Contents

Recommended Starting Material

To reach optimized results it is better to follow as listed below. The size of the recommended material to use with determined RNSol H and chloroform amount are written in Table 1.

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

Sample Size	RNSol H Amount	Chloroform Amount	Isopropanol
50-100 mg animal tissue	1 ml	200 µl	500 µl
1×10^5 - 10^7 Cells grown in monolayer	400 µl	80 µl	200 µl
5-10 $\times$ 10^6 Cells grown in suspension	750 µl	150 µl	375 µl
$\leq 5 \times 10^8$ bacteria cells	400 µl	200 µl	400 µl
5×10^8 - 10^9 bacteria cells	500 µl	300 µl	500 µl
0.5 – 1 ml whole blood	500 µl	100 µl	250 µl
1 – 5 ml whole blood	1000 µl	200 µl	400 µl
5 – 10 ml whole blood	1200 µl	200 µl	500 µl

Protocol 1

Isolation of Total RNA (based on solution)

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.
- If working with RNA for the first time, read Appendix 1, in main handbook, carefully.

Process

1. Remove the tissue from RNaseLag or use fresh tissue. Determine the weight of starting material and Add appropriate RNSol H to the tissue sample (refer to the Table 1).

2. Disrupt the tissue sample by selecting one of these ways:

    - After adding appropriate RNSol H 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 (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.

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

9. Centrifuge at 4˚C for 10 min at 13000 rpm.
10. Discard the supernatant and add 750 µl 75% Ethanol (nuclease free) to the pellet. Vortex the sample briefly then Centrifuge at 4˚C for 5 min at 12000 rpm.
11. Remove the supernatant completely, and briefly air-dry the RNA pellet (for 10 min).
12. Add 30-100 µl nuclease-free water. Pipetage several times to dislodge the RNA pellet then incubate 10 min at 60˚C. Afterward, pipetage until the pellet dissolved completely.

Protocol 2

Isolation of Total RNA (based on solution)

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.
- If working with RNA for the first time, read Appendix 1, in main handbook, carefully.

Process

1. Collect 0.5 to 10 ml 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.
2. Pulse vortex every 2 min during incubation to intersperse the sample.
3. Collect the WBCs by centrifugation at 2700 x g for 10 min at 4 ºC.
4. Discard the supernatant, add two volumes of RBC Lysis Buffer (RNase-free) to the pellet, vortex until the pellet is dissolved completely.
5. Collect the WBCs by centrifugation at 2700 x g for 10 min at 4ºc.
6. Discard the supernatant. Add appropriate amount of RNSol H to the sample (refer to the table 1).
7. Disrupt the cell pellet by vortex for 1 min then incubate at room temperature for 5 min.
8. Add appropriate amount of chloroform (refer to Table 1), then vigorously pulse vortex for 15 s and incubate at room temperature for 3 min.
9. Centrifuge at 4°C for 15 min at 13000 rpm.

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

Protocol 3

Isolation of Total RNA (based on solution)

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.
- If working with RNA for the first time, read Appendix 1, in main handbook, carefully.

Process

1. 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.

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

    Note: Before adding RNSol H, 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.

    b. For direct lysis of cells grown in a monolayer, add the appropriate amount of RNSol H 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.

3. Incubate for 5 min at room temperature.
4. Add appropriate amount of chloroform (refer to Table 1), vigorously then vigorously pulse vortex for 15 s and incubate at room temperature for 3 min.

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

Protocol 4

Isolation of RNA (based on solution)

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.
- If working with RNA for the first time, read Appendix 1, in main handbook, carefully.

Process

1. Calculate the bacteria cell number. Collect the cell by centrifugation at 13000 rpm for 10 min. Discard the supernatant.
2. Resuspend the pellet by vortexing. Add appropriate RNSol H to the sample (refer to the table 1). Incubate for 10-20 min at 60°C.
4. Add appropriate amount of chloroform (refer to Table 1), then vigorously pulse vortex for 15 s and incubate at room temperature for 3 min.
5. Centrifuge at 4°C for 15 min at 13000 rpm.
6. Transfer the aqueous phase (the upper phase) to a new tube. Be careful to avoid interfering the interphase.
7. Add appropriate amount of isopropanol (refer to Table 1). Invert several times.
8. Incubate at room temperature for 10 min.
9. Centrifuge at 4°C for 10 min at 13000 rpm.

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