Quick Protocol

RNSol H Reagent

RNA and total RNA isolation based on solution

- MiniPrep

For RNA Isolation from

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

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.

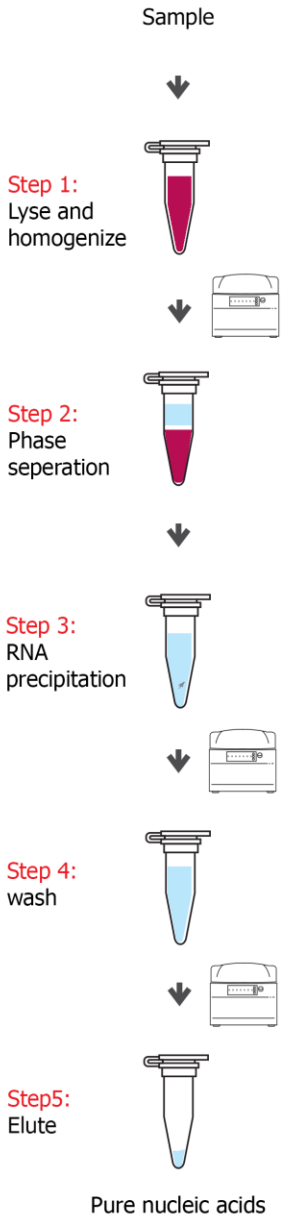
Table 1. Appropriate sample size and amount of RNSol H Reagent, chloroform and ethanol amount

Sample Size	RNSol H Reagent Amount	Chloroform Amount	Isopropanol
• 10-80 mg animal tissue, • (1×10^6)–(8×10^6) animal cells • 1-2 ml whole blood	800µl	200µl	400µl
• 90-100 mg animal tissue • (9×10^6) – (10^7) animal cells • 2.5-10 ml whole blood • 1 x 10^7 bacteria cell	1ml	200µl	500µl

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.

Procedure of solution -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:

- Do not allow non-stabilized tissues to thaw while weighing or handling prior to disruption.
- Set Thermoblock or water bath at 60°C before starting the process.

Process

- Remove the tissue from RNaseLag or use fresh tissue. Determine the weight of starting material and add appropriate RNSol H Reagent to the tissue sample (refer to the Table 1).
- Disrupt the tissue sample by selecting one of these ways:
- After adding appropriate RNSol H Reagent 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 RNSol H Reagent (refer to the Table 1).

  Optional: If samples have a high fat content, centrifuge the lysate for 5 min at 12000× g at 4–10°C, then transfer the clear supernatant to a new tube.

- Incubate for 5min at room temperature.
- Add appropriate amount of chloroform (refer to Table 1), then vigorously pulse vortex for 15 s and incubate at room temperature for 3min.
- Centrifuge at 4°C for 15min at 13000 rpm.
- Transfer the aqueous phase (the upper phase) to a new tube. Be careful to avoid interfering the interphase.
- Add appropriate amount of isopropanol (refer to Table 1). Invert several times.
- Incubate at room temperature for 10min.
- Centrifuge at 4°C for 10min at 13000 rpm.

- Discard the supernatant and add 750µl %75 ethanol (nuclease-free) to the pellet. Vortex the sample briefly then centrifuge at 4˚C for 5min at 12000 rpm.
- Remove the supernatant completely, and briefly air-dry the RNA pellet (for 10min).
- Add 30-100µl nuclease-free water. Pipetage several times to dislodge the RNA pellet then incubate 10min at 60˚C. Afterward, pipetage until the pellet dissolved completely.

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

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

Some tips to know:

- Set Thermoblock or water bath at 60˚C before starting the process.

Process

- Collect 0.5 to 10ml blood into EDTA tubes. Add three volumes of RBC Lysis Buffer (RNase-free). Invert the tube 5 times and incubate at 4 ºc for 10 min.
- Pulse vortex every 2min during incubation to intersperse the sample.
- Collect the WBCs by centrifugation at 2700 x g for 10min at 4 ºC.
- Discard the supernatant, add two volumes of RBC Lysis Buffer (RNase-free) 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 the cell pellet by vortex for 1min then incubate at room temperature for 5min.
- Add appropriate amount of chloroform (refer to Table 1), then vigorously pulse vortex for 15s and incubate at room temperature for 3min.
- Centrifuge at 4˚C for 15min at 13000 rpm.
- Transfer the aqueous phase (the upper phase) to a new tube. Be careful to avoid interfering the interphase.
- Add appropriate amount of isopropanol (refer to Table 1). Invert several times.
- Incubate at room temperature for 10min.
- Centrifuge at 4℃ for 10 min at 13000 rpm.

- Discard the supernatant and add 750µl %75 ethanol (nuclease-free) to the pellet. Vortex the sample briefly then Centrifuge at 4˚C for 5min at 12000 rpm.
- Remove the supernatant completely, and briefly air-dry the RNA pellet (for 10min).
- Add 30-100µl nuclease-free water. Pipetage several times to dislodge the RNA pellet then incubate 10min at 60˚C. Afterward, pipetage until the pellet dissolved completely.

Protocol 3: Isolation of Total RNA (Cultured cell)

Sample Type: Cells grown in monolayer, Cells grown in suspension

Some tips to know:

- Do not allow non-stabilized samples to thaw while weighing or handling prior to disruption.
- Set Thermoblock or water bath at 60˚C before starting the process.

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 2).

Table 2: 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 spin 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 2), 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.

  Optional: If samples have a high fat content, centrifuge the lysate for 5 minutes at 12000 × g at 4–10°C, then transfer the clear supernatant to a new tube.

- Incubate for 5min at room temperature.
- Add appropriate amount of chloroform (refer to Table 1), vigorously then vigorously pulse vortex for 15s and incubate at room temperature for 3min.
- Centrifuge at 4℃ for 15min at 13000 rpm.
- Transfer the aqueous phase (the upper phase) to a new tube. Be careful to avoid interfering the interphase.
- Add appropriate amount of isopropanol (refer to Table 1). Invert several times.
- Incubate at room temperature for 10 min.
- Centrifuge at 4°C for 10 min at 13000 rpm.
- Discard the supernatant and add 750µl %75 Ethanol (nuclease free) to the pellet. Vortex the sample briefly then Centrifuge at 4°C for 5 min at 12000 rpm.
- Remove the supernatant completely, and briefly air-dry the RNA pellet (for 10 min).
- Add 30-100µl nuclease-free water. Pipetage several times to dislodge the RNA pellet then incubate 10min at 60°C. Afterward, pipetage until the pellet dissolved completely.

Protocol 4: Isolation of RNA (Bacteria, gram negative and positive)

Sample Type: Bacteria (gram negative and positive)

Some tips to know:

- Set Thermoblock or water bath at 60°C before starting the process.
- All steps are carried out on ice.

Process

- Calculate the bacteria cell number. Collect the cell by centrifugation at 13000 rpm for 10min. Discard the supernatant.
- Resuspend the pellet by vortexing. Add appropriate RNSol H Reagent to the sample (refer to the table 1). Incubate for 10-20min at 60°C.
- Add appropriate amount of chloroform (refer to Table 1), then vigorously pulse vortex for 15s and incubate at room temperature for 3min.
- Centrifuge at 4°C for 15min at 13000 rpm.
- Transfer the aqueous phase (the upper phase) to a new tube. Be careful to avoid interfering the interphase.
- Add appropriate amount of isopropanol (refer to Table 1). Invert several times.
- Incubate at room temperature for 10min.
- Centrifuge at 4°C for 10min at 13000 rpm.
- Discard the supernatant and add 750µl 75% Ethanol (nuclease free) to the pellet. Vortex the sample briefly then Centrifuge at 4°C for 5min at 12000 rpm.
- Remove the supernatant completely, and briefly air-dry the RNA pellet (for 10 min).
- Add 30-100µl nuclease-free water. Pipetage several times to dislodge the RNA pellet then incubate 10min at 60℃. Afterward, pipetage until the pellet dissolved completely.