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We are still developing and optimizing this protocol

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DNA Extraction for Formalin Specimen

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ABSTRACT

DNA Extraction for Formalin Specimen (Qiagen Kit: QIAamp DNA FFPE Advanced Kit)

Deparaffinization process

1 Place the tissue in a 1.5 ml or 2 ml microcentrifuge tube. Add **300 µl Deparaffinization Solution**,

vortex vigorously for **10 s**, and centrifuge briefly to bring the sample to the bottom of the tube.

Note: For specimens stored only in alcohol (=not fixed in paraffin), "deparaffinization step" is not required. In this case, instead of 300 µl Deparaffinization Solution, add **300 µl ATL, 300 µl AL**, and **300 µl ethanol (96–100%)**, and proceed to the next **step 3**, without step 2 (incubation).

2 Incubate at **56°C** for **3 min**, then allow to **cool to room temperature**.

Preparation for Extraction 1

3 Add **25 µl Buffer FTB**, **55 µl RNase-free Water**, and **20 µl Proteinase K**. Mix by vortexing. Briefly centrifuge the tube to spin down any tissue that sticks to the tube wall or under the cap of the tube after vortexing.

Lysis

4 Incubate for **2 h** at **56°C** and **1000 rpm.**

Note: In general, more than one hour is required, and the results were most successful when the process was conducted for more than 2 hours. For relatively older samples, it can be proceed "overnight".

5 Incubate for **1 h** at **90°C** without shaking.

Preparation for Extraction 2

6 Carefully remove and discard the upper **blue phase**. Keep the lower aqueous lysate, add **150 µl RNase-free Water**, then vortex.

Note: If the deparaffinization process (step 1 and 2) is omitted, remove the **900 µl of the upper lysate**.

7 Add **2 µl RNase A**, vortex, and incubate for **2 min** at **room temperature** on the bench.

8 Add **20 µl Proteinase K**, vortex, and incubate for **15 min** at **65°C** and **450 rpm**.

9 Add **250 µl Buffer AL** and **250 µl ethanol (96–100%)** to each sample and mix thoroughly by vortexing

Extraction

10 Transfer **450 µl lysate** to the QIAamp UCP MinElute column (in a 2 ml collection tube), and centrifuge at **15,000 rpm** for **30 s**.

Note: Maximum speed is recommended.

11 Transfer the residual lysate to the same QIAamp UCP MinElute column, and centrifuge at **15,000 rpm** for **1 min**. Discard the flow-through from step 10 and 11 and reuse the collection tube.

Note: Maximum speed is recommended.

12 Add **500 µl Buffer AW1** to each spin column, and centrifuge at **15,000 rpm** for **30 s**. Discard the flow-through and reuse the collection tube.

Note: Maximum speed is recommended.

13 Add **500 µl Buffer AW2** to each spin column, and centrifuge at **15,000 rpm** for **30 s**. Discard the flow-through and reuse the collection tube.

Note: Maximum speed is recommended.

14 Add **250 µl ethanol (96–100%)** to the spin column, and centrifuge at **15,000 rpm** for **30 s**. Discard the flow-through and collection tube. Place the spin column into a new 2 ml collection tube and centrifuge for **3 min at full speed** to remove any residual liquid to dry the membrane.

Note: Maximum speed is recommended.

15 Place the QIAamp UCP MinElute column into a clean 1.5 ml microcentrifuge tube, and discard the collection tube containing the flow-through. Open the lid of the QIAamp MinElute column and apply **20 µl Buffer ATE** to the center of the membrane.

16 Close the lid and incubate at **room temperature** for **1 min**, then centrifuge at **full speed** for **1 min** to elute the DNA. **Step 15** is repeated **twice** more so that final volume is **60 µl (20 µl+20 µl+20 µl)**.

Note: The final volume can be up to 100 µl, but the sequence result in the case of 60 µl was the most successful.