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miRNeasy Tissue/Cells Advanced Mini Kit Handbook

For purification of total RNA, including miRNA from tissue and cells

Sample to Insight

QIAGEN

Protocol: Purification of Total RNA, Including Small RNAs, from Animal Tissues

Determining the correct amount of starting material

It is essential to use the correct amount of starting material to obtain optimal RNA yield and purity. A maximum amount of 30 mg fresh or frozen tissue or 15 mg RNAprotect stabilized tissue (which is partially dehydrated) can generally be processed. For most tissues, the DNA removal capacity of the gDNA Eliminator spin column, the RNA binding capacity of the RNeasy spin column, and the lysing capacity of Buffer RLT will not be exceeded by these amounts. However, smaller amounts may allow more efficient DNA removal. Average RNA yields from various tissues are given in table 3 (page14). Some tissues, such as spleen and thymus, contain very high amounts of DNA, which will overload the gDNA Eliminator spin column. For fibrous tissues, such as muscle and skin, that contain contractile proteins, connective tissue and collagen, we recommend the RNeasy Plus Universal Mini Kit, which efficiently removes these components in the phase separation.

Do not overload the gDNA Eliminator spin column, as this will lead to copurification of DNA with RNA. Do not overload the RNeasy spin column, as this will significantly reduce RNA yield and quality.

Important points before starting

- If using the miRNeasy Tissue/Cells Advanced Mini Kit for the first time, read "Important Notes" (page 14).
- It is important not to overload the RNeasy Mini spin column, as overloading will significantly reduce RNA yield and quality. Read "Determining the amount of starting material" (page 14).
- If working with RNA for the first time, read Appendix C (page 43).

- For optimal results, stabilize harvested tissues immediately in RNAprotect Tissue Reagent or Allprotect Stabilization Reagent. Tissues can be stored in the reagent for up to 1 day at 37°C, 7 days at 15–25°C, or 4 weeks at 2–8°C, or archived at –95°C to –15°C.
- Fresh, frozen, or RNAprotect- or AllProtect-stabilized tissues can be used. Tissues can be stored for several months at –90°C to –65°C. Do not allow tissues to thaw during weighing or handling prior to disruption in Buffer RLT. Homogenized tissue lysates (in Buffer RLT, step 3) can also be stored at –90°C to –65°C for several months. To process frozen homogenized lysates, incubate at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity. Continue with step 4.
- Generally, DNase digestion is not required since the combination of the RNeasy technologies in combination with the gDNA eliminator efficiently removes most of the DNA without DNase treatment. In addition, miRCURY Assays and most other assays for mature miRNA are not affected by the presence of small amounts of genomic DNA. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA. In these cases, small residual amounts of DNA can be removed by on-column DNase digestion (see Appendix B, page 40) or by DNase digestion after RNA purification (please contact QIAGEN Technical Service for a protocol).
- Buffer RLT und Buffer RWT may form a precipitate upon storage. If necessary, redissolve by warming and then place at room temperature (15–25°C).
- Buffer RLT, Buffer AL, and Buffer RWT contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 for Safety Information.
- All protocol and centrifugation steps should be performed at room temperature. During the procedure, work quickly.

Things to do before starting

- Buffers RWT and RPE are supplied as concentrates. Before using for the first time, add the required amounts of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.
- If performing optional on-column DNase digestion, prepare DNase I stock solution as described in Appendix B (page 40).

Notes before starting

- Equilibrate buffers to room temperature.
- All steps should be performed at room temperature. Work quickly.
- β-mercaptoethanol (β-ME) must be added 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 is stable at room temperature (15–25°C) for 1 month after addition of β-ME. Alternatively, add 20 µl 2 M dithiothreitol (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. Excise the tissue sample from the animal or remove it from storage. Remove RNAprotect-stabilized tissues from the reagent using forceps. Determine the amount of tissue. Do not use more than 30 mg.

   Weighing tissue is the most accurate way to determine the amount.

2. Follow either step 2a or 2b.

   2a. For RNAprotect or Allprotect-stabilized tissues: If tissue is still submerged in stabilizing reagent remove each tissue sample from the liquid. Remove residual reagent (e.g., by dabbing or rolling the tissue over a paper towel) If using the entire tissue, place it directly into a suitably sized vessel for disruption and homogenization, and proceed to step 3.

   If using only a portion of the tissue, cut it on a clean surface. Weigh the piece to be used, and place it into a suitably sized vessel for disruption and homogenization. Proceed to step 3. RNA in RNAprotect-stabilized tissues is protected during cutting and weighing of tissues at ambient temperature (18–25°C). It is not necessary to cut the tissues on ice or dry ice or in a refrigerated room. Remaining tissues can be stored in RNAprotect Tissue Reagent. Previously stabilized tissues can be stored at –80°C without the reagent.

   2b. For unstabilized fresh or frozen tissues:

   If using the entire tissue, place it directly into a suitably sized tube for disruption and homogenization, and proceed immediately to step 3.

   If using only a portion of the tissue, weigh the piece to be used, and place it into a suitably sized vessel for disruption and homogenization. Proceed immediately to step 3.

   RNA in harvested tissues is not protected until the tissues are treated with RNAprotect Tissue Reagent, flash-frozen, or disrupted and homogenized in step 3. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

   Note: Remaining fresh tissues can be placed into RNAprotect Tissue Reagent to stabilize RNA (see the *RNAprotect Tissue Handbook*). However, previously frozen tissues thaw too slowly in the reagent, preventing the reagent from diffusing into the tissues quickly enough to prevent RNA degradation.

3. Disrupt the tissue and homogenize the lysate in Buffer RLT (do not use more than 30 mg tissue) according to step 3a, 3b, 3c, or 3d.

   See "Determining the amount of starting material", page 14, for more details on disruption and homogenization.

   Optional: Ensure that β-ME is added to Buffer RLT before use (see "Important points before starting").

   Note: After storage in RNAprotect Tissue Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization using standard methods is usually not a problem. For easier disruption and homogenization, we recommended using 450 µl Buffer RLT.

   Note: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the RNeasy spin column. Homogenization with the TissueLyser and rotor–stator homogenizers generally results in higher RNA yields than with other methods.

Table 6. Volumes of buffer RLT for sample disruption and homogenization sample

	Amount	Buffer RLT	Buffer AL	Disruption and homogenization
Animal tissues	<20 mg	260 µl	80 µl	TissueLyser LT, TissueLyser II, TissueRuptor II, or mortar and pestle followed by QIAshredder or needle and syringe.
	20–30 mg	450 µl	140 µl	

3a. Disruption and homogenization using the TissueRuptor II: Place the weighed (fresh, frozen, or RNAprotect Tissue-stabilized) tissue in a suitably sized vessel. Add the appropriate volume of Buffer RLT (see Table 6). Immediately disrupt and homogenize the tissue until it is uniformly homogeneous (usually 20–40 s); see the *TissueRuptor II Handbook*. Proceed to step 4.

3b. Disruption using a mortar and pestle followed by homogenization using a QIAshredder homogenizer: Immediately place the weighed (fresh, frozen, or RNAprotect Tissue-stabilized) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw. Add the appropriate volume of Buffer RLT (see Table 6). Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at maximum speed. Proceed to step 4.

3c. Disruption using a mortar and pestle followed by homogenization using a needle and syringe: Immediately place the weighed (fresh, frozen, or RNAprotect Tissue-stabilized) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw. Add the appropriate volume of Buffer RLT (see Table 6), and homogenize by passing lysate at least 5 times through a 20-gauge needle fitted to an RNase-free syringe. Proceed to step 4.

3d. Disruption and homogenization using the TissueLyser II or TissueLyser LT: See the *TissueLyser Handbook* or the *TissueLyser LT Handbook*. Then proceed to step 4.

4. Centrifuge the lysate for 3 min at maximum speed. Carefully remove the supernatant by pipetting into a new tube.

   Note: This step is important, as it removes insoluble material that could clog the gDNA Eliminator spin column and interfere with DNA removal. In some preparations, very small amounts of insoluble material will be present after the 3 min centrifugation, making the pellet invisible.

5. Add the appropriate volume of Buffer AL (see Table 5) and mix thoroughly. Incubate at room temperature for 3 min.

6. Transfer the homogenized lysate to a gDNA Eliminator spin column placed in a 2 ml collection tube (supplied). Centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm). Discard the column and save the flow-through.

   Note: Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.

7. Transfer the flow-through to a new 2 ml reaction tube (not provided). Add 20 µl Buffer RPP. Close the tube cap and mix vigorously by vortexing for >20 s. Incubate at room temperature for 3 min.

8. Centrifuge at 12,000 x *g* for 3 min at room temperature to pellet the precipitate.

   Note: Supernatant should be clear and colorless. Transfer supernatant to a new 2 ml reaction tube.

9. Add 1 volume isopropanol and mix well by pipetting. Do not centrifuge.

   Note: When purifying RNA from certain tissues, precipitates may be visible after addition of isopropanol. This does not affect the procedure.

10. Transfer up to 700µl of the sample to an RNeasy Mini column placed in a 2ml collection tube (provided). Close the lid and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.

    Reuse the collection tube in step 11.

    If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.

11. Pipet 700 µl Buffer RWT to the RNeasy Mini spin column. Close the lid and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.

    Reuse the collection tube in step 12.

    Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.

12. Pipet 500 µl Buffer RPE onto the RNeasy Mini spin column. Close the lid and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.

    Reuse the collection tube in step 13.

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

13. Add 500 µl of 80% ethanol to the RNeasy Mini spin column. Close the lid and centrifuge for 2 min at ≥8000 x g. Discard the flow-through and the collection tube.

14. Place the RNeasy Mini spin column in a new 2 ml collection tube (supplied). Close the lid of the spin column and centrifuge at full speed for 1 min to dry the membrane. Discard the flow-through and the collection tube.

15. Place the RNeasy Mini spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the center of the spin column membrane and incubate for 1 min. Close the lid and centrifuge for 1 min at full speed to elute the RNA.

16. If the expected RNA yield is >30 µg, repeat step 15 using another 30–50 µl of RNase-free water, or using the eluate from step 15 (if high RNA concentration is required). Reuse the collection tube from step 15.

    If using the eluate from step 15, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.