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FormaPure Total:

Protocol for DNA and RNA Isolation from the Same 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

The FormaPure Total extraction and purification kit uses the patented Beckman Coulter SPRI paramagnetic bead-based technology to isolate both DNA and RNA 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 and RNA isolated with the FormaPure Total kit are compatible with the following downstream applications:

- Targeted NGS
- Whole exome sequencing
- Whole genome sequencing
- RNA-seq
- Endpoint or qPCR

FormaPure Total isolates both DNA and RNA from FFPE tissue sections totaling a thickness of up to 3 × 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. Half of the lysate is removed to perform RNA isolation, while the other half undergoes DNA isolation, which involves decrosslinking the remaining lysate at a high temperature. The rest of the protocol can be carried out in plates or tubes:

- ***For RNA isolation***, 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 eluting with water.
- ***For DNA isolation***, RNA is removed from the sample, and a binding solution is added to immobilize the DNA to the surface of the SPRI beads. Contaminants are rinsed away using a simple washing procedure, and the DNA is eluted with water.

Kit Specifications

Kit Type	Part Number	Number of Preps
Medium	C16676	96
Small	C16675	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 *techdocs.beckmancoulter.com*.

⚠ 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 Total 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.

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
Re-Bind		15 to 30 °C
RNase A	-	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

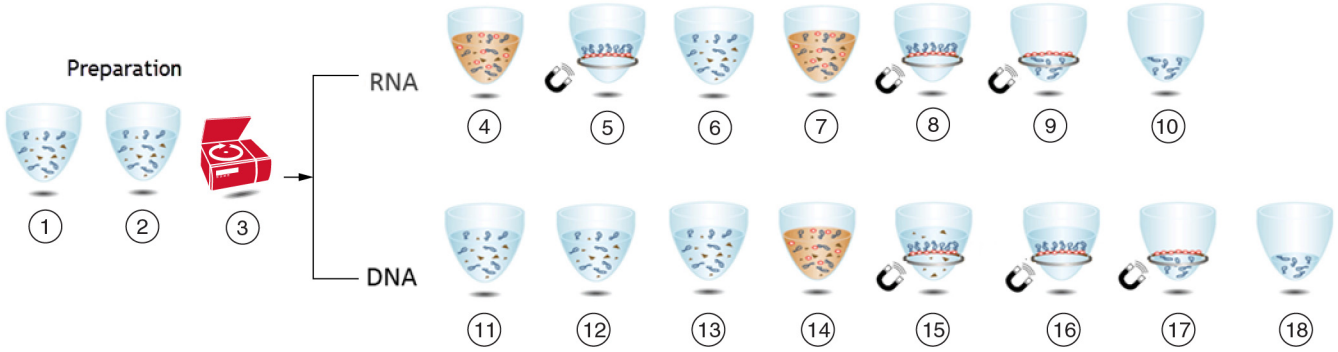
Item	Type
Microcentrifuge Tubes	1.5 mL
Cap Locks	for Microcentrifuge Tubes
96-Well Plate	1.2 mL, ThermoFisher Scientific, **PN AB1127** ***Or equivalent.***
96-Well Storage Plate	200 µL
PCR Adhesive Seals	for 96-Well Plate

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 at 80°C.
2. **Lysis**/decrosslink at 60°C.
3. Spin at high speed and remove half of the lysate for RNA workflow. Process the remaining lysate for DNA isolation.
4. **Bind**.
5. 80% **Ethanol** wash.
6. **DNase I** treatment.
7. **Re-Bind**.
8. 80% **Ethanol** wash.
9. Elution Buffer.
10. Transfer.
11. Extended lysis at 60°C. (*Optional for DNA-only workflow.*)
12. Decrosslink at 80°C.
13. **RNase A** treatment.
14. Bind.
15. Wash.
16. 80% **Ethanol** wash.
17. Elution Buffer.
18. Transfer.

Sample Preparation

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** (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** (LBA) to each sample.

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 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**.

4 To perform:

- ***DNA and RNA isolation from the same sample***, proceed to *Protocol for DNA and RNA Isolation from the Same FFPE Sample, page 9*.
- ***RNA isolation only***, skip to *Protocol for RNA-Only Isolation, page 14*.
- ***DNA isolation only***, skip to *Protocol for DNA-Only Isolation, page 17*.

Protocol for DNA and RNA Isolation from the Same FFPE Sample

This section contains complete instructions for performing DNA and RNA extractions and purifications from the same sample. To execute this protocol, complete the instructions in each of the following procedures:

1. *Lysate Splitting*, page 9
2. *RNA Isolation*, page 10
3. *DNA Isolation*, page 12

Lysate Splitting

To perform lysate splitting:

1 Take the tubes out of the heat source and centrifuge them at **10,000 × *g*** for **5 minutes**.

2 Split the lysate by transferring **100 µL** 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 any present pellet. This portion of the lysate will proceed through the RNA isolation protocol.

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.

IMPORTANT After RNA lysate removal, if there is a pellet of undigested tissue in the original tube, mix the lower aqueous phase by pipetting up and down 10 times, without disrupting the upper phase before incubation.

3 Incubate the original tube containing the remaining lysate (for DNA isolation) at **60°C** for an additional **60 minutes**.

NOTE If needed, a longer (or overnight) lysis can be done at **60°C** before proceeding to the decrosslinking step in *DNA Isolation*.

NOTE This step must be completed prior to performing the steps in *DNA Isolation*; however, while this step executes (i.e., the remaining lysate is undergoing extended lysis and decrosslinking incubations), the RNA extraction protocol can be completed in parallel; see *RNA Isolation* below for instructions.

RNA Isolation

To perform RNA isolation:

1 **First Bind**:

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

b. Add **150 µL** of **Bind** BBA 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.

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.

2 **Ethanol Wash**:

a. Remove the samples from the magnet.

b. Add **375 µL** of freshly prepared **80% Ethanol** to each sample.

c. Using a P1000 pipette set at 300 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.

3 **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 five 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. Seal the plate with an adhesive seal, or close the tubes, and incubate at **37°C** for **20 minutes**.

4 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.

5 Ethanol Wash:

a. Remove the samples from the magnet.

b. Add **375 µL** of freshly prepared **80% Ethanol** to each sample.

c. Using a P1000 pipette set at 300 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.

6 Elution:

a. Remove the samples from the magnet.

b. Add **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 an adhesive plate seal and incubate at **60°C** for **1 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.

DNA Isolation

To perform DNA isolation:

1 Decrosslinking:

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

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.

2 RNase A Treatment:

a. Add **2.5 µL** of **RNase A** to each sample.

b. Mix by pipetting up and down 5 times with a P200 pipette set at 75 µL to thoroughly distribute the enzyme. Mix gently to minimize the generation of bubbles.

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

3 Bind DNA:

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

b. Add **150 µL** of **Bind** BBA 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.

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.

4 Wash:

a. Remove the samples from the magnet.

b. Add **200 µL** of **Wash** WBA to each sample.

c. Using a P200 pipette set at 125 µL, mix by pipetting up and down 15 times or until the beads are fully re-suspended in the solution. Mix gently to minimize the generation of bubbles.

d. Place the samples on the magnet for 10 minutes; if the solution is not fully clear after 10 minutes, incubate until clear.

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

5 **Ethanol Wash**:

a. Remove the samples from the magnet.

b. Add **375 µL** of freshly prepared **80% Ethanol** to each sample.

c. Using a P1000 pipette set at 300 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.

6 **Elution**:

a. Remove the samples from the magnet.

b. Add **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 an adhesive plate 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.

Protocol for RNA-Only Isolation

To perform RNA extraction and purification:

1 **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.

2 **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.

3 **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.

4 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**.

5 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.

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 **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.

Protocol for DNA-Only Isolation

To perform DNA isolation:

NOTE If needed, an extended (up to overnight) lysis can be done at **60°C** before proceeding to the **Decrosslinking** step below.

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1 **Decrosslinking**:

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

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.

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2 **RNase A Treatment**:

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

b. Mix by pipetting up and down 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 five minutes.

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3 **Bind DNA**:

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.

4 **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 by pipetting up and down 15 times or until the beads are fully re-suspended in the solution. Mix gently to minimize the generation of bubbles.

d. Place the samples on the magnet for 10 minutes; if the solution is not fully clear after 10 minutes, incubate until clear.

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

5 **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.

6 **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.

Product Availability

FormaPure Total

REF C16675 — FormaPure Total, 50 Prep Kit

REF C16676 — FormaPure Total, 96 Prep Kit

Trademarks

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.

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

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