant will ensure complete removal of the mineral oil. • Some tissues are high in lipids and can result in cloudy eluents. Cloudy eluents from lipids should not affect the functionality in most downstream applications. **Causes that should be resolved:** • Ensure that all of the paraffin is solubilized after the **Deparaffinization** step. A fully deparaffinized tissue should be completely immersed in the bottom lysate layer after centrifugation. See *Excess Paraffin/Insufficient Deparaffinization* below. • Ensure that wash steps are performed properly and sufficiently. Cloudy eluents from paraffin carryover should not affect most downstream applications, but may lower yields due to inefficient tissue digestion.

Table 1 Troubleshooting Low Yield

Problem	Possible Solution(s) and Comment(s)
Bead Clumping	• See *Over-Dried Beads* below. • Insufficient washing and removal of impurities. Ensure that the **Wash** steps are performed sufficiently. View video to gain a better understanding of proper washing technique. • See *Incomplete Tissue Digestion* above.
Inaccurate Ethanol Percentage Used	Ethanol is hygroscopic and may become more dilute over time; 80% ethanol should be prepared fresh. Lower ethanol concentrations may increase solubilization of nucleic acids during **Wash** steps.
Excess Paraffin/Insufficient Deparaffinization	After addition of lysis buffer and subsequent centrifugation, confirm that the tissue is fully immersed in the bottom lysate layer. If the paraffin is not fully dissolved, the tissues may tend to migrate toward the mineral oil layer even after centrifugation. If this is observed, place samples back in **80°C** for and additional **3 minutes** before adding Proteinase K.
Incomplete Lysate Transfer	Ensure that the entire lysate is transferred, including the white precipitate that may form near the interface. It is okay to carry over some mineral oil if it ensures all of the lysate is transferred.
Over-Dried Beads	Ensure that the beads are not over-dried after the **Ethanol Wash** steps. If cracking of the bead pellet is observed, it is a sign of over-drying and the next step should be carried out immediately.
Incomplete Elution	Ensure that the recommended time and temperature is used during the **Elution** step to completely elute the nucleic acids off of the beads.
Using Non-Recommended Tubes or Plates	• Different types of plastics can have variable rate of heat transfer resulting in unexpected in-well incubation temperatures. • Different types of plastics can cause variation on the impact of the applied magnetic field to the paramagnetic beads. • Adjust settings on heat sources to maintain specified in-well/tube temperatures and settling times during bead separation steps.
Using Non-Recommended Magnet	Development of FormaPure chemistries was performed with the specific magnets listed in *Hardware & Accessories*. If using a non-recommended magnet, settling times may vary. Adjust settling times during bead separation steps; supernatant should be clear and pellet should be visible on the side wall of tube or well.

Table 2 Troubleshooting Poor Quality of Extracted Nucleic Acids

Problem	Possible Solution(s) and Comment(s)
Nucleic Acid Appears Degraded	• The processes of formalin fixation, paraffin embedding and/or storage of FFPE tissues cause degradation of nucleic acids. • If nucleic acids are more degraded than expected, use sterile techniques to ensure that DNase and RNase are not a source of contamination during the isolation processes. • Store the nucleic acids at -20°C, or -80°C for long-term storage.
RNAse and/or DNAse Contamination	• Use sterile techniques to ensure that DNase and RNase are not a source of contamination during the isolation processes. • Filter tips should be used for RNA workflows so buffer sources are not contaminated. • If all other sources of contamination are ruled out, replace reagents.
DNA Contamination with RNA	While RNase A should be active in sample lysates that contain cellular debris and Lysis buffer components, these components will inhibit DNase activity. Make sure that the ethanol washes are performed properly before DNase treatments, and remove the ethanol as much as possible as excess ethanol may also prevent DNase activity.
RNA Contaminated with DNA	Ensure temperatures are appropriate for full nuclease activity: RNase A treatments should be carried out at room temperature and DNase I treatments should be carried out at 37°C.
Poor Performance in Downstream Assays	• Ensure that the **Wash** steps are performed properly and sufficiently. View video to gain a better understanding of proper washing technique. • Residual ethanol should be removed and/or air-dried before proceeding to subsequent steps. • During supernatant removal steps after magnetic separation, make sure to remove as much of the supernatant as possible without disturbing the beads. • Some more fibrous tissues, such as muscle, will form more extensive or tighter crosslinks upon fixation, so longer **Decrosslinking** incubations may increase nucleic acid functionality. For DNA isolations, **Decrosslinking** incubations can be performed for up to **3 hours** at **80°C**. We do not recommend extending the **Decrosslinking** incubations for RNA isolations as this can further degrade the RNA.

Revision History

Go to www.beckman.com/techdocs to download the most recent manual for this product.

- **Initial Issue AA, 02/2019**
- **Revision AB, 04/2019**

  Updates were made to the following sections:

  — *Before You Begin*
  — *Procedure*

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