Quick-Start Protocol April 2022

RNeasy® Protect Animal Blood Kit

The RNeasy Protect Animal Blood Kit (cat. no. 73224) is shipped at ambient temperature. RNeasy MinElute® spin columns and the RNase-Free DNase Set box should be stored immediately upon receipt at 2–8°C. The remaining components of the kit can be stored dry at room temperature (15–25°C) for up to 9 months.

Further information

- *RNeasy Protect Animal Blood Handbook*: **www.qiagen.com/HB-1256**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Add 4 volumes of ethanol (96–100%) to Buffer RPE before use.
- Blood samples must be collected in RNAprotect® Animal Blood Tubes (supplied separately).
- To purify **total RNA**, **including miRNA**, refer to the *RNeasy Protect Animal Blood Handbook*. Buffer RWT (ordered separately; cat. no. 1067933) is required.

1. Centrifuge the RNAprotect Animal Blood Tube for 3 min at 5000 x *g*.

   Note: Be sure to incubate the tube for at least 2 h at room temperature (15–25°C) after blood collection to achieve complete lysis of blood cells.
2. Remove the supernatant by decanting or pipetting, taking care not to disturb the pellet. Add 1 ml RNase-free water to the pellet and close the tube.
3. Vortex until the pellet is dissolved and centrifuge for 3 min at 5000 x *g*. Remove the entire supernatant by decanting or pipetting and discard.
4. Add 240 µl Buffer RSB and vortex until the pellet is visibly dissolved.
5. Pipet the sample into a 1.5 ml collection tube (supplied). Add 200 µl Buffer RBT and 20 µl proteinase K. Mix by vortexing for 5 s and incubate 10 min at 55°C in a shaker–incubator set at 400–1400 rpm.
6. Pipet the sample into a QIAshredder (lilac) spin column placed in a 2 ml collection tube and centrifuge for 3 min at full speed (≤20,000 x *g*).
7. Transfer the entire flow-through to a new 1.5 ml collection tube (supplied).

8. Add 240 µl ethanol (96–100%) and mix by vortexing.
9. Pipet the sample into an RNeasy MinElute spin column (pink) placed in a 2 ml collection tube. Centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through. Reuse the collection tube in step 10.
10. Add 350 µl Buffer RW1 to the RNeasy MinElute spin column. Centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through. Reuse the collection tube in step 13.
11. Add 10 µl DNase I stock solution to 70 µl Buffer RDD in a 1.5 ml microcentrifuge tube. Mix by gently inverting the tube and centrifuge briefly to collect residual liquid from the sides of the tube.
12. Pipet the DNase I incubation mix (80 µl) directly onto the RNeasy MinElute spin column membrane and incubate on the bench top for 15 min.
13. Add 350 µl Buffer RW1 to the RNeasy MinElute spin column. Centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through. Reuse the collection tube in step 14.
14. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through. Reuse the collection tube in step 15.
15. Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm).
16. Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column and centrifuge at full speed for 5 min. Discard the flow-through and collection tube.
17. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube and pipet 14–30 µl Buffer REB directly onto the spin column membrane. Centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the RNA.
18. Incubate the RNA eluate for 5 min at 65°C. After incubation, chill immediately on ice. Do not exceed the incubation time or temperature. If not used immediately, store RNA at –20°C to –70°C.

Document Revision History

Date	Changes
04/2022	Corrected the time for centrifugation in step 13.

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