April 2019

Quick-Start Protocol

AllPrep® RNA/Protein Kit

The AllPrep RNA/Protein Kit (cat. no. 80404) should be stored at room temperature (15–25°C) and is stable for at least 6 months under these conditions.

Further information

- *AllPrep RNA/Protein Handbook*: **www.qiagen.com/HB-0907**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Buffer APL and Buffer RLT may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature (15–25°C).
- Buffer RLT and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See the "Safety Information" section in the *AllPrep RNA/Protein Handbook*.
- Unless otherwise indicated, all protocol steps, including centrifugation, should be performed at room temperature. During the procedure, work quickly. Cells should be processed immediately after harvesting.
- β-mercaptoethanol (β-ME) must be added to Buffer RLT before use. Add 10 µl ß-ME per 1 ml buffer RLT. Buffer RLT containing ß-ME can be stored at room temperature for up to 1 month.
- Buffer RPE is supplied as concentrate. Before using it for the first time, add the appropriate volume of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.

1. Vortex the Protein Cleanup spin column gently to resuspend the resin.
2. Loosen the cap of the Protein Cleanup spin column by a quarter turn.
3. Snap off the bottom closure of the Protein Cleanup spin column, and then place the spin column in a 2 ml collection tube (supplied).
4. Centrifuge for 3 min at 750 x *g*.
5. Equilibrate the Protein Cleanup spin column by adding 500 µl of equilibration buffer (not supplied), vortexing gently, and then centrifuging for 3 min at 750 x *g*.
6. Carefully transfer Protein Cleanup spin column to a clean microcentrifuge tube (not supplied).
7. Remove the cell-culture medium. Wash the cells with an appropriate volume of PBS or other physiological salt solution.
8. Remove the PBS. Add an appropriate volume of Buffer APL (see Table 1) and incubate for 5 min.

Table 1. Recommended volumes of Buffer APL for cell lysis and recommended centrifuging for the Protein Cleanup spin column

Cell-culture plate	Volume of Buffer APL per well	Centrifugation speed (RCF)
12-well plate	200 µl	240 x *g*
24-well plate	150 µl	240 x *g*
48-well plate	100 µl	420 x *g*
96-well plate	50 µl	420 x *g*

9. Homogenize by pipetting up and down several times.
10. Pipet the lysate into an AllPrep spin column that is inside a 2 ml collection tube, and then centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm).

    Note: The AllPrep spin column contains bound RNA. Keep at room temperature for later use in step 14.

    The flow-through contains total protein; proceed immediately to step 11. If there will be a slight delay, keep the flow-through on ice.

11. Slowly (dropwise) pipet the flow-through from step 10 directly onto the center of the slanted gel bed in the Protein Cleanup spin column.
12. Centrifuge for 3 min at either 240 x *g* or 420 x *g* (see Table 1 for details).
13. Remove the Protein Cleanup spin column from the microcentrifuge tube. The flow-through contains purified total protein and is ready either for use in downstream applications or for further purification/fractionation.

    Note: The purified protein contains buffer components that interfere with some quantification methods, including Bradford and Lowry assays. However, protein can be quickly and easily quantified by measuring absorbances at 260 nm and 280 nm (see Appendix C of the *AllPrep RNA/Protein Handbook*). Alternatively, a bicinchoninic acid (BCA) assay can be performed instead.

    Note: The functionality of proteins after lysis and cleanup was tested by measuring β-galactosidase, or beta-gal, activity.
14. Place the AllPrep spin column in a new 2 ml collection tube (supplied). Add 350 µl Buffer RLT, and then centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm).
15. Add 1 volume of 70% ethanol (usually 350 µl) to the flow-through. Mix well by pipetting up and down several times.
16. Add 700 µl of the sample, including any precipitate that may have formed, to an RNeasy spin column inside a 2 ml collection tube. Close the lid gently, and then centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.*

    Reuse the collection tube in step 17.
17. Add 700 µl Buffer RW1 to the RNeasy® spin column. Close the lid gently, and centrifuge for 30 s at ≥8000 x g (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.*

    Reuse the collection tube in step 18.

* Flowthrough contains RLT or RWT Buffer and is therefore not compatible with bleach.

18. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and then centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.

    Reuse the collection tube in step 19.

19. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and then centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.

    Reuse the collection tube in step 20.

20. Centrifuge the RNeasy spin column for 1 min at full speed to remove residual wash buffer.

    Recommended: Perform a second dry centrifugation: Place the spin column in a new 2 ml collection tube (not supplied), discard the old collection tube with the flow-through, and then centrifuge at full speed for 1 min.

21. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Pipet 30–50 µl RNase-free water directly onto the spin column membrane. Close the lid gently, and then centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute RNA.

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