Quick Protocol

MiRJia Lipid Kit

RNA and total RNA isolation based on silica technology

- MiniPrep

For RNA Isolation from

Animal Adipose Tissue

Kit Content

Component	50 preps	100 preps
RNSol H Reagent	50ml	100ml
TWB1 (concentrate)	16ml	2 x 16ml
TWB2 (concentrate)	15ml	2 x 15ml
Nuclease-free Water	5ml	10ml
HiPure DR Column	50	100
Collection Tube	50	100

Recommended Starting Material

To reach optimized results it is better to follow as listed below. The size of the recommended starting material to use with determined RNSol H Reagent, chloroform and ethanol volumes are written in Table 1.

Table 1. Appropriate sample size and amount of RNSol H Reagent, 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	
30 mg Animal Adipose tissue	300 µl	
40 mg Animal Adipose tissue	400 µl	
50 mg Animal Adipose tissue	500 µl	200 µl
60 mg Animal Adipose tissue	600 µl	
70 mg Animal Adipose tissue	700 µl	
80 mg Animal Adipose tissue	800 µl	
90 mg Animal Adipose tissue	900 µl	
100 mg Animal Adipose tissue	1ml	

Before start

- Not forget to add the appropriate amount of ethanol (%96–100) to TWB1 and TWB2 as indicated on the bottle, before using for the first time.

- Do not forget to prepare %70 ethanol (nuclease-free).

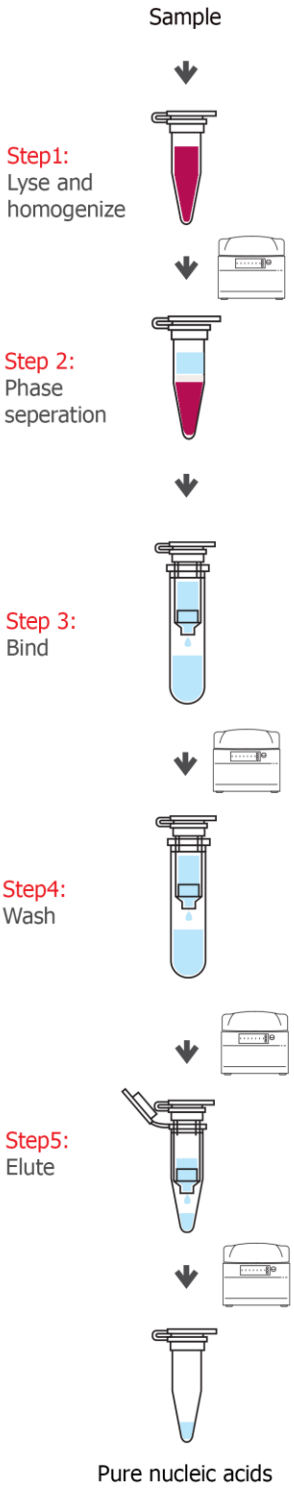
Washing Buffer Preparation

Before the first use, add appropriate amount of ethanol (96-100%) to each washing buffer tube, then mix thoroughly to prepare washing buffer, refer to Table 2. Do not forget to tick the check box on the bottle label to indicate that ethanol has been added. Before each use mix reconstituted buffer by shaking. Storing at room temperature.

Table 2: Washing buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
TWB1	16ml	24ml	40ml
TWB2	15ml	45ml	60ml

Procedure of silica-based RNA isolation in quick look



Protocols

Protocols Phenol-Chloroform Based Protocols

Protocol: Isolation of Total RNA (Animal adipose tissues fresh and frozen)

Sample Type: Animal adipose tissues (fresh and frozen)

Some Tips to Know:

- All steps, before applying sample into HiPure DR Column, are carried out on ice.
- Not forget to add the appropriate amount of ethanol (%96–100) to 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 carefully.

Process

- Remove the tissue from RNaseLag or use fresh tissue. Determine the Weight of starting material. Add appropriate amount of RNSol H Reagent to the tissue sample (refer to the Table 1).
- Disrupt and homogenize the tissue sample by selecting one of these ways:
  - After adding appropriate amount of RNSol H Reagent, use micropestle followed by homogenizer or syringe needle to homogenize the tissue.
  - After adding appropriate amount of RNSol H Reagent, 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, 1.5ml microcentrifuge tube. Let the liquid nitrogen to evaporate completely (but do not allow the tissue to thaw). Add the appropriate volume of RNSol H Reagent (refer to the Table 1) 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.
- Incubate at room temperature for 5min.
- Add appropriate amount of chloroform (refer to Table 1), vigorously shake it for 30s. Then pulse vortex for 15s and incubate at room temperature for 3min.
- Centrifuge at 4°C for 15min at 13000 rpm.

- Transfer the aqueous phase (the upper phase) to a new tube. Be careful to avoid interfering the interphase.
- Add one volume of absolute ethanol to the separated aqueous phase. Pulse vortex for 30s.
- Transfer the solution to a HiPure DR Column placed in a 2ml collection tube (supplied in the kit box). Centrifuge for 1min at 13000 rpm at room temperature. Discard the flow-through.
- Add 700µl TWB1 to the HiPure DR Column. Centrifuge for 1min at 13000 rpm at room temperature. Discard the flow-through.
- Add 500µl TWB2 to the HiPure DR Column. Centrifuge for 1min at 13000 rpm at room temperature. Discard the flow-through.
- Add 500µl TWB2 to the HiPure DR Column. Centrifuge for 3min at 14000 rpm at room temperature. Discard the collection tube with the flow-through.
- Place the HiPure DR Column in a new 1.5ml microtube. Add 30-100µl RNase-free water directly to the HiPure DR Column membrane. Centrifuge for 1min at 12000 rpm to elute the RNA.

  Note: If the expected RNA yield is more than the yield from pervious step, put the HiPure DR Column on a new microtube and add another 30-100µl RNase-free water. Centrifuge for 1min 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.