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 and chloroform amount are written in Table 1.

Table 1. Appropriate sample size and amount of RNSol and Chloroform amount

Sample Size	RNSol Amount	Chloroform Amount
10 mg animal tissue, 1 × 10^6 animal cells	100 µl	100 µl
20 mg animal tissue 2 × 10^6 animal cells	200 µl	100 µl
30 mg animal tissue 3 × 10^6 animal cells 0.5-1 ml whole blood	300 µl	200 µl
40 mg animal tissue 4 × 10^6 animal cells	400 µl	200 µl
50 mg animal tissue 5 × 10^6 animal cells 1-3 ml whole Blood	500 µl	300 µl
60 mg animal tissue 6 × 10^6 animal cells	600 µl	300 µl
70 mg animal tissue 7 × 10^6 animal cells 3-5 ml whole Blood	700 µl	400 µl
80 mg animal tissue 8 × 10^6 animal cells	800 µl	400 µl
90 mg animal tissue 9 × 10^6 animal cells	900 µl	500 µl
100 mg animal tissue 10^7 animal cells 5-10 ml whole Blood	1ml	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 Reagent to the tissue sample (refer to the Table 1).
2. Disrupt and homogenize the tissue sample by selecting one of these ways:
    - After adding appropriate RNSol amount, use Micropestle followed by homogenizer or syringe needle to homogenize the tissue.
    - After adding appropriate RNSol 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 (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.

3. Vortex for 30-60 s.
4. Add appropriate amount of chloroform (refer to Table 1), vigorously shake it for 30 s. Then pulse vortex for 15 s and incubate at -20°C for 2 min.
5. Centrifuge at 4°C for 12 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 an equal volume of isopropanol to it. Invert several times, then vortex for 10 s.
8. Incubate at -20°C for 5-30 min.
9. Centrifuge at 4°C for 15 min at 13000 rpm.
10. Discard the supernatant and add 750 µl 75% Ethanol (nuclease free) to the pellet. Centrifuge at 4°C for 5 min at 12000 rpm.
11. Discard the supernatant and add 750 µl 75% Ethanol (nuclease free) to the pellet. Centrifuge at 4°C for 5 min at 12000 rpm.
12. Remove the supernatant completely, and briefly air-dry the RNA pellet (for 10 min).
13. Add 30-100 µl RNase 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 10min at 4ºc.
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 Reagent to the sample (refer to the table 1).
7. Disrupt and homogenize the sample by selecting one of these ways:
    - After adding appropriate amount of RNSol, use Micropestle followed by homogenizer or syringe needle to homogenize the cell pellet.
    - After adding appropriate amount of RNSol, 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.

8. After passing through the syringe for 5-10 times, pulse vortex for 1 min. Incubate for 5 min 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.

9. Add appropriate amount of chloroform (refer to Table 1), vigorously shake it for 30 s. Then pulse vortex for 15 s and incubate at -20°C for 2 min.
10. Centrifuge at 4°C for 12 min at 13000 rpm.
11. Transfer the aqueous phase (the upper phase) to a new tube. Be careful to avoid interfering the interphase.
12. Add an equal volume of isopropanol to it. Invert several times, then vortex for 10 s.
13. Incubate at -20°C for 5-30 min.
14. Centrifuge at 4°C for 15 min at 13000 rpm.
15. Discard the supernatant and add 750 µl 75% Ethanol (nuclease free) to the pellet. Centrifuge at 4°C for 5 min at 12000 rpm.
16. Discard the supernatant and add 750 µl 75% Ethanol (nuclease free) to the pellet. Centrifuge at 4°C for 5 min at 12000 rpm.
17. Remove the supernatant completely, and briefly air-dry the RNA pellet (for 10 min).
18. Add 30-100 µl RNase 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: Animal Cultured Cells (fresh and frozen)

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 (refer to Table 1), Vortex to mix.

        Note: Before adding RNSol, 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 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.
3. Homogenize the lysate by:
    a. Homogenize the lysate for 30 s using a homogenizer.
    b. Pass the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNase-free syringe.
    c. Pass the lysate through a shredder spin column by centrifuging at full speed for 2 min.

        Optional: Homogenizing step is optional. It means that using homogenizer or syringe needle to homogenize the lysate can be omitted from the process.
4. Add appropriate amount of chloroform (refer to Table 1), vigorously shake it for 30 s. Then pulse vortex for 15 s and incubate at -20˚C for 2 min.
5. Centrifuge at 4˚C for 12 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 an equal volume isopropanol to it. Invert several times, then vortex for 10 s.
8. Incubate at -20˚C for 5-30 min.

9. Centrifuge at 4˚C for 15 min at 13000 rpm.
10. Discard the supernatant and add 750 µl 75% Ethanol (nuclease free) to the pellet. Centrifuge at 4˚C for 5 min at 12000 rpm.
11. Discard the supernatant and add 750 µl 75% Ethanol (nuclease free) to the pellet. Centrifuge at 4˚C for 5 min at 12000 rpm.
12. Remove the supernatant completely, and briefly air-dry the RNA pellet (for 10 min).
13. Add 30-100 µl RNase free water. Pipetage several times to dislodge the RNA pellet then incubate 10 min at 60˚C. Afterward, pipetage until the pellet dissolved completely.