Quick-Start Protocol April 2016

AllPrep® DNA/RNA/miRNA Universal Kit, Part 2

The AllPrep DNA/RNA/miRNA Universal Kit (cat. no. 80224) should be stored dry at room temperature (15–25°C) and is stable for at least 9 months under these conditions if not otherwise stated on label. DNase I should be stored at 4–8°C upon arrival.

Further information

- *AllPrep DNA/RNA/miRNA Universal Handbook*: **www.qiagen.com/HB-1295**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Unless otherwise stated, centrifugation is performed at full speed (maximum 20,000 x *g*) and the flow-through is discarded.
- Symbols: ■ processing 350 µl lysate; ▲ processing 600 µl lysate.

Total RNA purification

1. Add ■ 50 µl or ▲ 80 µl Proteinase K to the flow-through from step 3 of the *Quick-Start Protocol AllPrep DNA/RNA/miRNA Universal Kit, Part 1*, and mix well.
2. Add ■ 200 µl or ▲ 350 µl of 96–100% ethanol to the mixture from step 1, and mix well. Incubate at room temperature (15–25°C) for 10 min.
3. Add ■ 400 µl or ▲ 750 µl of 96–100% ethanol and mix well.
4. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to an RNeasy® spin column placed in a 2 ml collection tube (supplied). Centrifuge for 15 s. Repeat until the complete lysate is used.
5. Add 500 µl Buffer RPE to the RNeasy spin column. Centrifuge for 15 s.
6. Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix gently by inverting the tube. Add the DNase I incubation mix (80 µl) directly to the RNeasy spin column membrane, and place on the bench top for 15 min.

7. Add 500 µl Buffer FRN to the RNeasy spin column. Centrifuge for 15 s. Save the flow-through. Place the RNeasy spin column in a new 2 ml collection tube (supplied). Reapply the flow-through to the spin column and centrifuge for 15 s.
8. Add 500 µl Buffer RPE to the RNeasy spin column. Centrifuge for 15 s.
9. Add 500 µl of 96–100% ethanol to the RNeasy spin column. Centrifuge for 2 min.
10. **Optional**: Place the RNeasy spin column in a new 2 ml collection tube (supplied) and centrifuge for 2 min.
11. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA. Repeat this step to further elute the RNA. To avoid dilution, reapply the eluate from step 11.

Genomic DNA purification

1. Add 350 µl Buffer AW1 to the AllPrep DNA spin column from step 4 of the *Quick-Start Protocol AllPrep DNA/RNA Mini Kit, Part 1*. Centrifuge for 15 s.
2. Add 20 µl Proteinase K to 60 µl Buffer AW1, mix gently and apply the mixture to the AllPrep DNA spin column membrane. Incubate for 5 min at room temperature (15–25°C).
3. Add 350 µl Buffer AW1 to the AllPrep DNA spin column. Centrifuge for 15 s.
4. Add 500 µl Buffer AW2 to the AllPrep DNA spin column. Centrifuge for 2 min.
5. Place the AllPrep DNA spin column in a new 1.5 ml collection tube (supplied). Add 100 µl Buffer EB directly to the spin column membrane. Centrifuge for 1 min at ≥8000 x *g* (10,000 rpm) to elute the DNA.
6. Repeat step 5 to elute further DNA. To achieve a higher DNA concentration, elute with 2 x 50 µl Buffer EB. The final DNA yield, however, may be reduced.

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