May 2020

Quick-Start Protocol

QIAamp® DNA FFPE Advanced Kit and QIAamp DNA FFPE Advanced UNG Kit

QIAamp DNA FFPE Advanced UNG Kit (cat. no. 56704) consists of the QIAamp DNA FFPE Advanced Kit (cat. no. 56604) and Uracil-N-glycosylase (UNG, cat. no. 19160).

UNG is shipped on dry ice and should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. Under these conditions, UNG is stable until the expiration date printed on the UNG tube label.

Store QIAamp UCP MinElute® columns at 2–8°C. All other components of the QIAamp DNA FFPE Advanced Kit and QIAamp DNA FFPE Advanced UNG Kit should be stored dry at room temperature (15–25°C). Under these conditions, they are stable for at least 12 months, unless otherwise stated on the label.

Further information

- *QIAamp DNA FFPE Advanced Kit and QIAamp DNA FFPE Advanced UNG Kit Handbook*: **www.qiagen.com/HB-2730**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Deparaffinization Solution solidifies at temperatures below 18°C. Incubate at 30°C to redissolve.
- If Buffer AL contains precipitates, dissolve by heating at 70°C with gentle agitation.
- Add 25 ml ethanol (96–100%) to the bottle containing 19 ml Buffer AW1 concentrate, and add 30 ml ethanol (96–100%) to the bottle containing 13 ml Buffer AW2 concentrate.
- Preheat a thermomixer at 56°C for use in steps 2 and 4, and preheat a second thermomixer at 90°C for use in step 5.
- The kit can process FFPE tissue sections of 5–10 µm thickness, totaling up to 4 mm^3 of tissue. In cases where calculating the exact amount is impossible, use no more than 2 sections of 5–10 µm thickness.

Procedure

1. Place the FFPE sections in a 1.5 ml or 2 ml microcentrifuge tube (not supplied). Add 300 µl Deparaffinization Solution, vortex vigorously for 10 s, and centrifuge briefly to bring the sample to the bottom of the tube.
2. Incubate at 56°C for 3 min, then allow to cool to room temperature.

   Note: If too little Deparaffinization Solution is used or if too much paraffin is carried over with the sample, Deparaffinization Solution may become waxy or solid after cooling. If this occurs, add additional Deparaffinization Solution and repeat the incubation at 56°C.
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 FFPE tissue that sticks to the tube wall or under the cap of the tube after vortexing.

   Note: A master mix that comprises the respective components may be prepared in advance.

4. Incubate for 1 h at 56°C and 1000 rpm.

   Note: After incubation, set the thermomixer to 50°C for incubation in step 6a if using the QIAamp DNA FFPE Advanced UNG Kit. If using the QIAamp DNA FFPE Advanced Kit, set the thermomixer to 65°C for incubation in step 8.

5. Incubate for 1 h at 90°C.

   Optional: After incubation, briefly centrifuge the tube to remove drops from inside the lid.

6. Carefully remove and discard the upper blue phase. Keep the lower aqueous lysate, and proceed with step 6a or 6b.

   6a. **QIAamp DNA FFPE Advanced UNG Kit**: Add 115 µl RNase-free water and 35 µl UNG, vortex, and incubate at 50°C for 5 min.

   Optional: After incubation, briefly centrifuge the tube to remove drops from inside the lid.

   Note: After incubation, set the thermomixer to 65°C for incubation in step 8.

   6b. **QIAamp DNA FFPE Advanced Kit**: Add 150 µl RNase-free water, and then vortex.

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

   Optional: After incubation, briefly centrifuge the tube to remove drops from inside the lid.

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

   Optional: After incubation, briefly centrifuge the tube to remove drops from inside the lid.

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

   Optional: Centrifuge briefly to remove drops from inside the lid.

10. Transfer 450 µl lysate to the QIAamp UCP MinElute column (in a 2 ml collection tube), and centrifuge at 15000 x *g* for 30 s.

11. Transfer the residual lysate to the same QIAamp UCP MinElute column, and centrifuge at 15000 x *g* for 1 min. Discard the flow-through and reuse the collection tube.

12. Add 500 µl Buffer AW1 to each spin column, and centrifuge at 15000 x *g* for 30 s. Discard the flow-through and reuse the collection tube.

13. Add 500 µl Buffer AW2 to each spin column, and centrifuge at 15000 x *g* for 30 s. Discard the flow-through and reuse the collection tube.
14. Add 250 µl ethanol (96–100%) to the spin column, and centrifuge at 15000 x *g* for 30 s. Discard the flow-through and collection tube. Place the spin column into a new 2 ml collection tube (supplied) and centrifuge for 3 min at full speed to remove any residual liquid to dry the membrane.
15. Place the QIAamp UCP MinElute column into a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the flow-through. Open the lid of the QIAamp UCP MinElute column and apply 20–100 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.

Document Revision History

Date	Changes
05/2020	Initial release.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.