## Recommended Starting Material

To reach optimized results it is better to follow as listed below. The size of the recommended material to use with determined lysis volumes are written in Table.

**Table.** Appropriate sample size and amount of RLB

| SAMPLE TYPE | SAMPLE SIZE | AMOUNT OF RLB |
|---|---|---|
| Animal Fibrous Tissues | 0.5-30 mg | 300 µl |

## Protocol

## Isolation of RNA (based on silica technology)

**Sample Type:** Animal tissues (fibrous tissue)

### Some Tips to Know:

- For isolation of RNA from skeletal muscle use MiRJia or RNSol.
- Set Thermoblock or water bath at 55℃ before starting the process.
- All centrifugation steps are carried out at room temperature (15–25 °C) in a microcentrifuge.
- All steps, before applying sample into spin column, are carried out on ice.
- Before using the protocol for the first time, add 2-Mercaptoethanol to RLB buffer, 2% of RLB volume. 2-Mercaptoethanol is commercially available and due to safety issue, is not provided in the kit. It can be order separately, by ROJETechnologies (Cat No BU983034) or Sigma-Aldrich (Cat No M3148).

    **Attention!** It is recommended to prepare it freshly. It is not stable more than 2 weeks.

- If RLB buffer forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage condition and will not influence the efficiency of the buffer.
- Do not forget to add the appropriate amount of Ethanol (96–100%) to TW1 and TW2 buffers (TWB1 and TWB2), as indicated on the bottle, before using for the first time.
- For frozen samples in RNaseLag, thaw them to room temperature. Avoid repeated freezing and thawing of samples, since it may cause RNA degradation.
- If working with RNA for the first time, read Appendix 1, in main handbook, carefully.
- Consider that the provided nuclease-free water in kit is intended to use as rehydration solution. It is recommended to buy nuclease-free water for consuming in lyses step.

**Process**

1. Remove the tissue from RNaseLag or use fresh tissue or snap-freeze tissue. Weight the intended tissue, up to 30 mg. Add 300 µl RLB buffer to the tissue sample.
2. Disrupt and homogenize the tissue sample by selecting one of these ways:

   - After adding appropriate RLB buffer amount, use Micropestle followed by homogenizer or syringe needle to homogenize the tissue.

- After adding appropriate RLB buffer amount, using TissueLyser or homogenizer to disrupt and homogenize the sample simultaneously.
- Grind the weighed tissue in liquid nitrogen carefully with a cold mortar and pestle. Transfer tissue powder into an RNase-free, liquid-nitrogen–cooled, 1.5 ml microcentrifuge tube. Let the liquid nitrogen to evaporate completely (but do not allow the tissue to thaw). Add the appropriate volume of RLB buffer (see Table) and homogenize by passing the lysate 5-10 times through a blunt 20-gauge needle fitted to an RNase-free syringe.

  **Note:** Make sure that the disruption and homogenization is complete.

3. Add 600 µl nuclease-free water and 15 µl RJ-Protease. pulse vortex for 15 s and incubate the microtube at 55˚C for 15 min.
4. Centrifuge the lysate for 3 min at 15000 rpm. Carefully remove the supernatant by pipetting, and transfer it to a new RNase-free microcentrifuge tube.

   **Note:** Be careful not to disrupt the pellet by pipette tip.

5. Add half volume of Absolute Ethanol to the cleared lysate, and mix immediately by pulse vortexing for 15 s. Do not centrifuge.

   **Note:** Sometimes, precipitates may appear after Ethanol addition. This does not disturb the procedure.

6. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to a spin column placed in a 2 ml collection tube

(supplied in the kit box). Centrifuge for 1 min at 13000 rpm. Discard the flow-through.

> **Note:** If the lysate does not pass the column, repeat the centrifugation once more at full speed for 1 min.

7. Repeat the previous step by the remaining sample from step 5.

> **Note:** If the lysis does not pass the column, repeat the centrifugation once more at full speed for 1 min.

8. Add 700 µl TWB1 (TW1 buffer) to the spin column. Centrifuge for 1 min at 13000 rpm. Discard the flow-through.
9. Add 500 µl TWB2 (TW2 buffer) to the spin column. Centrifuge for 1 min at 13000 rpm. Discard the flow-through.
10. Add 500 µl TWB2 (TW2 buffer) to the spin column. Centrifuge for 3 min at 14000 rpm. Discard the collection tube with the flow-through.
11. Place the spin column in a new 1.5 ml microtube. Add 30-50 µl RNase-free water directly to the spin column membrane. Centrifuge for 1 min at 12000 rpm to elute the RNA.

> **Note:** If the expected RNA yield is more than the yield from pervious step, put the spin column on a new microtube and add another 30-50 µl RNase-free water. Centrifuge for 1 min at 12000 rpm. The yield will be nearly same as previous step. However, it is possible to pass the follow-through from step 11 once more to obtain RNA with higher concentration.