Quick Protocol

MiRJia Kit

Total RNA isolation based on silica technology

- MiniPrep

For RNA Isolation from

Animal Tissue (Fibrous and non-fibrous tissue)
Animal Cells
PBMC (Peripheral Blood Mononuclear Cell)
Whole Blood
WBC (White Blood Cell)

Kit Content

Component	100 preps
RNSol H Reagent	100ml
TWB1 (concentrate)	2 x 16ml
TWB2 (concentrate)	2 x 15ml
Nuclease-free Water	10ml
HiPure DR Column	100
Collection Tube	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 and chloroform volumes, are written in Table 1.

Table 1: Appropriate sample size and amount of RNSol H Reagent, chloroform and ethanol amount

Sample Size	RNSol H Reagent Amount	Chloroform Amount
10-80 mg animal tissue, $(1\times10^6) – (8\times10^6)$ animal cells 1-2 ml whole blood	800µl	200µl
90-100 mg animal tissue $(9\times10^6) – (10^7)$ animal cells 2.5-10 ml whole blood	1ml	300µl

Washing Buffer Preparation

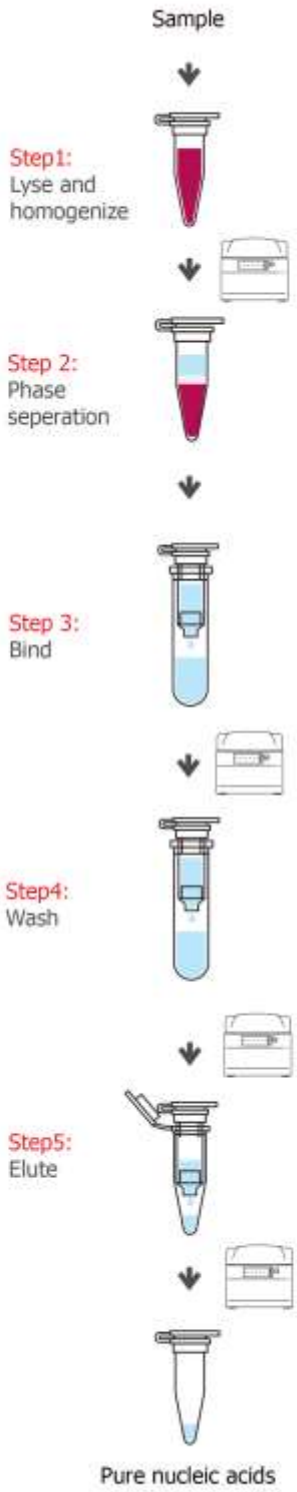
Before the first use, add appropriate amount of ethanol (96-100%) to each washing buffer tube, then mix thoroughly to prepare washing buffer, refer to Table 2. Do not forget to tick the check box on the bottle label to indicate that ethanol has been added. Before each use mix reconstituted buffer by 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 1: Isolation of Total RNA (Animal Tissues, fresh and frozen)

Sample Type: Animal 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 (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, 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 and 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.

- Add appropriate amount of chloroform (refer to Table 1), vigorously shake it for 30s. then pulse vortex for 15 s and incubate at -20°C for 2min.
- Centrifuge at 4℃ for 12min at 13000 rpm.

- Transfer the aqueous phase (the upper phase) to a new tube. Be careful to avoid interfering the interphase.
- Add one and half volume of absolute ethanol to separated aqueous phase. Pulse vortex for 30s.
- Transfer the solution to a HiPure DR Column placed in a 2 ml 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 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 11 once more to obtain RNA with higher concentration.

Protocol 2: Isolation of Total RNA (PBMC, WBC and Whole blood)

Sample Type: PBMC (Peripheral Blood Mononuclear Cell), Whole blood, WBC (white Blood Cell)

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 (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, 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

- Collect 0.5 to 10ml blood into EDTA tubes. Add three volume of Nuclease-free RBC Lysis Buffer. Invert the tube 5 times and incubate at 4ºC for 10min.
- Pulse vortex every 2min during incubation to intersperse the sample.
- Collect the WBCs by centrifugation at 2700 x g for 10min at 4ºC.
- Discard the supernatant, add two volume of Nuclease-free RBC Lysis to the pellet, vortex until the pellet is dissolved completely.
- Collect the WBCs by centrifugation at 2700 x g for 10min at 4ºC.
- Discard the supernatant. Add appropriate amount of RNSol H Reagent to the sample (refer to the Table 1).
- Disrupt and homogenize the 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 cell pellet.
- After adding appropriate amount of RNSol H Reagent, use TissueLyser or homogenizer to disrupt and homogenize the sample simultaneously.

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

- After passing through the syringe for 5-10 times, pulse vortex for 1min, incubate for 10min at room temperature.

  Note: During isolating RNA from PBMC, it is necessary to thoroughly homogenize the sample and it is recommended to homogenize by passing the lysate 5-10 times through a blunt 20-gauge needle fitted to an RNase-free syringe.
- Add appropriate amount of chloroform (refer to Table 1), vigorously shake it for 30s. Then pulse vortex for 15 s and incubate at room temperature for 5min.
- Centrifuge at 4℃ for 12min at 13000 rpm.
- Transfer the aqueous phase (the upper phase) to a new tube. Be careful to avoid interfering the interphase.
- Add 400µl 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 1 min 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 nuclease-free 1.5 ml 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 17 once more to obtain RNA with higher concentration.

Protocol 3: Isolation of Total RNA (Animal cultured cell)

Sample Type: Animal cultured cell

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 (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, 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 cultured cells from RNaseLag or use fresh cultured cells. Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5min at 300 x g. Carefully remove all supernatant by aspiration (for information about sample preparation refer to table 1).

  Note: Incomplete removal of cell-culture medium will inhibit complete lysis and binding to HiPure DR Column, which finally reduce RNA yield.

- Disrupt the cells by selecting one of these ways:
  - Adding appropriate volume of RNSol H Reagent (refer to Table 1), Vortex to mix.

Note: Before adding RNSol H Reagent, flick the tube to loosen the cell pellet thoroughly. Make sure that loosening the cell occurs completely to avoid inefficient lysis and reduced RNA yields.

- For direct lysis of cells grown in a monolayer, add the appropriate amount of RNSol H Reagent to the cell-culture dish (refer to Table 1). Collect the lysate with a rubber policeman. Transfer the lysate into a microcentrifuge tube. Vortex to mix, and ensure that no cell clumps are visible.

- Homogenize the lysate by:
  - Homogenize the lysate for 30s using a homogenizer.
  - Pass the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNase-free syringe.
  - Pass the lysate through a shredder HiPure DR Column by centrifuging at full speed for 2min.

    Optional: Homogenizing step is optional. It means that using homogenizer or syringe needle to homogenize the lysate can be omitted from the process.
- Add appropriate amount of chloroform (refer to Table 1), vigorously shake it for 30s. Then pulse vortex for 15 s and incubate at -20℃ for 2min.
- Centrifuge at 4℃ for 12min at 13000 rpm.
- Transfer the aqueous phase (the upper phase) to a new tube. Be careful to avoid interfering the interphase.
- Add one and half 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.