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Introduction

The E.Z.N.A.® SQ Tissue RNA Kit is designed for isolating total RNA from animal tissue and cultured cells. The solution based system can be easily scale up and down based on the starting material. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation are eliminated.

RNA purified using the E.Z.N.A.® SQ Tissue RNA method can be directly used for applications such as RT-PCR*, Nouthern blotting, and other enzymatic reactions.

Principle

E.Z.N.A.® SQ Tissue RNA uses a highly efficient solution based system to provide a convenient, fast, reliable and non-toxic method to isolate high quality RNA from various samples. Samples are first lysed with RCL buffer. Cellular proteins and genomic DNA are removed by precipitation with PNP Buffer while RNA will remain in solution. RNA is further purified by isopropanol precipitation.

Storage and Stability

All components of the E.Z.N.A.® SQ Tissue RNA should be stored at 22°C-25°C. Under these conditions, RNA has successfully been purified and used for RT-PCR after 12 months of storage. Under cool ambient conditions, a precipitate may form in the Buffer RCL and PNP. In case of such an event, heat the bottle at 55°C to dissolve.

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*The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 (and international equivalents) owned by Hoffmann-LaRoche, Inc.

RNA Yields from Various Samples

Species and Sample	Amount of Sample	Typical Yield
Human or Animal Solid Tissue	1-2 mg	0.4-10 µg
	10-20 mg	4 -120 µg
	100-200 mg	200-450µg
	1 gram	1-2 mg
Cultured Cells	100 -10,000 cells	20-250 µg
	2×10^5 - 5×10^5	1-5 µg
	3×10^6 - 5×10^6	25-50 µg
	1×10^8	0.5-1 mg

Kit Contents

Product No.	R3053-00	R3053-05	R3053-40
Amount of tissue or number of cells can be processed per kit	0.5 gram or 1×10^8 cells	5 gram or 1×10^9 cells	40 gram or 6.7×10^9 cells
RCL Buffer	25 mL	250 mL	2 x 1000 mL
PNP Buffer	9 mL	90 ml	750 mL
DEPC Water	5 mL	50 mL	400 mL
Instruction Manual	1	1	1

Starting material

The SQ Tissue RNA protocol can be easily adjusted based on different amount of starting material. Use the following table to prepare required reagent and tube to process the extraction.

Reagent volumes required for processing various amount of tissues

Reagent	Amount of Tissue (mg)				
	1-2	5-10	10-20	100-200	500
Process Tube	1.5 ml	1.5 ml	1.5 ml	15 ml	50 ml
RCL Buffer	100µl	300µl	600µl	6 ml	30 ml
PNP Buffer	33 µl	100µl	200µl	2 ml	10 ml
100% isopropanol	100µl	300µl	600µl	6 ml	30 ml
70% ethanol	100µl	300µl	600µl	6 ml	30 ml
DEPC Water	15µl	25µl	100µl	500µl	1.5 ml

Reagent volumes required for processing various number of cells

Reagent	Number of Cells			
	$100\text{-}10^4$	**$2\text{-}5 \times 10^5$**	**$3\text{-}5 \times 10^6$**	**$1\text{-}2 \times 10^7$**
Process Tube	**0.5 ml**	**1.5 ml**	**15 ml**	**50 ml**
RCL Buffer	**100µl**	**150µl**	**600µl**	**15 ml**
PNP Buffer	**33 µl**	**50µl**	**200µl**	**5 ml**
100% isopropanol	**100µl**	**150µl**	**600µl**	**15 ml**
70% ethanol	**100µl**	**150µl**	**600µl**	**15 ml**
DEPC Water	**10µl**	**20µl**	**100µl**	**250 ml**

A. RNA Purification Protocol for 0.1-2.5 mg of Animal Tissue

Materials to be supplied by user

- Microcentrifuge capable of 13,000 x g
- Sterile 0.5 mL microcentrifuge tubes
- Ice bath
- Isopropanol
- 70% ethanol
- Microfuge tube pestle

1. Dissect tissue quickly and freeze in liquid nitrogen. Storage at -70°C. Fresh tissue can also be used. Work quickly and keep the sample on ice at all times.

2. Add 0.1-2.5 mg frozen ground tissue or fresh tissue to a 0.5 mL microtube containing 100µl RCL Buffer. Homogenize throughly using a microfuge tube pestle (Product # SSI-1015-39).

3. Add 33µl of PNP Buffer to the cell lysate. Mix the sample gently by inverting the tube 10 times.

4. Place the tube on ice for 5 minutes.

5. Centrifuge at max speed (≥13,000 x g) for 3 minutes at room temperature. The precipitated protein and DNA will form a tight pellet.

6. Transfer the supernatant to a new sterile 0.5 mL centrifuge tube that containing 100µl of 100% isopropanol. If the RNA yield is expected to be low, add total 1µg Linear Polyacrylamide (Cat#PR033) or glycogen (Cat # AC122) to the 100µl isopropanol.

7. Gently mix the solution by inverting the tube 30-40 times.

8. Centrifuge at 13, 000 x g for 5 minutes at room temperature.

9. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 100µl of 70% ethanol and invert the tube few times to wash the RNA pellet.

10. Centrifuge at 13, 000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

11. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes.

12. Add 15 µL of DEPC Water and vortex for 1 minutes to mix.

13. Incubate sample on ice for at least 30 minutes. Vortex sample vigorously for 10 seconds and pulse spin.

14. Store RNA at -70°C.

B. RNA Purification Protocol for 5-10 mg of Animal Tissue

Materials to be supplied by user

- Microcentrifuge capable of 13,000 x g
- Sterile 1.5 mL microcentrifuge tubes
- Ice bath
- Isopropanol
- 70% ethanol
- Microfuge tube pestle

1. Dissect tissue quickly and freeze in liquid nitrogen. Storage at -70°C. Fresh tissue can also be used. Work quickly and keep the sample on ice at all time.

2. Add 5-10 mg frozen ground tissue or fresh tissue to a 1.5 mL microtube containing 300 µl RCL Buffer. Homogenize throughly using a microfuge tube pestle (Product # SSI-1015-39).

3. Add 100 µl of PNP Buffer to the cell lysate. Mix the sample gently by inverting the tube 10 times.

4. Place the tube on ice for 5 minutes.

5. Centrifuge at max speed (≥13,000 x g) for 3 minutes at room temperature. The precipitated protein and DNA will form a tight pellet.

6. Transfer the supernatant to a new sterile 1.5 mL centrifuge tube that containing 300µl of 100% isopropanol. If the RNA yield is expected to be low, add total 1µg Linear Polyacrylamide (Cat# PR033) or glycogen (Cat # AC122) to the 100µl isopropanol.

7. Gently mix the solution by inverting the tube 30-40 times.

8. Centrifuge at 13, 000 x g for 5 minutes at room temperature.

9. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 300µl of 70% ethanol and invert the tube few times to wash the RNA pellet.

10. Centrifuge at 13, 000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

11. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes**.**

12. Add 25-30 µL of DEPC Water and vortex for 1 minutes to mix.

13. Incubate sample on ice for at least 30 minutes. Vortex sample vigorously for 10 seconds and pulse spin.

14. Store RNA at -70^{o}C.

C. RNA Purification Protocol for 10-20mg of Animal Tissue

Materials to be supplied by user

- Microcentrifuge capable of 13,000 x g
- Sterile 1.5 mL microcentrifuge tubes
- Ice bath
- Isopropanol
- 70% ethanol
- Microfuge tube pestle

1. Dissect tissue quickly and freeze in liquid nitrogen. Storage at -70°C. Fresh tissue can also be used. Work quickly and keep the sample on ice at all time.

2. Add 10-20 mg frozen ground tissue or fresh tissue to a 1.5 mL microtube containing 600µl RCL Buffer. Homogenize throughly using a microfuge tube pestle (Product # SSI-1015-39).

3. Add 200 µl of PNP Buffer to the cell lysate. Mix the sample gently by inverting the tube 10 times.

4. Place the tube on ice for 5 minutes.

5. Centrifuge at max speed (≥13,000 x g) for 3 minutes at room temperature. The precipitated protein and DNA will form a tight pellet.

6. Transfer the supernatant to a new sterile 1.5 mL centrifuge tube that containing 600µl of 100% isopropanol. If the RNA yield is expected to be low, add total 1µg Linear Polyacrylamide (Cat# PR033) or glycogen (Cat # AC122) to the 100µl isopropanol.

7. Gently mix the solution by inverting the tube 30-40 times.

8. Centrifuge at 13, 000 x g for 5 minutes at room temperature.

9. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 600µl of 70% ethanol and invert the tube few times to wash the RNA pellet.

10. Centrifuge at 13, 000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

11. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes**.**

12. Add 100 µL of DEPC Water and vortex for 1 minutes to mix.

13. Incubate sample on ice for at least 30 minutes. Vortex sample vigorously for 10 seconds and pulse spin.

14. Store RNA at -70^{o}C.

D. RNA Purification Protocol for 100-200 mg of Animal Tissue

Materials to be supplied by user

- Centrifuge capable of 3,000-5,000 x g
- High speed centrifuge tube with capacity of at least 15 ml
- Ice bath
- Isopropanol
- 70% ethanol
- Ground pestle

1. Dissect tissue quickly and freeze in liquid nitrogen. Storage at -70°C. Fresh

tissue can also be used. Work quickly and keep the sample on ice at all times.

2. Add 100-200 mg frozen ground tissue or fresh tissue to a 15 mL centrifuge tube containing 6 ml RCL Buffer. Homogenize the sample using 5-10 strokes with tube pestle.

3. Add 2 ml of PNP Buffer to the cell lysate. Mix the sample gently by inverting the tube 10 times.

4. Place the tube on ice for 5 minutes.

5. Centrifuge at 3,000-5,000 g for 10 minutes at room temperature. The precipitated protein and DNA will form a tight pellet.

6. Transfer the supernatant to a new 15 ml sterile centrifuge tube that containing 6 ml of 100% isopropanol.

7. Gently mix the solution by inverting the tube 30-40 times.

8. Centrifuge at 3,000-5,000 x g for 15 minutes at room temperature.

9. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 6 ml of 70% ethanol and invert the tube few times to wash the RNA pellet.

10. Centrifuge at 3,000-5,000 x g for 10 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

11. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes**.**

12. Add 250 µL of DEPC Water and vortex for 1 minutes to mix.

13. Incubate sample on ice for at least 30 minutes. Vortex sample vigorously for 10 seconds and pulse spin.

14. Store RNA at -70^{o}C.

E. RNA Purification Protocol for 100 - 10,000 cultured cells

Materials to be supplied by user

- Microcentrifuge capable of 13,000 x g
- 0.5 or 1.5 ml centrifuge tube
- Ice bath
- Isopropanol
- 70% ethanol
- Ground pestle

1. Remove the media and collect cells.

   For cells grown in suspension, pellet the cell by centrifugation and discard the media, add 100µl RCL. Pipetting up and down 3-5 times to lyse the cell.

   For cells grown in monolayer, cells can be directly lysed by adding 100µl of RCL Buffer directly into the culture plate or flask. lyse and homogenize the sample by shaking or vortexing. Transfer the cell lysate into a 0.5 or 1.5 ml centrifuge tube

2. Add 33 µl of PNP Buffer to the cell lysate. Mix the sample gently by inverting the tube 10 times.

3. Place the tube on ice for 5 minutes.

4. Centrifuge at max speed (≥13,000 x g) for 5 minutes at room temperature. The precipitated protein and DNA will form a tight pellet.

5. Transfer the supernatant to a new 1.5 ml . centrifuge tube that containing 100 µl of 100% isopropanol.

6. Gently mix the solution by inverting the tube 30-40 times.

7. Centrifuge at 13, 000 x g for 10 minutes at room temperature.

8. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 100 µl of 70% ethanol and invert the tube few times to wash the RNA pellet.

9. Centrifuge at 13, 000 x g for 3 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

10. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes**.**

11. Add 10 µl of DEPC Water and vortex for 1 minutes to mix.

12. Incubate sample on ice for at least 30 minutes. Vortex sample vigorously for 10 seconds and pulse spin.

13. Store RNA at -70^{o}C.

F. RNA Purification Protocol for 2x 10^5 -5 x 10^5 cultured cells

Materials to be supplied by user

- Microcentrifuge capable of 13,000 x g
- 0.5 or 1.5 ml centrifuge tube
- Ice bath
- Isopropanol
- 70% ethanol
- Ground pestle

1. Remove the media and collect cells.

   For cells grown in suspension, pellet the cell by centrifugation and discard the media, add 150µl RCL. Pipetting up and down 3-5 times to lyse the cell.

   For cells grown in monolayer, cell can be directly lysed by directly adding the 150µ RCL Buffer directly into the culture plate or flask. lyse and homogenize the sample by shaking or vortexing. Transfer the cell lysate into a 0.5 or 1.5 ml centrifuge tube

2. Add 50 µl of PNP Buffer to the cell lysate. Mix the sample gently by inverting the tube 10 times.

3. Place the tube on ice for 5 minutes.

4. Centrifuge at max speed (≥13,000 x g) for 5 minutes at room temperature. The precipitated protein and DNA will form a tight pellet.

5. Transfer the supernatant to a new 1.5 ml sterile centrifuge tube that containing

150 µl of 100% isopropanol.

6. Gently mix the solution by inverting the tube 30-40 times.

7. Centrifuge at 13, 000 x g for 10 minutes at room temperature.

8. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 150 µl of 70% ethanol and invert the tube few times to wash the RNA pellet.

9. Centrifuge at 13, 000 x g for 3 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

10. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes**.**

11. Add 15 µl of DEPC Water and vortex for 1 minutes to mix.

12. Incubate sample on ice for at least 30 minutes. Vortex sample vigorously for 10 seconds and pulse spin.

13. Store RNA at -70^{o}C.

G. RNA Purification Protocol for 3-5 x 10^6 Cells

Materials to be supplied by user

- Microcentrifuge capable of 13,000 x g
- 1.5 ml centrifuge tube
- Ice bath
- Isopropanol
- 70% ethanol
- Ground pestle

1. Remove the media and collect cells.

   For cells grown in suspension, pellet the cell by centrifugation and discard the media, add 600µl RCL. Pipetting up and down 3-5 times to lyse the cell.

   For cells grown in monolayer, cells can be directly lysed by adding the 600µl

of RCL Buffer directly into the culture plate or flask. lyse and homogenize the sample by shaking or vortexing. Transfer the cell lysate into a 1.5 ml centrifuge tube

2. Add 200 µl of PNP Buffer to the cell lysate. Mix the sample gently by inverting the tube 10 times.

3. Place the tube on ice for 5 minutes.

4. Centrifuge at max speed (≥13,000 x g) for 5 minutes at room temperature. The precipitated protein and DNA will form a tight pellet.

5. Transfer the supernatant to a new 1.5 ml sterile centrifuge tube that containing 600 µl of 100% isopropanol.

6. Gently mix the solution by inverting the tube 30-40 times.

7. Centrifuge at 13, 000 x g for 10 minutes at room temperature.

8. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 600 µl of 70% ethanol and invert the tube few times to wash the RNA pellet.

9. Centrifuge at 13, 000 x g for 3 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

10. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes**.**

11. Add 100 µl of DEPC Water and vortex for 1 minutes to mix.

12. Incubate sample on ice for at least 30 minutes. Vortex sample vigorously for 10 seconds and pulse spin.

13. Store RNA at -70^{o}C.

H. RNA Purification Protocol for 1-2 x 10^{7} cultured cells

Materials to be supplied by user

- Centrifuge capable of 3,000 g

- 15 ml high speed centrifuge tube
- Ice bath
- Isopropanol
- 70% ethanol
- Ground pestle

1. Remove the media and collect cells.

   For cells grown in suspension, pellet the cell by centrifugation and discard the media, add 3 ml RCL. Pipetting up and down 3-5 times to lyse the cell.

   Note: Leave 0.1-0.2 ml media or salt balance buffer to fully resuspend the cell pellet before addition of RCL Buffer.

   For cells grown in monolayer, cell can be directly lysed by directly adding the 3 ml of RCL Buffer directly into the culture plate or flask. lyse and homogenize the sample by shaking or vortexing. Transfer the cell lysate into a 15 ml centrifuge tube

2. Add 1 ml of PNP Buffer to the cell lysate. Mix the sample gently by inverting the tube 10 times.

3. Place the tube on ice for 5 minutes.

4. Centrifuge at max speed 3,000-5,000 x g for 15 minutes at room temperature. The precipitated protein and DNA will form a tight pellet.

5. Transfer the supernatant to a new 15 ml high speed centrifuge tube that containing 3 ml of 100% isopropanol.

6. Gently mix the solution by inverting the tube 30-40 times.

7. Centrifuge at 3,000-5,000 x g for 15 at room temperature.

8. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 3 ml of 70% ethanol and invert the tube few times to wash the RNA pellet.

9. Centrifuge at 3,000-5,000 x g for 10 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

10. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes**.**

11. Add 250 µl of DEPC Water and vortex for 1 minutes to mix.

12. Incubate sample on ice for at least 30 minutes. Vortex sample vigorously for 10 seconds and pulse spin.

13. Store RNA at -70°C.

I. RNA Purification Protocol for 3-5 x 10^7 cultured cells

Materials to be supplied by user

- Centrifuge capable of 3,000 g
- 15 ml high speed centrifuge tube
- Ice bath
- Isopropanol
- 70% ethanol
- Ground pestle

1. Remove the media and collect cells.

   For cells grown in suspension, pellet the cell by centrifugation and discard the media, add 6 ml RCL. Pipetting up and down 3-5 times to lyse the cell.

   Note: Leave 0.1-0.2 ml media or salt balance buffer to fully resuspend the cell pellet before addition of RCL Buffer.

   For cells grown in monolayer, cell can be directly lysed by directly adding the 3 ml of RCL Buffer directly into the culture plate or flask. lyse and homogenize the sample by shaking or vortexing. Transfer the cell lysate into a 15 ml centrifuge tube

2. Add 2 ml of PNP Buffer to the cell lysate. Mix the sample gently by inverting the tube 10 times.

3. Place the tube on ice for 5 minutes.

4. Centrifuge at 3,000-5,000 for 10 minutes at room temperature. The precipitated protein and DNA will form a tight pellet.

5. Transfer the supernatant to a new 15 ml high speed centrifuge tube that containing 6 ml of 100% isopropanol.

6. Gently mix the solution by inverting the tube 30-40 times.

7. Centrifuge at 3,000-5,000 x g for 15 minutes at room temperature.

8. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 6 ml of 70% ethanol and invert the tube few times to wash the RNA pellet.

9. Centrifuge at 3,000-5,000 x g for 3 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

10. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes**.**

11. Add 500 µl of DEPC Water and vortex for 1 minutes to mix.

12. Incubate sample on ice for at least 30 minutes. Vortex sample vigorously for 10 seconds and pulse spin.

13. Store RNA at -70°C

J. RNA Purification from 10-20 mg Paraffin-embedded Tissue

Materials to be supplied by user

- Microcentrifuge capable of 13,000 x g
- Sterile 1.5 mL microcentrifuge tubes
- Ice bath
- Isopropanol
- 70% ethanol
- Microfuge tube pestle

1. Place 10-20 mg minced tissue into a 1.5 ml centrifuge tube. Add 300µl xylene and mix throughly by vortexing for 20 seconds. . Incubate at room temperature for 5 minutes with constant shaking.

2. Centrifuge at 13,000 x g for 5 minutes. Carefully remove the xylene with

pipette.

3. Wash the tissue sample two more times by repeating step 1-2.

4. Add 600µl ethanol into the tube and mix throughly by vortexing the tube for 10 seconds. Incubate at room temperature for 5 minutes.

5. Centrifuge at 13,000 x g for 3 minutes. Discard the ethanol.

6. Wash the sample with ethanol 2 more times by repeating step 4-5 twice.

7. Add 600 µl of RCL Buffer to the tube. Homogenize throughly with 5-10 strokes using a microfuge tube pestle (Product # SSI-1015-39).

8. Add 200 µl of PNP Buffer to the cell lysate. Mix the sample gently by inverting the tube 10 times.

9. Place the tube on ice for 5 minutes.

10. Centrifuge at max speed (≥13,000 x g) for 3 minutes at room temperature. The precipitated protein and DNA will form a tight pellet.

11. Transfer the supernatant to a new sterile 1.5 mL centrifuge tube that containing 600µl of 100% isopropanol. If the RNA yield is expected to be low, add total 1µg Linear Polyacrylamide (Cat# PR033) or glycogen (Cat # AC122) to the 100µl isopropanol.

12. Gently mix the solution by inverting the tube 30-40 times.

13. Centrifuge at 13, 000 x g for 5 minutes at room temperature.

14. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 600µl of 70% ethanol and invert the tube few times to wash the RNA pellet.

15. Centrifuge at 13, 000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

16. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes**.**

17. Add 100 µL of DEPC Water and vortex for 1 minutes to mix.

18. Incubate sample on ice for at least 30 minutes. Vortex sample vigorously for 10 seconds and pulse spin.

19. Store RNA at -70^{o}C.