Quick-Start Protocol March 2016

AllPrep® DNA/RNA FFPE Kit, Part 1

The AllPrep DNA/RNA FFPE Kit (cat. no. 80234) can be stored for at least 9 months if not otherwise stated on label: buffers at room temperature (15–25°C); other components at 2–8°C.

Further information

- *AllPrep DNA/RNA FFPE Handbook*: **www.qiagen.com/HB-0373**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Unless otherwise stated, perform all steps quickly at room temperature (15–25°C).
- Unless otherwise stated, centrifugation is performed at ≥8000 x *g* (≥10,000 rpm).
- Reconstitute Buffer FRN, Buffer RPE, Buffer AW1, Buffer AW2 and RNase-Free DNase I as described in the handbook. Mix by shaking before use.
- Set a thermal mixer, heated orbital incubator, or heating block to 56°C.
- Flow-through from steps 8, 9 and 12 contains Buffer RLT and Buffer FRN and is therefore not compatible with bleach.
- Symbols: ■ purification of total RNA that **does not include small RNAs**; ● purification of total RNA that **does include small RNAs**.

1. Prepare sections and remove paraffin as described in the handbook.
2. Resuspend the pellet obtained after deparaffinization by adding 150 µl Buffer PKD and flicking the tube to loosen the pellet. Add 10 µl proteinase K and mix by vortexing.
3. Incubate at 56°C for 15 min. Incubate on ice for 3 min.
4. Centrifuge for 15 min at 20,000 x *g* (14,000 rpm). Carefully transfer the supernatant, without disturbing the pellet, to a new ■ 1.5 ml or ▲ 2 ml Safe-Lock microcentrifuge tube (see handbook for ordering information) for RNA purification (below).

5. Keep the pellet for DNA purification; see "Genomic DNA purification" in the *Quick-Start Protocol AllPrep DNA/RNA FFPE Kit, Part 2*.

RNA purification

6. Incubate the supernatant at 80°C for 15 min.
7. Add 320 µl Buffer RLT, and mix by vortexing or pipetting. Add ■ 720 µl or ▲ 1120 µl ethanol (96–100%), and mix well by vortexing or pipetting.
8. Transfer 700 µl sample, including any precipitate, to an RNeasy® MinElute® spin column placed in a 2 ml collection tube (supplied). Centrifuge for 15 s.

   Repeat step until complete lysate is used.
9. Add 350 µl Buffer FRN to the spin column. Centrifuge for 15 s.
10. Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube. Add the DNase I incubation mix (80 µl) directly to the spin column membrane, and place on the benchtop (20–30°C) for 15 min.
11. Add 500 µl Buffer FRN to the spin column. Centrifuge for 15 s. Save the flow-through for use in step 12.
12. Place the spin column in a new 2 ml collection tube (supplied). Apply the flow-through from step 11 to the spin column. Centrifuge for 15 s.
13. Add 500 µl Buffer RPE to the spin column. Centrifuge for 15 s. Repeat step.
14. Place the spin column in a new 2 ml collection tube (supplied). Open the lid, and centrifuge at full speed for 5 min to dry spin column membrane.
15. Place the spin column in a new 1.5 ml collection tube (supplied). Add 14–30 µl RNase-free water directly to the spin column membrane. Close lid, and incubate for 1 min at room temperature (15–25°C). Centrifuge at full speed for 1 min to elute the RNA.

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