Instructions For Use

FormaPure DNA:

Extended Protocol for DNA Isolation from FFPE Sample

PN B44690KF
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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 B89230 — FormaPure DNA, 50 Prep Kit

REF B89231 — FormaPure DNA, 96 Prep Kit

REF B89232 — FormaPure DNA, 384 Prep Kit

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Protocol for DNA Isolation

Contents

Introduction

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

- Targeted amplicon NGS
- Targeted capture NGS
- Whole exome sequencing
- Whole genome sequencing
- Endpoint or qPCR

FormaPure DNA isolates DNA from tissue sections totaling a thickness of up to 3 x 10 microns. 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, followed by decrosslinking at a high temperature. The remaining protocol can be carried out in plates or tubes. RNA is removed from the sample and 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 and the nucleic acids are eluted with water.

Kit Specifications

Kit Type	Number of Preps
Small kit, PN B89230	50
Medium kit, PN B89231	96
Large kit, PN B89232	384

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 DNA 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
Wash		15 to 30 °C
RNase A	-	15 to 30 °C
Proteinase K	-	15 to 30 °C

Materials Required but not Supplied

Required Reagents

Reagent	Supplier	Part Number
100% ethanol	AmericanBio	AB00138 (or equivalent)
Nuclease-free water	Thermo Fisher	AM9932 (or equivalent)

Required Equipment

FormaPure DNA processing can be done in a 96-well plate or tube format. Refer to the tables below for the hardware and consumables required for this procedure.

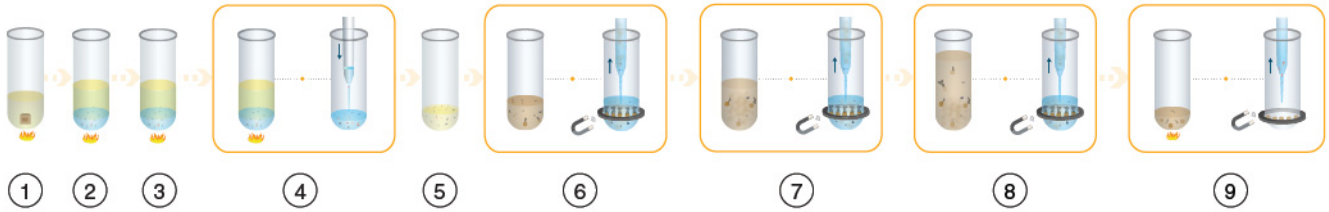
Table 1 Required Hardware and Accessories

Hardware and Accessories	Format
Pipettes (P20, P200, P1000 multi or single channel as needed)	plate and tube
Adjustable heat source (for example, a water bath or a heat block). Two are recommended.	plate and tube
Vortexer	plate and tube
Beckman Coulter Microcentrifuge 16, or equivalent	plate and tube
Beckman Coulter Agencourt SPRIPlate 96R Ring Super Magnet Plate, PN A32782	plate
Beckman Coulter Agencourt SPRIStand Magnetic 6-tube Stand (for 1.5, 1.7, or 2.0 mL tubes), PN A29182	tube

Table 2 Required Consumables

Consumables	Format
Barrier tips for P20, P200, and P1000 pipettes	plate and tube
1.5-1.7 mL microcentrifuge tubes	plate and tube
Microcentrifuge tube cap locks	plate and tube
Thermo Fisher 1.2 mL 96-well Plate, PN AB1127, or equivalent	plate
200 µL 96-well storage plate	plate
PCR Adhesive Seals	plate

Process Overview



1. Deparaffinization
2. Tissue digestion
3. Extended lysis for DNA (optional)
4. Decrosslinking
5. RNase A treatment
6. Bind DNA
7. Wash
8. Ethanol wash
9. Elution

DNA Extraction Protocol

Before You Start

- Preheat adjustable heat sources to 80°C and 55°C.
- Prepare 80% ethanol from 100% stock using nuclease-free water. Do not use a previously prepared solution.
- 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** (MO) to each sample and immerse the sections completely with a pipette tip.

b. ⚠ Incubate at **80°C** for **5 minutes**.

c. 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** (LBA) to each sample.

NOTE Do not vortex the tubes as this may cause the mineral oil and lysis to emulsify.

b. Centrifuge the tubes at **10,000 × *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 starts turning cloudy or 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 pipette mix 10 times without disrupting the upper phase.

d. Incubate the tubes for a minimum of **60 minutes** at **55°C** (up to 16 hours) to achieve complete lysis.

4 Decrosslinking:

a. ⚠ Incubate the tubes at **80°C** for **60 minutes.**

b. Remove the tubes from the heat source.

c. Transfer as much of the lysate (lower phase) as possible to a 96-well plate, or to 1.5 mL tubes, without disrupting the upper phase.

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 RNase A Treatment:

a. Add **5 µL** of **RNase A** to each sample.

b. Pipette mix five times with a P200 pipette set at 150 µL to thoroughly distribute the enzyme. Mix gently to minimize the generation of bubbles.

c. Incubate at room temperature for **5 minutes.**

6 Bind DNA:

a. Fully resuspend the **Bind** BBA solution by shaking or vortexing.

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

NOTE DNA binds to the magnetic particles during this step. When mixing, use a mix volume that is slightly less than the total volume in the well and pipette slowly to minimize the formation of air bubbles. Air bubbles can trap magnetic beads and prevent them from being pulled to the bottom of the plate, thus decreasing yield.

c. Incubate at room temperature for **5 minutes**.

d. Place the samples on the magnet for 10 minutes, or until the solution is clear, to allow the beads to separate. Use a SPRIPlate 96R Ring Super Magnet Plate if working in a 96-well plate, or place the tubes in an Agencourt SPRIStand Magnetic 6-tube Stand if using tubes.

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

NOTE When aspirating, place the pipette at the center of the ring, or away from the beads in the tube, to avoid disturbing the magnetic beads. Bead loss will result in lower yields.

7 Wash:

a. Remove the samples from the magnet.

b. Add **400 µL** of **Wash** WBA to each sample.

c. Using a P1000 pipette set at 250 µL, mix the samples 15 times or until the beads are fully resuspended in the solution. Mix gently to minimize the generation of bubbles.

d. Place the samples back on the magnet for 10 minutes, or until the solution is clear, to allow the beads to separate.

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

NOTE When aspirating, place the pipette at the center of the ring, or away from the beads in the tube, to avoid disturbing the magnetic beads. Bead loss will result in lower yields.

8 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 the sample 20 times, or until the beads are fully resuspended.

d. Place the samples back on the magnet for three minutes, or until the solution is clear, to allow the beads to separate.

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

NOTE Remove as much ethanol as possible, without disturbing the magnetic beads, before drying. Dispose of Ethanol waste in accordance with the local regulations and acceptable laboratory practices.

f. Air dry the samples on the magnet for 10 minutes.

9 **Elution**:

a. Remove the samples from the magnet.

b. Add a minimum of 40 µL of nuclease free water to each sample and mix 10 times with a P200 pipette set at 30 µL or until beads are fully resuspended.

c. Cap tubes or cover the plate with a PCR adhesive seal and incubate at 55°C for one minute.

d. Place the samples back on the magnet for one minute, or until the solution is clear, to allow the beads to separate.

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.

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 3 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 3 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 3 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 Table 1, Required Hardware and 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 4 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, 12/2017

Revision AB, 1/2018

Revision AC, 5/2018
Updates were made to the following sections:

- *Process Overview*
- *Troubleshooting Guide*

Revision AD, 3/2019
Updates were made to the following section:

- *Materials Supplied*

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