BECKMAN COULTER **FormaPure Total**

1 Start here

Be sure to read the full instructions for use before proceeding with this checklist. Video links are provided throughout the protocol demonstrating best practices. FormaPure.com/IFU

1. Sample Preparation

- ☐ a. Place **one to three 10 µm** FFPE tissue sections into a **1.5 mL** tube

2. Deparaffinization

- ☐ a. Add **450 µL** of mineral oil - MO - and immerse the tissue completely — FormaPure.com/oil
- ☐ b. Incubate at **80°C** for **5 mintues**
- ☐ c. Vortex **two times** for **5 seconds**

3. Tissue Digestion

- ☐ a. Add **200 µL** of Lysis buffer - LBA
- ☐ b. Centrifuge at **10,000 x g** for **15 seconds**
- ☐ c. Add **20 µL** of **Proteinase K** to the lower phase. Pipette mix **10 times**.
- ☐ d. Incubate at **60°C** for **120 minutes** Start _____ End _____

Continued on reverse side

1 continued

The protocol splits at this point depending on which extractions you'd like to perform.

For RNA isolation only: Proceed to **"Card 4 - RNA Only isolation"**

For DNA isolation only: Proceed to **"Card 5 - DNA Only isolation"**

For Total isolation: Continue below

4. Lysate Splitting

☐ a. Centrifuge at **10,000 x g** for **5 minutes**

☐ b. Transfer **100 µL** of the Lysate (bottom layer) to a new tube or plate for RNA isolation. Avoid disturbing the upper phase (mineral oil) or any pellet that may be present.

The transferred 100 µL should proceed to:

"Card 2 - Total Isolation - RNA"

The remaining content should proceed to:

"Card 3 - Total Isolation - DNA"

Note: The RNA portion should be processed right away. The DNA portion can remain at **60°C** up to overnight.

2 Total isolation - RNA

5. First Bind

- ☐ a. Add **150 µL** of Bind buffer - BBA — FormaPure.com/bind
- ☐ b. Pipette mix **10 times**
- ☐ c. Incubate for **5 minutes**
- ☐ d. Separate on the magnet for **10 minutes** — FormaPure.com/separate
- ☐ e. Aspirate and discard supernatant — FormaPure.com/supernatant

6. Ethanol Wash

- ☐ a. Remove samples from the magnet
- ☐ b. Add **375 µL** of **80% ethanol**
- ☐ c. Pipette mix **20 times** to resuspend the beads — FormaPure.com/resuspend
- ☐ d. Separate on the magnet for **3 minutes** — FormaPure.com/separate
- ☐ e. Aspirate and discard supernatant — FormaPure.com/supernatant
- ☐ f. Air dry on the magnet for **10 minutes** — FormaPure.com/dry

7. DNase I Treatment

- ☐ a. Add **80 µL** of water
- ☐ b. Add **10 µL** of **10x DNase I buffer** and **10 µL** of **DNase I** and pipette mix **5 times**
- ☐ c. Incubate at **37°C** for **20 minutes**

Continued on reverse side

2 continued

8. Re-bind

- ☐ a. Add **150 µL** of Re-bind buffer - RBA
- ☐ b. Pipette mix **10 times**
- ☐ c. Incubate for **5 minutes**
- ☐ d. Separate on the magnet for **10 minutes** — FormaPure.com/separate
- ☐ e. Aspirate and discard supernatant — FormaPure.com/supernatant

9. Ethanol Wash

- ☐ a. Remove samples from the magnet
- ☐ b. Add **375 µL** of **80% ethanol**
- ☐ c. Pipette mix **20 times** to resuspend the beads — FormaPure.com/resuspend
- ☐ d. Separate on the magnet for **3 minutes** — FormaPure.com/separate
- ☐ e. Aspirate and discard supernatant — FormaPure.com/supernatant
- ☐ f. Air dry on the magnet for **10 minutes** — FormaPure.com/dry

10. Elution

- ☐ a. Remove samples from the magnet
- ☐ b. Add **40 µL** water
- ☐ c. Pipette mix **10 times** to resuspend the beads — FormaPure.com/resuspend
- ☐ d. Incubate at **60°C** for **1 minute**
- ☐ e. Separate on the magnet for **1 minute** — FormaPure.com/separate
- ☐ f. Transfer eluate to a new plate or tube
- ☐ g. Store at **-20°C**, or **-80°C** for long-term storage

3 Total isolation - DNA

5. Lysis

- ☐ a. If there is a pellet, pipette mix **10 times**
- ☐ b. Incubate at **60°C** for **60 minutes** (or up to **overnight** if needed)

Start _____ End _____

6. Decrosslinking

- ☐ a. Incubate at **80°C** for **60 minutes**
- ☐ b. Transfer lysate to a new tube or plate

Start _____ End _____

▶ FormaPure.com/lysate

7. RNase Treatment

- ☐ a. Add **2.5 µL** of **RNase A**
- ☐ b. Pipette mix **5 times**
- ☐ c. Incubate for **5 minutes**

8. Bind DNA

- ☐ a. Add **150 µL** of Bind buffer - BBA
- ☐ b. Pipette mix **10 times**
- ☐ c. Incubate for **5 minutes**
- ☐ d. Separate on the magnet for **10 minutes**
- ☐ e. Aspirate and discard supernatant

▶ FormaPure.com/bind

▶ FormaPure.com/separate

▶ FormaPure.com/supernatant

Continued on reverse side

3 continued

9. Wash

- ☐ a. Remove samples from the magnet
- ☐ b. Add **200 µL** of Wash buffer - WBA
- ☐ c. Pipette mix **15 times** to resuspend the beads — FormaPure.com/resuspend
- ☐ d. Separate on the magnet for **10 minutes** — FormaPure.com/separate
- ☐ e. Aspirate and discard supernatant — FormaPure.com/supernatant

10. Ethanol Wash & Air Dry

- ☐ a. Remove samples from the magnet
- ☐ b. Add **375 µL** of **80% ethanol**
- ☐ c. Pipette mix **20 times** to resuspend the beads — FormaPure.com/resuspend
- ☐ d. Separate on the magnet for **3 minutes** — FormaPure.com/separate
- ☐ e. Aspirate and discard supernatant — FormaPure.com/supernatant
- ☐ f. Air dry on the magnet for **10 minutes** — FormaPure.com/dry

11. Elution

- ☐ a. Remove samples from the magnet
- ☐ b. Add **40 µL** water
- ☐ c. Pipette mix **10 times** to resuspend the beads — FormaPure.com/resuspend
- ☐ d. Incubate at **60°C** for **1 minute**
- ☐ e. Separate on the magnet for **1 minute** — FormaPure.com/separate
- ☐ f. Transfer eluate to a new plate or tube
- ☐ g. Store at **-20°C**

4 RNA-only isolation

4. Lysis Transfer

- ☐ a. Centrifuge at **10,000 x g** for **5 minutes**
- ☐ b. Transfer all of the lysate to a new tube or plate

FormaPure.com/lysate

5. First Bind

- ☐ a. Add **300 µL** of Bind buffer - BBA
- ☐ b. Pipette mix **10 times**
- ☐ c. Incubate for **5 minutes**
- ☐ d. Separate on the magnet for **10 minutes**
- ☐ e. Aspirate and discard supernatant

FormaPure.com/bind
FormaPure.com/separate
FormaPure.com/supernatant

6. Ethanol Wash

- ☐ a. Remove samples from the magnet
- ☐ b. Add **750 µL** of **80% ethanol**
- ☐ c. Pipette mix **20 times** to resuspend the beads
- ☐ d. Separate on the magnet for **3 minutes**
- ☐ e. Aspirate and discard supernatant
- ☐ f. Air dry on the magnet for **10 minutes**

FormaPure.com/resuspend
FormaPure.com/separate
FormaPure.com/supernatant
FormaPure.com/dry

7. DNase I Treatment

- ☐ a. Add **80 µL** of water
- ☐ b. Add **10 µL** of **10x DNase I buffer** and **10 µL** of **DNase I** and pipette mix **5 times**
- ☐ c. Incubate at **37°C** for **20 minutes**

Continued on reverse side

4 continued

8. Re-bind

- ☐ a. Add **150 µL** of Re-bind buffer - RBA
- ☐ b. Pipette mix **10 times**
- ☐ c. Incubate for **5 minutes**
- ☐ d. Separate on the magnet for **10 minutes** — FormaPure.com/separate
- ☐ e. Aspirate and discard supernatant — FormaPure.com/supernatant

9. Ethanol Wash

- ☐ a. Remove samples from the magnet
- ☐ b. Add **750 µL** of **80% ethanol**
- ☐ c. Pipette mix **20 times** to resuspend the beads — FormaPure.com/resuspend
- ☐ d. Separate on the magnet for **3 minutes** — FormaPure.com/separate
- ☐ e. Aspirate and discard supernatant — FormaPure.com/supernatant
- ☐ f. Air dry on the magnet for **10 minutes** — FormaPure.com/dry

10. Elution

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- ☐ b. Add **40 µL** water
- ☐ c. Pipette mix **10 times** to resuspend the beads — FormaPure.com/resuspend
- ☐ d. Incubate at **60°C** for **1 minute**
- ☐ e. Separate on the magnet for **1 minute** — FormaPure.com/separate
- ☐ f. Transfer eluate to a new plate or tube
- ☐ g. Store at **-20°C**, or **-80°C** for long-term storage

5 DNA-only isolation

4. Lysis

- ☐ a. If needed, extended **60°C** lysis (up to **overnight**) — Start _____ End _____

5. Decrosslinking

- ☐ a. Incubate at **80°C** for **60 minutes** — Start _____ End _____
- ☐ b. Transfer all of the lysate to a new tube or plate — FormaPure.com/lysate

6. RNase Treatment

- ☐ a. Add **5 µL** of **RNase A**
- ☐ b. Pipette mix **5 times**
- ☐ c. Incubate for **5 minutes**

7. Bind DNA

- ☐ a. Add **300 µL** of Bind buffer - BBA — FormaPure.com/bind
- ☐ b. Pipette mix **10 times**
- ☐ c. Incubate for **5 minutes**
- ☐ d. Separate on the magnet for **10 minutes** — FormaPure.com/separate
- ☐ e. Aspirate and discard supernatant — FormaPure.com/supernatant

Continued on reverse side

5 continued

8. Wash

- ☐ a. Remove samples from the magnet
- ☐ b. Add **400 µL** of Wash buffer - WBA
- ☐ c. Pipette mix **15 times** to resuspend the beads — FormaPure.com/resuspend
- ☐ d. Separate on the magnet for **10 minutes** — FormaPure.com/separate
- ☐ e. Aspirate and discard supernatant — FormaPure.com/supernatant

5. Ethanol Wash & Air Dry

- ☐ a. Remove samples from the magnet
- ☐ b. Add **750 µL** of **80% ethanol**
- ☐ c. Pipette mix **20 times** to resuspend the beads — FormaPure.com/resuspend
- ☐ d. Separate on the magnet for **3 minutes** — FormaPure.com/separate
- ☐ e. Aspirate and discard supernatant — FormaPure.com/supernatant
- ☐ f. Air dry on the magnet for **10 minutes** — FormaPure.com/dry

10. Elution

- ☐ a. Remove samples from the magnet
- ☐ b. Add **40 µL** water
- ☐ c. Pipette mix **10 times** to resuspend the beads — FormaPure.com/resuspend
- ☐ d. Incubate at **60°C** for **1 minute**
- ☐ e. Separate on the magnet for **1 minute** — FormaPure.com/separate
- ☐ f. Transfer eluate to a new plate or tube
- ☐ g. Store at **-20°C**