# Protocol: RNA Cleanup and Concentration

The RNeasy Micro Kit can be used to clean up and concentrate RNA previously isolated by different methods or after enzymatic reactions, such as labeling or DNase digestion. For concentration of total cellular RNA purified using the PAXgene® Blood RNA Kit, we recommend using the RNeasy MinElute Cleanup Kit (cat. no. 74204).

## Determining the correct amount of starting material

A maximum of 45 µg RNA in a maximum volume of 200 µl can be cleaned up in this protocol. This amount corresponds to the binding capacity of the RNeasy MinElute spin column.

## Important points before starting

- If preparing RNA for the first time, read Appendix A (page 61).
- Generally, DNase digestion is not required since RNeasy MinElute silica-membrane technology efficiently removes most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA (e.g., TaqMan® RT-PCR analysis with a low-abundance target). In these cases, DNA can be removed by a DNase digestion before starting RNA cleanup (see Appendix D, page 73).
- Buffer RLT and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for Safety Information.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.
- In the procedure below, ▲ refers to use of starting volumes ≤100 µl and ● refers to use of starting volumes of 100–200 µl.

## Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Before using the kit for the first time, prepare 80% ethanol by mixing 24 ml ethanol (96–100%) and 6 ml RNase-free water (supplied).
- Buffer RLT may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature (15–25°C).
- **Optional**: If cleaning up crude RNA preps (e.g., after salting-out methods) or samples rich in RNases, we recommend adding β-ME to Buffer RLT before use. Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature for up to 1 month. Alternatively, add 20 µl of 2 M DTT per 1 ml Buffer RLT. The stock solution of 2 M DTT in water should be prepared fresh or frozen in single-use aliquots. Buffer RLT containing DTT can be stored at room temperature for up to 1 month.

## Procedure

1. Adjust the sample to a volume of▲ 100 µl or ● 200 µl with RNase-free water. Add ▲ 350 µl or ● 700 µl Buffer RLT, and mix well.

   If starting with an RNA pellet, be sure that the pellet is dissolved in the RNase-free water (supplied) before adding Buffer RLT.

   **Optional**: Add β-ME (or DTT) to Buffer RLT before use (see "Things to do before starting").
2. Add ▲ 250 µl or ● 500 µl of 96–100% ethanol to the diluted RNA, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 3.

3. Transfer the sample (700 µl) to an RNeasy MinElute spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.*

   For ● samples >700 µl, transfer the remaining sample (up to 700 µl) and repeat the centrifugation. Discard the flow-through.*

4. **Optional**: Add 700 µl Buffer RW1 to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and collection tube.*

5. Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Add 500 µl Buffer RPE to the spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through. Reuse the collection tube in step 6.

   **Note**: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting").

6. Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and collection tube.

   Prepare the 80% ethanol with ethanol (96–100%) and the RNase-free water supplied with the kit.

   **Note**: After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

* Flow-through contains Buffer RLT or Buffer RW1 and is therefore not compatible with bleach. See page 6 for Safety Information.

7. 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.

   To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

   It is important to dry the spin column membrane because residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

8. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.

   As little as 10 µl RNase-free water can be used for elution if a higher RNA concentration is required, but the yield will be reduced by approximately 20%. Do not elute with less than 10 µl RNase-free water, as the spin column membrane will not be sufficiently hydrated.

   The dead volume of the RNeasy MinElute spin column is 2 µl: elution with 14 µl RNase-free water results in a 12 µl eluate.

   For RT-PCR and real-time RT-PCR with the purified RNA, QIAGEN offers a range of optimized, ready-to-use kits that provide highly specific and sensitive results. For details, visit **www.qiagen.com/PCR**. For WTA of limited amounts of RNA, we recommend the QuantiTect Whole Transcriptome Kit. For details, visit **www.qiagen.com/goto/WTA**.