WelPrep Cell / Tissue RNA Kit

www.welgene.com.tw

Content

Kit contents

Items	Quantity
RNA Lysis buffer	14.5 ml
Shredder column	40
RNA spin column	40
RNA Wash buffer 1*	22 ml
RNA Wash buffer 2*	9 ml
Nuclease-free H_2O	1.5 ml
Hand book	1

1*. Before use, please add 9.5 ml of 100% EtOH first. The mild turbid wash buffer 1 normally becomes clear after add alcohol.

2*. Before use, please add 36 ml of 100% EtOH first.

Storage

The buffers , Shredder columns and RNA Spin Columns can be stored dry at room temperature (15–25°C) for up to 2 years. Proteinase K solution that is stable for at least one year after delivery when stored at 4°C.

Product Use Limitations

For research use only. Not for use in diagnostic procedures. The performance characteristics of this product have not been fully established.

Principle

The *WelPrep* Cell / Tissue RNA Kit allows the isolation of total RNA from various biological samples. The procedure is simple on the selective binding properties of a silica-based membrane. RNA lysis buffer, which contain guanidine thiocyanate disrupts cells, denatures cellular proteins. After lysis with buffer, samples are centrifuged through a Shredder column to remove insoluble material to reduce the viscosity of the lysates by disrupting gelatinous material. Ethanol is added to optimize the binding conditions, and the solution is applied to a *WelPrep* RNA spin column. During a brief centrifugation, RNA longer than 200 bases is adsorbed by the silica membrane of the spin column and other impurities pass through. Remaining contaminants are removed in three wash steps, and pure RNA is then eluted by nuclease-free water.

Equipment and Reagents to Be Supplied by Users

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

- chloroform
- Ethanol (70%)
- DNase I (Welgene, Cat.No.R005) (For certain RNA applications that are interfered with low amount of DNA, residual DNA can be removed by optional oncolumn DNase digestion using the RNase-Free DNase. Alternatively, residual DNA can be removed by a DNase digestion after RNA purification)
- 1.5 ml or 2 ml microcentrifuge tubes
- Sterile, RNase-free pipet tips
- Variable-speed microcentrifuge with a rotor for 2 ml microcentrifuge tubes
- Centrifuge capable of attaining 3000–5000 x g and equipped with a fixed angel rotor for 50-ml centrifugal tubes
- Vortex mixer
- Disposable gloves
- Trizol® Reagent (Invitrogen, Cat.No. 15596-026) (Trizol Reagent is applied to the tissues which contains abundance of proteins, such as skin, heart, muscles and most of the tumor samples. To lyze the cells more efficiently, the standard protocol has been adapted to use Trizol Reagent as lysis reagent.)

Protocol I: Isolation of RNA from cell line

I. Before start :

- The protocol is for isolating total RNA from about 1 x 10^6 cells and do not use more than 1 x 10^7 cells.

II. Protocol :

1.Harvest cells:

A. Cells grown in suspension

A-1. Centrifuge the cell at 300 x g for 15 min.

A-2. Remove the medium by aspiration, add the appropriate volume of RNA lysis buffer, mix thoroughly, and proceed to the step 2 of the protocol.

Number of cells	RNA lysis buffer (μl)
< 5 x 10^6	350 μl
5 x 10^6 – 1 x 10^7	600 μl

B. cells grown in a monolayer

B-1. Remove the medium by aspiration.

B-2. Lyze the cells with RNA lysis buffer:

Dish diameter (cm)	RNA lysis buffer (μl)
< 10 cm	350 μl
> 10 cm	600 μl

2. Homogenize the sample by pipetting the lysate directly onto a Shredder column placed in a 2 ml collection tube, and centrifuge at maximal speed for 2 min.
3. Remove the Shredder column. Add 1 volume of 70% ethanol to the lysate and mix well by pipetting. Do not centrifuge.
4. Pipet lysate onto RNA spin column, centrifuge at 13000 rpm for 30 sec. (Discard the flow-through from the collection tube).
5. Pipette 700 μl RNA wash buffer 1 onto the column, centrifuge at 13000 rpm for 60 sec. (Discard the flow-through from the collection tube).

6. [Optional] Preparing DNase I solution:
   Add 10 µl DNase I stock solution to 70 µl DNase Digestion Buffer and mix by gently flicking the tube (***DO NOT VORTEX!***), centrifuge briefly to collect residual liquid the sides of the tube.
7. [Optional] Pipette the 80 µl DNase I solution mix directly onto the spin-column membrane, and incubate at room temperature for 15 min.
8. [Optional] Pipette 350 µl of wash buffer 1 onto the column, centrifuge at 13000 rpm for 30 sec. (Discard the flow-through from the collection tube).
9. Pipette 500 µl of wash buffer 2 onto the column, centrifuge at 13000 rpm for 2 min. (Discard the flow-through from the collection tube).
10. To elute the RNA, transfer the column on a new 1.5 ml elution tube, and pipette 30 µl nuclease-free water onto the column membrane and incubate for 1 min, then centrifuge at 13000 rpm for 2 min.
11. Store the RNA samples at -80°C.

Protocol IIa: Isolation of RNA from tissues

I. Before start :

● The protocol is for isolating total RNA from tissues no more than 30 mg.

II. Protocol :

1. Homogenize the starting material using an appropriate volume of RNA lysis buffer.

Amount of starting material	RNA lysis buffer (µl)
< 20 mg	350 µl
> 20-30 mg	600 µl

2. Centrifuge the lysate at 12000 g for 10 min at 2-8°C.
3. Carefully transfer the clear supernatant onto a Shredder column placed in a 2 ml collection tube, and centrifuge at maximal speed for 2 min.
4. Remove the Shredder column. Add 1 volume of 70% ethanol to the lysate and mix well by pipetting. Do not centrifuge.
5. Pipet lysate onto RNA spin column, centrifuge at 13000 rpm for 30 sec. (Discard the flow-through from the collection tube).
6. Pipette 700µl RNA wash buffer 1 onto the column, centrifuge at 13000 rpm for 60 sec. (Discard the flow-through from the collection tube).
7. [Optional] Preparing DNase I solution:
   Add 10µl DNase I stock solution to 70 µl DNase Digestion Buffer and mix by gently flicking the tube (***DO NOT VORTEX!***), centrifuge briefly to collect residual liquid the sides of the tube.
8. [Optional] Pipette the 80 µl DNase I solution mix directly onto the spin-column membrane, and incubate at room temperature for 15 min.
9. [Optional] Pipette 350 µl of wash buffer 1 onto the column, centrifuge at 13000 rpm for 30 sec. (Discard the flow-through from the collection tube).
10. Pipette 500 µl of wash buffer 2 onto the column, centrifuge at 13000 rpm for 30 sec. (Discard the flow-through from the collection tube).

11. Pipette 500 µl of wash buffer 2 onto the column, centrifuge at 13000 rpm for 2 min. (Discard the flow-through from the collection tube).
12. To elute the RNA, transfer the column on a new 1.5 ml elution tube, and pipette 30 ul nuclease-free water onto the column membrane and incubate for 1 min, then centrifuge at 13000 rpm for 2 min.
13. Store the RNA samples at -80°C.

Protocol IIb: Isolation of RNA from tissues

I. Before start :

● The protocol is suitable for the tissues which are rich in contractile and adhesive proteins. Total RNA isolation from heart, muscle and skin are relatively difficult because of containing high abundance of these proteins. To remove these proteins, the standard *WelPrep* RNA isolation protocol has been integrated a Trizol Reagent (Invitrogen, Cat.No : 15596-018) lysis step. In the initial lysis step, samples are treated with Trizol Reagent instead of RNA lysis buffer.

II. Protocol :

1. Homogenize the starting material using an appropriate volume of Trizol Reagent. Keep homogenate at RT for 5 min.

Amount of starting material (mg)	Trizol (ml) - Standard tissue	Trizol (ml) for difficult tissue
100	1	2
500	5	10
1000	10	20

2. Add chloroform to the homogenate (0.2 ml chloroform per ml of Trizol used) and shake vigorously for 15 sec, then allow sample to sit at RT for 2-3 min.
3. Spin at 12000 x g for 15 min at 4°C.
4. Carefully transfer aqueous phase (top) to new tube.
   *It is **extremely important** not to get any of the material from the aqueous/organic interface; it is suggested to sacrifice aqueous material rather than risk taking this precipitate.*
   Measure the volume of the aqueous sample.
5. Add 1 volume of 70% ethanol to the lysate and mix well by pipetting. Do not centrifuge.
6. Pipet lysate onto RNA spin column, centrifuge at 13000 rpm for 30 sec.
   (Discard the flow-through from the collection tube).
7. Pipette 700 µl RNA wash buffer 1 onto the column, centrifuge at 13000 rpm for 60 sec.
   (Discard the flow-through from the collection tube).

8. [Optional] Preparing DNase I solution:
   Add 10 µl DNase I stock solution to 70 µl DNase Digestion Buffer and mix by gently flicking the tube (***DO NOT VORTEX!***), centrifuge briefly to collect residual liquid the sides of the tube.
9. [Optional] Pipette the 80 µl DNase I solution mix directly onto the spin-column membrane, and incubate at room temperature for 15 min.
10. [Optional] Pipette 350 µl of wash buffer 1 onto the column, centrifuge at 13000 rpm for 30 sec. (Discard the flow-through from the collection tube).
11. Pipette 500 µl of wash buffer 2 onto the column, centrifuge at 13000 rpm for 30 sec. (Discard the flow-through from the collection tube).
12. Pipette 500 µl of wash buffer 2 onto the column, centrifuge at 13000 rpm for 2 min. (Discard the flow-through from the collection tube).
13. To elute the RNA, transfer the column on a new 1.5 ml elution tube, and pipette 30 µl nuclease-free water onto the column membrane and incubate for 1 min, then centrifuge at 13000 rpm for 2 min.
14. Store the RNA samples at -80°C.

Protocol III: RNA cleanup

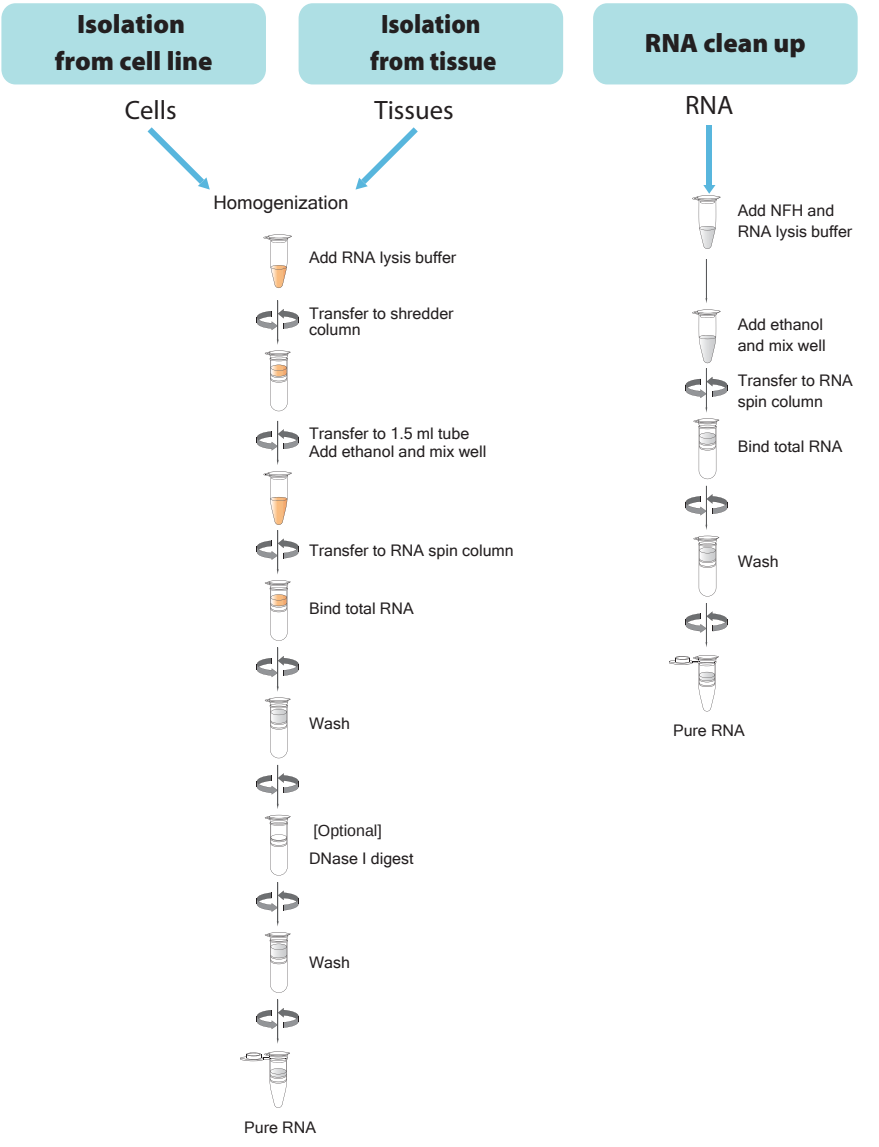
I. Before start :

● The protocol is for cleanup RNA up to 50 ug.

II. Protocol :

1. Adjust sample to a volume of 100 ul with nuclease free water. Add 350 µl of RNA lysis buffer, and mix thoroughly.
2. Add 1 volume of 70% ethanol to the solution and mix well by pipetting. Do not centrifuge.
3. Pipet sample onto RNA spin column, centrifuge at 13000 rpm for 30 sec. (Discard the flow-through from the collection tube).
4. Pipette 500 µl of wash buffer 2 onto the column, centrifuge at 13000 rpm for 30 sec. (Discard the flow-through from the collection tube).
5. Pipette 500 µl of wash buffer 2 onto the column, centrifuge at 13000 rpm for 2 min. (Discard the flow-through from the collection tube).
6. To elute the RNA, transfer the column on a new 1.5 ml elution tube, and pipette 30 µl nuclease-free water onto the column membrane and incubate for 1 min, then centrifuge at 13000 rpm for 2 min.
7. Store the RNA samples at -80°C.

WelPrep Cell / Tissue RNA procedure



Troubleshooting Guide

	Comments and suggestions
RNA degraded	Be careful any RNases contamination in the container or tubes during the whole isolation procedure. Cleaning equipments by RNAZap (Ambion) inactivates contaiminated RNase.
RNA does not perform well in downstream experiments	
a) Salt carryover during elution	Be sure that Wash Buffer 1 is at room temperature.
b) Ethanol carryover during wash	Be sure that during the second wash with Wash Buffer 2, the *WelPrep* spin column is spun at maximum speed for 3 min to dry the spin column membrane. After centrifugation, remove the *WelPrep* spin column from the collection tube carefully so that the flow-through does not come into contact with the spin column, causing carryover of ethanol.
Low RNA yield	
a) Incomplete disruption and homogenization	Use Trizol Reagent for cell disruption and homogenization.
b) RNA still bound to the membrane	Repeat the elution step by incubating the *WelPrep* spin column on the heatblock for 10 min with RNase-free water before centrifugation.
c) Ethanol carryover	In the RNA wash buffer 2, be sure to dry the *WelPrep* RNA spin column by centrifugation at ≥ 8000 x g (≥10,000 rpm) for 2 min at room-temperature.
Low OD260/280 raio	Low OD 260 absorbance, usually lower than 0.3, may cause the low ***OD260/OD280***.

Troubleshooting Guide

	Comments and suggestions
RNA degraded	
a) Tissues are not appropriate collected	After excised from the organism, tissues should be stored in liquid nitrogen in 20 min.
b) Inappropriate volume of RNA lysis buffer (or Trizol Reagent) added	Reduce the amount of tissue or increase the amount of RNA lysis buffer (or Trizol Reagent).
c) RNase contamination	During the isolation process, RNase is introduced during operation. Apply RNaseZap (Ambion) or RNaseAway (Invitrogen) evenly over the pipetman or other equipments in the operation area to eliminate unwanted RNase.
DNA contamination	
a) Not use shredder column	For the cell line and standard tissue samples, it is suggested to use shredder column step to eliminate genomic DNA contamination. To remove the residue DNA, the procedures are: ***Step 1.*** Mix the following in a microcentrifuge tube: • 87.5 µl RNA solution (contaminated with genomic DNA) • 10 µl DNase digestion buffer • 2.5 µl DNase I stock solution Make the volume up to 100 µl with RNase-free water. ***Step 2.*** Incubate at room temperature (20–25°C) for 10 min. ***Step 3.*** Clean up the RNA according to "Protocol: RNA Cleanup".

Troubleshooting Guide

	Comments and suggestions
b) No incubation with RNA Wash Buffer 1	In subsequent preparations, incubate the RNeasy spin column for 5 min at room temperature (15–25°C) after addition of RNA Wash Buffer 1 and before centrifuging. To remove the residue DNA, the procedures are: ***Step 1.*** Mix the following in a microcentrifuge tube: • 87.5 µl RNA solution (contaminated with genomic DNA) • 10 µl DNase digestion buffer • 2.5 µl DNase I stock solution Make the volume up to 100 µl with RNase-free water. ***Step 2.*** Incubate at room temperature (20–25°C) for 10 min. ***Step 3.*** Clean up the RNA according to "Protocol: RNA Cleanup".

Appendix: Storage, Quantification, and Determination of Quality of Total RNA

Storage of RNA

Purified RNA should be stored at –80°C in nuclease-free water.

Quantification of RNA

The concentration of RNA should be meatured the absorbance at 260 nm (A260) in a spectrophotometer. An absorbance of 1 unit at 260 nm corresponds to 40 µg of RNA per ml.

Purity of RNA

Good purity RNA will have an OD 260/280 ratio of 1.8 to 2 and an OD 260/230 of 1.8 or greater. This is because nucleic acid is detected at 260 nm, whereas protein, salt and solvents are detected at 230 and 280 nm.A high OD 260/280 and OD 260/230 ratio therefore indicates theRNA purified are free of any of these contaminants.

Integrity of RNA

The integrity and size distribution of total RNA purified using the WelPrep Blood RNA System can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining or by Agilent 2100 Bioanalyzer. The 28S:18S rRNA ratio has traditionally been viewed as the primary indicator of RNA quality, with a ratio of 2.0 considered to be indicative of high quality, intact RNA. However, with widespread use of the Agilent 2100 bioanalyzer, it has become increasingly clear that the long time standard of a 2.0 rRNA ratio is difficult to meet, especially in RNA derived from clinical samples. This has led researchers to question the wisdom of using the ratio of the 28S and 18S rRNAs, two highly structured and long-lived molecules, as the sole measure of the quality of the underlying mRNA. It is found that total RNAs with 28S:18S rRNA ratios of 1.2 or greater usually provide high quality intact mRNA, and perform well in a variety of applications.

Note

Note