Quick Protocol

RNJia Kit

RNA isolation based on silica technology

- MiniPrep

For RNA Isolation from

Animal Tissue (Fibrous and non-fibrous tissue)
Animal Cells
Bacteria Cells
PBMC
WBC
Whole blood

Kit Content

Component	100 preps
RLB	2 x 50ml
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 RLB are written in Table 1.

Table 1. Appropriate sample size and amount of RLB

Sample Type	Sample Size	Amount of RLB
Animal cells	≤5 $X10^6$	350µl
Animal cells	5 $X\ 10^6$ To 1 $X\ 10^7$	600µl
Animal not fibrous tissues	0.5-10 Mg	350µl
Animal not fibrous tissues	10-30 Mg	600µl
Animal fibrous tissues	0.5-30 Mg	300µl
Bacteria cells	≤5 $X10^8$	500µl
Bacteria cells	5 $X\ 10^8$ - 1 $X\ 10^9$	800µl
Bacteria cells	1 $X\ 10^9$ – 2 $X\ 10^9$	1000µl
Whole blood	Up to 2 x 10^6 cells	350µl
Whole blood	2 x 10^6 to 1 x 10^7 cells	600µl

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 1: Isolation of Total RNA (Animal tissues, fresh and frozen)

Sample Type: Animal tissues (fresh and frozen)

Some Tips to Know:

- 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 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.

Process

- Remove the tissue from RNaseLag or use fresh tissue or snap-freeze tissue. Weight the intended tissue. Add appropriate RLB to the tissue sample (refer to the Table 3).

Table 3. The appropriate amount of RLB

Sample type	Storage Condition	Weight	RLB amount
Soft tissue (brain, liver and etc.)	Fresh or snap freeze	0.5-10mg	350µl
Soft tissue (brain, liver and etc.)	Treated with RNaseLag	0.5-10mg	600µl

Soft tissue (brain, liver and etc.)	Fresh or snap freeze and treated with RNaseLag	10-30mg	600µl
Other tissue types	Fresh, snap freeze or treated with RNaseLag	0.5-30mg	600µl

- Disrupt and homogenize the tissue sample by selecting one of these ways:
- After adding appropriate amount of RLB, use Micropestle followed by homogenizer or syringe needle to homogenize the tissue.
- After adding appropriate amount of RLB, use 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 RLB (refer to the Table 3) 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. For soft tissues like brain, the tissue will be disappeared completely, however for hard tissue like spleen or kidney; a little non-homogenized tissue might remain at the end of lysis step, which will not influence the other steps.

- 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: For some tissues the pellet is recognizable, however for others like brain, the pellet is very little or very clear, and is hard to be recognized. So be careful not to disrupt the pellet by pipette tip.

- Add one volume of %70 ethanol (molecular biology grade, nuclease-free) to the cleared lysate, and mix immediately by pulse vortexing for 15 s. Do not centrifuge.

  Note: If total RNA is required, add absolute ethanol instead of 70% ethanol to the cleared lysate.

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

- Transfer up to 700µl of the lysate, 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 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 1.5 ml microtube. Add 30-50µl 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 30-50µl 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 10 once more to obtain RNA with higher concentration.

Protocol 2: Isolation of Total RNA (Animal tissues, fibrous tissue)

Sample Type: Animal tissues (fibrous tissue)

Some Tips to Know:

- It is possible to Isolate RNA from Fibrous tissue like lung, heart, and skin, however RJ-Protease (Cat No EB983121) and nuclease-free water (Cat No WA983014, WA983008, WA983009 and WA983010) should be ordered separately. However, RNJia Fibrous kit (Cat No RN983024, RN983025 and RN983026) is designed specifically to isolate RNA from fibrous tissue.

Note: For isolation of RNA from skeletal muscle use RNSol H Reagent instead of RLB.

- 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 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.
- 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 30 mg. Add 300 µl 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 600 µl nuclease-free water and 15 µl RJ-Protease. Pulse vortex for 15s and incubate the microtube at 55°C for 15min.
- 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 15 s. 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 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 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 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. 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 3: Isolation of Total RNA (Animal cultured cell)

Sample Type: Animal cultured cell

Some tips to know:

- 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 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.

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 5 min at 300 x g. Carefully remove all supernatant by aspiration (for information about sample preparation refer to table 4).

Table 4: Lysis of attached monolayer and suspension cells

Cultured cell type	Lysis type	Description
Cells grown in a monolayer	Lyse directly in the cell-culture vessel	Determine the number of cells. Completely aspirate the cell-culture medium.
	trypsinize and collect cells at 300 x g	Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.1–0.25% trypsin in PBS. After the cells detach from the surface, add

		medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free centrifuge tube, and centrifuge at 300 x g for 5 min. Completely aspirate the supernatant, and continue the process.
Cells grown in suspension	Collect cells at 300 x g	Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5 min at 300 x g. Carefully remove all supernatant by aspiration.

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 RLB (refer to Table 1), Vortex to mix.

    Note: Before adding RLB, 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 RLB 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.
- 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: The pellet is very little or very clear, and is hard to be recognized. So be careful not to disrupt the pellet by pipette tip.

- Add one volume of %70 ethanol (molecular biology grade, nuclease-free) to the cleared lysate, and mix immediately by pulse vortexing for 15 s. Do not centrifuge.

  Note: If total RNA is required, add absolute ethanol instead of %70 ethanol (molecular biology grade, nuclease-free) to the cleared lysate.

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

- Transfer up to 700µl of the lysate, 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 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 1 min.
- 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 1 min.

- 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 1.5 ml microtube. Add 30-50µl 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 30-50µl 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.

Protocol 4: Isolation of RNA (PBMC, WBC and Whole blood)

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

Some tips to know:

- 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 won't influence the efficiency of the buffer.
- 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.

Process

- Collect 0.5 to 1.5ml blood into EDTA tubes. Add three volume of RBC Lysis Buffer (RNase-free). Invert the tube 5 times and incubate at 4ºC for 10min.
- Pulse vortex every 2 min 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 RBC Lysis Buffer (RNase-free) to the pellet, vortex until the pellet is dissolved completely.
- Collect the WBCs by centrifugation at 2700 g for 10min at 4ºC.
- Discard the supernatant. Disrupt the cells by adding RLB (Supplemented with 2-βME). Loosen the cell pellet thoroughly by flicking the tube. Add the appropriate volume of RLB (see Table 1) and vortex or pipet to mix. After adding appropriate amount of RLB, use Micro pestle followed by homogenizer or syringe needle to homogenize the sample.

  Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields.

  Note: For previously isolated PBMCs and WBCs, start from step 6.

- Centrifuge the lysate for 3min at 15000 rpm. Carefully remove the supernatant by pipetting, and transfer it to a new RNase-free microcentrifuge tube.
- Add one volume of %100 ethanol to the homogenized lysate, and mix well by pulse vortexing for 15 s.

  Note: When purifying RNA from isolated cells, precipitates may be visible after addition of ethanol. This does not affect the procedure.
- Transfer up to 700µl of the sample, including any precipitate that may have formed, to HiPure DR Column placed in a 2ml collection tube (supplied in the kit box). Close the lid gently, and centrifuge for 1min at 13000 rpm. Discard the flow-through. Reuse the collection tube in step 10.
- 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 3 min 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 30-50 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 30-50µl RNase-free water. Centrifuge for 1min at 12000 rpm. However, it is possible to pass the flow-through from step 13 once more to obtain RNA with higher concentration.

Protocol 5: Isolation of RNA (Bacteria, gram negative and positive)

Sample Type: Bacteria (gram negative and positive)

Protocol Type: Enzymatic lysis

Some Tips to Know:

- It is possible to Isolate RNA from bacteria by RNJia Kit, however RJ-Protease (Cat No EB983121) and Lysozyme (Cat No EB983017) should be ordered separately. RNJia Bacteria Kit (Cat No RN983020, RN983021, RN983022 and RN983023) is designed specifically to isolate RNA from Bacteria cells.
- 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 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 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 10min. Discard the supernatant.
- Add 20µl RJ-Protease and 200 µl of prepared lysozyme solution to the pellet. Resuspend by pulse vortexing for 15 s 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 5).
- 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 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 1 min.

Table 5. Appropriate absolute ethanol amount for Bacteria Cell Number

Bacteria Cell Number	Absolute Ethanol Amount
$<5 \times 10^8$	300µl
$5 \times 10^8 - 1 \times 10^9$	480µl
$>1 \times 10^9$	600µl

- 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 1 min.

- 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℃ for 5min. Centrifuge for 1 min 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 6: 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:

- It is possible to Isolate RNA from bacteria by RNJia Kit, however RJ-Protease (Cat No EB983121) and Lysozyme (Cat No EB983017) should be ordered separately.

RNJia Bacteria Kit (Cat No RN983020, RN983021, RN983022 and RN983023) is designed specifically to isolate RNA from Bacteria cells.

- 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 25–50mg 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 15 s 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 1min then incubate at 40˚C for 5min.
- Transfer the suspension into the 2 ml Safe-Lock tube containing the acid washed glass beads prepared in step 1. Disrupt the cells in the Tissuelyser for 5 min at maximum speed.
- Centrifuge at 14000 rpm for 1min. Transfer the supernatant to a new microcentrifuge tube. Add appropriate amount of absolute ethanol (refer to Table 5), 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 1 min at 13000 rpm. Discard the flow-through.
- Add 500µl TWB2 to the HiPure DR Column. Centrifuge for 1 min at 13000 rpm. Discard the flow-through.
- Add 500µl TWB2 to the HiPure DR Column. Centrifuge for 3 min 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.