Quick Protocol

RNJia Fibrous Kit

RNA isolation based on silica technology

- MiniPrep

For RNA Isolation from

Animal Tissue (Fibrous)

Kit Content

Component	50 preps	100 preps
RLB	15ml	30ml
TWB1 (concentrate)	16ml	2 x 16ml
TWB2 (concentrate)	15ml	2 x 15ml
Nuclease-free Water	5ml	10ml
RJ-Protease	750µl	2 x 750µl
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 RLB are written in Table 1.

Table 1. Appropriate sample size and amount of RLB

Sample Type	Sample Size	Amount of RLB
Animal Fibrous Tissues	0.5-30mg	300µl

Before start

- Not forget to add the appropriate amount of ethanol (molecular biology grade, %96–100) to TWB1 and TWB2 as indicated on the bottle, before using for the first time. Refer to washing buffer preparation section.

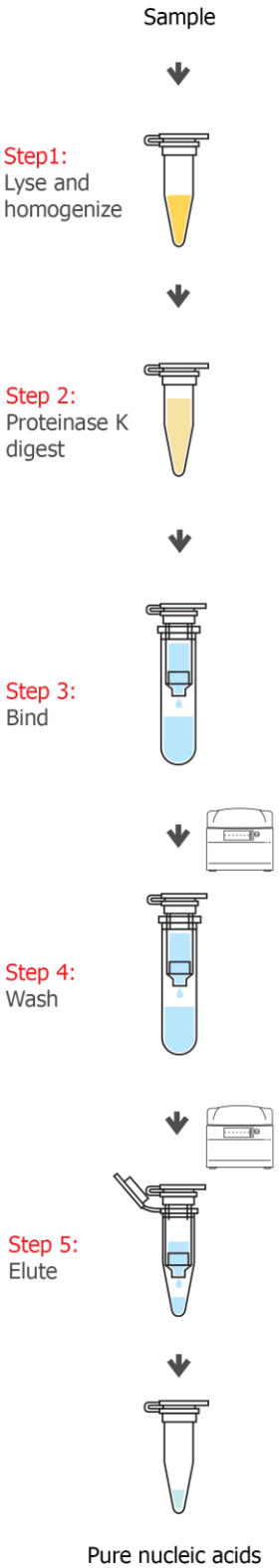
Washing Buffer Preparation

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

Phenol-Chloroform Free Based Protocols

Protocol: Isolation of Total RNA (Animal fibrous tissue)

Sample Type: Animal tissues (fibrous tissue)

Some Tips to Know:

- 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 HiPure DR Column, are carried out on ice.
- Before using the protocol for the first time, add 2-Mercaptoethanol to RLB, %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 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 (molecular biology grade, %96–100) to TWB1 and TWB2, as indicated on the bottle, before using for the first time. Refer to washing buffer preparation section.
- For frozen samples in RNaseLag, thaw them to room temperature. Avoid repeated freezing and thawing of samples, since it may cause RNA degradation.
- 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

- Remove the tissue from RNaseLag or use fresh tissue or snap-freeze tissue. Weight the intended tissue, up to 30mg. Add 300µl RLB to the tissue sample.
- Disrupt and homogenize the tissue sample by selecting one of these ways:
  - After adding appropriate RLB amount, use Micropestle followed by homogenizer or syringe needle to homogenize the tissue.

- After adding appropriate RLB 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 (see Table 1) 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.

- Add 600µl nuclease-free water and 15µl RJ-Protease. Pulse vortex for 15s and incubate the microtube at 55˚C for 15 min.
- Centrifuge the lysate for 3min 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.

- Add half volume of absolute ethanol to the cleared lysate, and mix immediately by pulse vortexing for 15s. Do not centrifuge.

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

- Transfer up to 700µl of the sample, including any precipitate that may have formed, to a HiPure DR Column placed in a 2ml collection tube (supplied in the kit box). Centrifuge for 1min 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 1min.

- 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 1min.

- Add 700µl TWB1 to the HiPure DR Column. Centrifuge for 1min at 13000 rpm. Discard the flow-through.
- Add 500µl TWB2 to the HiPure DR Column. Centrifuge for 1min at 13000 rpm. Discard the flow-through.
- Add 500µl TWB2 to the HiPure DR Column. Centrifuge for 3min at 14000 rpm. Discard the collection tube with the flow-through.

- Place the HiPure DR Column in a new 1.5ml microtube. Add 30-50µl 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 30-50µl 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 11 once more to obtain RNA with higher concentration.