Quick Protocol

RNJia Bacteria Kit

RNA isolation based on silica technology

- MiniPrep

For RNA Isolation from

Bacteria Cells

Kit Content

Component	50 preps	100 preps
RLB	50ml	2 x 50ml
TWB1 (concentrate)	16ml	2 x 16ml
TWB2 (concentrate)	15ml	2 x 15ml
Nuclease-free Water	5ml	10ml
RJ-Protease	1ml	2 x 1ml
Lysozyme	200mg	400mg
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	Absolute Ethanol Amount
Bacteria Cells	$\leq 5 \times 10^8$	500µl	300µl
Bacteria Cells	$5 \times 10^8 - 1 \times 10^9$	800µl	480µl
Bacteria Cells	$1 \times 10^9 - 2 \times 10^9$	1000µl	600µl

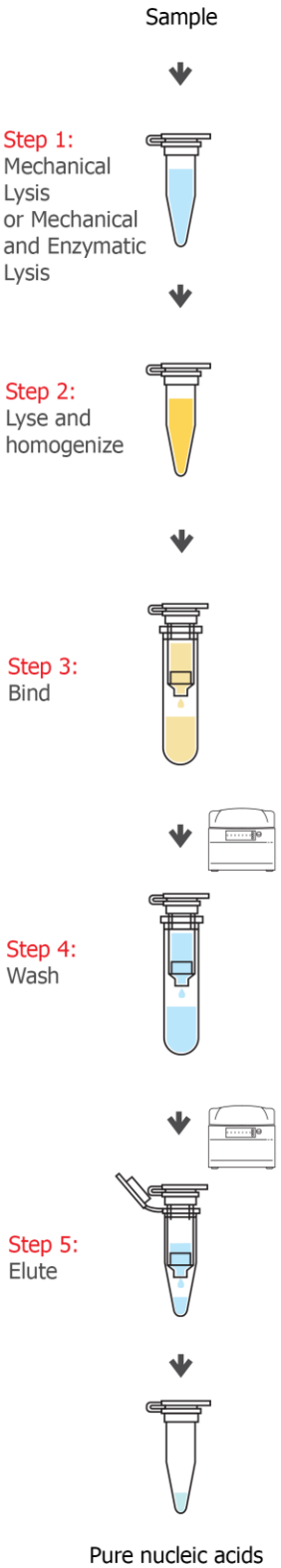
Washing Buffer Preparation

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 1: Isolation of RNA (Bacteria, gram negative and positive)

Sample Type: Bacteria (gram negative and positive)

Protocol Type: Enzymatic lysis

Some Tips to Know:

- This protocol needs to be improved by the user for intended bacterial species.
- Set Thermoblock or water bath at both temperature, 40℃ and 60℃ before starting the process.
- 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.
- In this protocol, the bacteria cell lysis is accomplished by lysozyme and RJ-Protease. Lysozyme should be prepared as a 20 mg/ml solution in nuclease-free TE buffer.

  Attention! It is recommended to prepare lysozyme freshly. It is not stable more than 3 weeks at 4°C.

Process

- Calculate the bacteria cell number. Collect the cell by centrifugation at 10000 rpm for 10 min. Discard the supernatant.
- Add 20µl RJ-Protease and 200 µl of prepared lysozyme solution to the pellet. Resuspend by pulse vortexing for 15s to completely solve the pellet. Incubate the lysate at 40°C for 15min. During the incubation, pulse vortex every 2min for 10s.

- Add appropriate amount of RLB (refer to Table 1), to the pellet. Vortex for 1 min and incubate at 40˚C for 5min.
- Add an appropriate absolute ethanol, invert several times (refer to Table 1).
- Transfer up to 700µl of the sample to a HiPure DR Column placed in a 2 ml collection tube (supplied in the kit box). Centrifuge for 1 min 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 4.

  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 30-50 µl RNase-free water directly to the HiPure DR Column membrane. Incubate at 60˚C for 5min. 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. Incubate at 60˚C for 5min. 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 10 once more to obtain RNA with higher concentration.

Protocol 2: Isolation of RNA (Bacteria, gram negative and positive)

Sample Type: Bacteria (gram negative and positive)

Protocol Type: Simultaneously mechanical and enzymatic lysis

Some tips to know:

- This protocol needs to be improved by the user for intended bacterial species.

- Set Thermoblock or water bath at both temperature, 40℃ and 60℃ before starting the process.
- 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 BU983041) 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 won't 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.
- In this protocol, the bacteria cell lysis is accomplished by lysozyme and RJ-Protease. Lysozyme should be prepared as a 20mg/ml solution in nuclease-free TE buffer.

  Attention! It is recommended to prepare lysozyme freshly. It is not stable more than 3 weeks at 4°C.

Process

- For each sample, weigh 25–50 mg acid-washed glass beads (150–600µm diameter) in a 2ml Safe-Lock tube.
- Calculate the bacteria cell number. Collect the cell by centrifugation at 10000 rpm for 10min. Discard the supernatant.
- Add 20µl RJ-Protease and 200µl of prepared lysozyme solution to the pellet. Resuspend by pulse vortexing for 15s to completely dissolve the pellet. Incubate the lysate at 40°C for 15min. During the incubation, pulse vortex every 2min for 10s.
- Add appropriate amount of RLB (refer to Table 1), to the pellet. Vortex for 1 min then incubate at 40°C for 5min.

- Transfer the suspension into the 2ml Safe-Lock tube containing the acid washed glass beads prepared in step 1. Disrupt the cells in the Tissuelyser for 5min at maximum speed.
- Centrifuge at 14000 rpm for 1 min. Transfer the supernatant to a new microcentrifuge tube. Add appropriate amount of absolute ethanol (refer to Table 1), invert several times.
- Transfer up to 700µl of the sample 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 6.

  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. Incubate at 60˚C for 5min. 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 30-50µl RNase-free water. Incubate at 60˚C for 5min. 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.