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 RNSol and chloroform amount are written in Table.

Table. Appropriate sample size and amount of RNSol and Chloroform amount for Animal Adipose tissue

Sample Size	RNSol Amount	Chloroform Amount
10 mg Animal Adipose tissue	100 µl	100 µl
20 mg Animal Adipose tissue	200 µl	100 µl
30 mg Animal Adipose tissue	300 µl	100 µl
40 mg Animal Adipose tissue	400 µl	100 µl
50 mg Animal Adipose tissue	500 µl	200 µl
60 mg Animal Adipose tissue	600 µl	200 µl
70 mg Animal Adipose tissue	700 µl	200 µl
80 mg Animal Adipose tissue	800 µl	200 µl
90 mg Animal Adipose tissue	900 µl	200 µl
100 mg Animal Adipose tissue	1ml	200 µl

Protocol

Protocol: Isolation of RNA (based on silica technology)

Sample Type: Animal adipose tissues (fresh and frozen)

Some Tips to Know:

- All steps, before applying sample into spin column, are carried out on ice.
- 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, thaw them to room temperature. Avoid repeated thawing and freezing of samples due to decreasing in RNA yield.
- If working with RNA for the first time, read Appendix 1, in main handbook, carefully.

Process

1. Remove the tissue from RNaseLag or use fresh tissue. Determine the Weight of starting material. Add appropriate amount of RNSol Reagent to the tissue sample (refer to the Table).
2. Disrupt and homogenize the tissue sample by selecting one of these ways:
   - After adding appropriate amount of RNSol, use micropestle followed by homogenizer or syringe needle to homogenize the tissue.
   - After adding appropriate amount of RNSol, 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 RNSol (refer to the Table) and homogenize by passing the lysate 5-10 times through a blunt 20-gauge needle fitted to an RNase-free syringe.

Optional: Homogenizing step is optional. It means that using homogenizer or syringe needle to homogenize the lysate can be omitted from the process.

3. Incubate at room temperature for 5 min.
4. Add appropriate amount of chloroform (refer to Table), vigorously shake it for 30 s. Then pulse vortex for 15 s and incubate at room temperature for 3 min.
5. Centrifuge at 4°C for 15 min at 13000 rpm.
6. Transfer the aqueous phase (the upper phase) to a new tube. Be careful to avoid interfering the interphase.
7. Add one volume of absolute Ethanol to the separated aqueous phase. Pulse vortex for 30 s.
8. Transfer the solution to a spin column placed in a 2ml collection tube (supplied in the kit box). Centrifuge for 1 min at 13000 rpm at room temperature. Discard the flow-through.
9. Add 700 µl TWB1 (TW1 buffer) to the spin column. Centrifuge for 1 min at 13000 rpm at room temperature. Discard the flow-through.
10. Add 500 µl TWB2 (TW2 buffer) to the spin column. Centrifuge for 1 min at 13000 rpm at room temperature. Discard the flow-through.

11. Add 500 µl TWB2 (TW2 buffer) to the spin column. Centrifuge for 3 min at 14000 rpm at room temperature. Discard the collection tube with the flow-through.
12. Place the spin column in a new 1.5 ml microtube. Add 30-100 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 30-100 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 flow-through from step 12 once more to obtain RNA with higher concentration.