

# Quick Protocol

## RNJia 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
<b>RNSol H Reagent</b>	50ml	100ml
<b>TWB1 (concentrate)</b>	16ml	2 x 16ml
<b>TWB2 (concentrate)</b>	15ml	2 x 15ml
<b>Nuclease-free Water</b>	5ml	10ml
<b>HiPure DR Column</b>	50	100
<b>Collection Tube</b>	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
10mg Animal Adipose tissue	100µl	100µl
20mg Animal Adipose tissue	200µl	
30mg Animal Adipose tissue	300µl	
40mg Animal Adipose tissue	400µl	
50mg Animal Adipose tissue	500µl	200µl
60mg Animal Adipose tissue	600µl	
70mg Animal Adipose tissue	700µl	
80mg Animal Adipose tissue	800µl	
90mg Animal Adipose tissue	900µl	
100mg Animal Adipose tissue	1ml	

## Washing Buffer Preparation

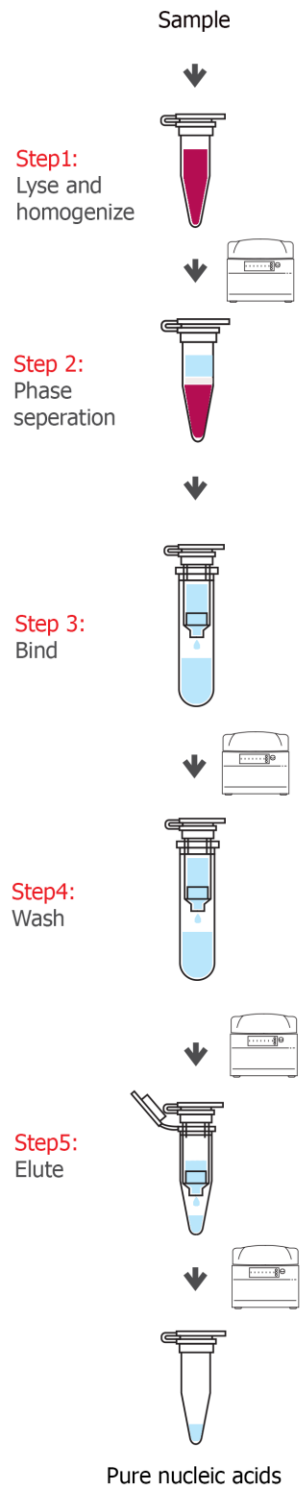
Before the first use, add appropriate amount of ethanol (%96-100) to each washing buffer tube, then mix thoroughly to prepare washing buffer, refer to Table 2. Do not forget to tick

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

<b>Buffer Name</b>	<b>Concentrated Volume</b>	<b>Amount of Ethanol</b>	<b>Final Volume</b>
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.

#### 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, 1.5 ml microcentrifuge tube. Let the liquid nitrogen to evaporate completely (but do not allow the tissue to thaw). Add the appropriate volume of RNSol 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 5 min.
- 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 15 min 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 30-100 µl RNase-free water directly to the HiPure DR 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 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.