Quick-Start Protocol March 2016

# GeneRead™ DNA FFPE Kit, Part 1

Uracil-N-glycosylase is shipped on dry ice and should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. When the product is stored under these conditions and handled correctly, performance is guaranteed until the expiration date (see the quality control label on Uracil-N-glycosylase tubes). Store QIAamp® MinElute® Columns at 2–8°C. All other components of the GeneRead DNA FFPE Kit (cat. no. 180134) should be stored dry at room temperature (15–25°C). Under these conditions, they are stable for at least 12 months if not otherwise stated on label.

## Further information

- *GeneRead DNA FFPE Handbook*: **www.qiagen.com/HB-1757**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

## Notes before starting

- One section with a thickness up to 10 µm can be processed using this kit. Avoid using too much starting material as this severely affects kit performance.
- Preheat a thermomixer to 56°C for use in steps 2 and 4. If possible, preheat a second thermomixer to 90°C for use in step 5, and a third to 50°C for use in step 8. A heating block can also be used.
- Deparaffinization Solution solidifies at temperatures below 18°C. Incubate at 30°C to resolve. If Buffer FTB contains precipitates, dissolve by heating to 30°C.
- Unless stated otherwise, all centrifugation steps should be performed for 1 min at full speed (maximum 20,000 x *g*) in a conventional, table-top centrifuge.

1. Using a scalpel, trim excess paraffin off the sample block and cut up one section, up to 10 µm thick. If the sample surface has been exposed to air, discard the first 2–3 sections. Immediately place the section in a 1.5 ml or 2 ml microcentrifuge tube (not supplied).

Add 160 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. 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 56°C incubation.
3. Add 55 µl RNase-free water, 25 µl Buffer FTB and 20 µl proteinase K (a master mix comprising these components may be prepared in advance). Vortex and briefly centrifuge the sample. Deparaffinization Solution will form a layer above Buffer FTB with the addition of proteinase K.
4. Incubate at 56°C for 1 hour.
5. Incubate at 90°C for 1 hour. If using only one thermomixer, leave the sample at room temperature (15–25°C) after the 56°C incubation in step 4, until the heating block has reached 90°C for step 5.
6. Briefly centrifuge the tube to remove drops from inside the lid and transfer the lower, clear phase into a new microcentrifuge tube (not provided).
7. Add 115 µl RNase-free water and mix.
8. Add 35 µl UNG to the sample, vortex and incubate at 50°C for 1 hour in a thermomixer. If using only one thermomixer, leave the sample at room temperature (15–25°C) after the 90°C incubation in step 5, until the thermomixer has reached 50°C for step 8.
9. Briefly centrifuge the tube to remove drops from inside the lid.
10. Add 2 µl RNase A (100 mg/ml), mix, and incubate for 2 min at room temperature. Continue with step 11 in part 2 of the protocol.

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