omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® SQ Total RNA Kit

R3053-00	5 preps
R3053-05	50 preps

Manual Date: June 2020
Revision Number: v3.0

For Research Use Only

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Introduction

The E.Z.N.A.® SQ Total RNA Kit is designed to reliably isolation of total RNA from animal tissue and cultured cells. The isolation systems follow salt-out precipitation based method and can be easily scaled up or down based on the amount of starting material. The protocol eliminates the need for phenol/chloroform extractions and other time-consuming steps such as as CsCl gradient ultracentrifugation. RNA purified using the E.Z.N.A.® SQ Total RNA method can directly used for applications such as RT-PCR, Northern blotting, and other enzymatic reactions.

The E.Z.N.A.® SQ Total RNA Kit uses a salt-out precipitation based extraction protocol for extracting high quality RNA from a variety of sample sources. Samples are lysed in RCL Buffer under denaturing conditions. Cellular proteins and genomic DNA are removed by precipitation with PNP Buffer leaving RNA in the solution. RNA is recovered by isopropanol precipitation and eluted in Nuclease-free Water.

New in this Edition:

June 2020:

- This manual has been edited for content and redesigned to enhance user readability.
- Nuclease-free Water has replaced DEPC Water. DEPC Water is no longer supplied in this kit and is no longer available for purchase.

Kit Contents

Product	R3053-00	R3053-05
Total Amount of Tissue	0.5 g or 1 x 10^8 cells	5 g or 1 x 10^9 cells
RCL Buffer	25 mL	2 x 125 mL
PNP Buffer	9 mL	90 mL
Nuclease-free Water	15 mL	60 mL
User Manual	✓	✓

Storage and Stability

All of the E.Z.N.A.® SQ Total RNA Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve any deposits by warming the solution at 37°C and gently shaking.

Typical RNA Yields

Sample Type	Amount of Sample	Typical Yield
Human/Animal Tissue	1-2 mg	0.4-10 µg
	10-20 mg	4-120 µg
	100-200 mg	200-450 µg
	1 g	1-2 mg
Cultured Cells	100-10,000 cells	20-250 µg
	2 x 10^5 - 5 x 10^5 cells	1-5 µg
	3 x 10^6 - 5 x 10^6 cells	25-50 µg
	1x10^7 to 5x10^7 cells	0.5-1 mg

Starting Materials

The E.Z.N.A.® SQ Total RNA protocol can be easily adjusted based on the amount of starting material. Use the following tables to prepare the required reagents and tubes to process the samples.

Reagent	Amount of Tissue (mg)				
	1-2	5-10	10-20	100-200	500
Tube Required	1.5 mL	1.5 mL	1.5 mL	15 mL	50 mL
RCL Buffer	100 µL	300 µL	600 µL	6 mL	30 mL
PNP Buffer	33 µL	100 µL	200 µL	2 mL	10 mL
100% isopropanol	100 µL	300 µL	600 µL	6 mL	30 mL
70% ethanol	100 µL	300 µL	600 µL	6 mL	30 mL
Nuclease-free Water	15 µL	25 µL	100 µL	500 µL	1.5 mL

Reagent	Number of Cells			
	100-10^4	2-5x10^5	3-5x10^6	1-5x10^7
Tube Required	0.5 mL	1.5 mL	15 mL	50 mL
RCL Buffer	100 µL	150 µL	600 µL	15 mL
PNP Buffer	33 µL	50 µL	200 µL	5 mL
100% isopropanol	100 µL	150 µL	600 µL	15 mL
70% ethanol	100 µL	150 µL	600 µL	15 mL
Nuclease-free Water	10 µL	20 µL	100 µL	250 µL

E.Z.N.A.® SQ Total RNA Kit - 0.1-20 mg Fresh or Frozen Tissue

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000*g*
- Nuclease-free 0.5 mL, 1.5 mL, or 2 mL microcentrifuge tubes
- Ice bucket
- 70% ethanol
- 100% isopropanol
- Vortexer
- Grinding and Homogenization Materials
  - For frozen tissue:
    - Liquid nitrogen
    - Mortar and pestle
  - Microcentrifuge tube pestle compatible with the microcentrifuge tubes used

Before Starting:

- Prepare an ice bucket.

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

2. Add RCL Buffer to a 1.5 mL microcentrifuge tube (not provided) according to the table below.

Amount of Tissue	RCL Buffer
0.1-2.5 mg	100 µL
5-10 mg	300 µL
10-20 mg	600 µL

3. Add the appropriate amount of frozen ground tissue or fresh tissue to the tube of RCL Buffer prepared in Step 2. Homogenize thoroughly using a microcentrifuge tube pestle.

4. Add PNP Buffer according to the table below. Invert the tube 10 times to gently mix.

Amount of Tissue	PNP Buffer
0.1-2.5 mg	33 µL
5-10 mg	100 µL
10-20 mg	200 µL

5. Let sit on ice for 5 minutes.

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

7. Add 100% isopropanol to a new microcentrifuge tube according to the table below.

Amount of Tissue	100% Isopropanol
0.1-2.5 mg	100 µL
5-10 mg	300 µL
10-20 mg	600 µL

Note: If the RNA yield is expected to be low, add a total of 1 µg linear polyacrylamide or glycogen (Cat# AC122) to isopropanol.

8. Transfer the supernatant from Step 6 to the tube of 100% isopropanol prepared in Step 7.

9. Invert the tube 30-40 times to gently mix.

10. Centrifuge at maximum speed for 5 minutes at room temperature. Remove the supernatant and drain the tube briefly on clean absorbent paper.

11. Add 70% ethanol according to the table below. Invert the tube a few times to wash the RNA pellet.

Amount of Tissue	70% Ethanol
0.1-2.5 mg	100 µL
5-10 mg	300 µL
10-20 mg	600 µL

12. Centrifuge at maximum speed for 2 minutes at room temperature. Carefully remove the ethanol. The pellet may be very loose at this point so pour slowly and watch the pellet.

13. Invert the tube on clean absorbent paper and air dry the pellet for 10-15 minutes.

14. Add Nuclease-free Water according to the table below. Vortex for 1 minute to mix.

Amount of Tissue	Nuclease-free Water
0.1-2.5 mg	15 µL
5-10 mg	25-30 µL
10-20 mg	100 µL

15. Let sit on ice for at least 30 minutes. Vortex sample vigorously for 10 seconds and pulse spin.

16. Store RNA at -70°C.

E.Z.N.A.® SQ Total RNA Kit - 100-200 mg Fresh or Frozen Tissue

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 3,000*g*
- Nuclease-free 15 mL microcentrifuge tubes
- Ice bath
- 70% ethanol
- 100% isopropanol
- Vortexer
- Grinding and Homogenization Materials
  - For frozen tissue:
    - Liquid nitrogen
    - Mortar and pestle
  - Centrifuge tube pestle compatible with the centrifuge tubes used

Before Starting:

- Prepare an ice bucket.

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

2. Add 6 mL RCL Buffer to a 15 mL centrifuge tube.

3. Add 100-200 mg frozen ground tissue or fresh tissue to the tube of RCL Buffer prepared in Step 2. Homogenize thoroughly using a centrifuge tube pestle.

4. Add 2 mL PNP Buffer. Invert the tube 10 times to gently mix.

5. Let sit on ice for 5 minutes.

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

7. Add 6 mL100% isopropanol to a new 15 mL centrifuge tube.

8. Transfer the supernatant from Step 6 to the tube of 100% isopropanol prepared in Step 7.

9. Invert the tube 30-40 times to gently mix.

10. Centrifuge at 3,000-5,000*g* for 15 minutes at room temperature. Remove the supernatant and drain the tube briefly on clean absorbent paper.

11. Add 6 mL 70% ethanol. Invert the tube a few times to wash the RNA pellet.

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

13. Invert the tube on clean absorbent paper and air dry the pellet for 10-15 minutes.

14. Add 250 µL Nuclease-free Water. Vortex for 1 minute to mix.

15. Let sit on ice for at least 30 minutes. Vortex sample vigorously for 10 seconds and pulse spin.

16. Store RNA at -70°C.

E.Z.N.A.® SQ Total RNA Kit - 100 to 5 x 10^6 Cultured Cells

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000*g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Ice bucket
- 70% ethanol
- 100% isopropanol
- Vortexer

Before Starting:

- Prepare an ice bucket.

1. Collect cells by one of the methods below.

   A. Cells grown in suspension
      1. Pellet the cells by centrifugation.
      2. Discard the media.
      3. Add RCL Buffer according to the table below.
      4. Pipet up and down 3-5 times to lyse cells.
      5. Proceed to Step 2.

   B. Cells grown in monolayer
      1. Add RCL Buffer directly to the culture plate or flask according to the table below.
      2. Shake or vortex the culture plate or tube to lyse cells.
      3. Transfer lysate to a new microcentrifuge tube (not provided).
      4. Proceed to Step 2.

Amount of Cells	RCL Buffer
100-10,000 cells	100 µL
2 x 10^5 - 5 x 10^5	150 µL
3 x 10^6 - 5 x 10^6	600 µL

2. Add PNP Buffer according to the table below. Invert the tube 10 times to gently mix.

Amount of Cells	PNP Buffer
100-10,000 cells	33 µL
2×10^5 - 5×10^5	50 µL
3×10^6 - 5×10^6	200 µL

3. Let sit on ice for 5 minutes.

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

5. Add 100% isopropanol to a new microcentrifuge tube according to the table below.

Amount of Cells	100% Isopropanol
100-10,000 cells	100 µL
2×10^5 - 5×10^5	150 µL
3×10^6 - 5×10^6	600 µL

6. Transfer the supernatant from Step 4 to the 100% isopropanol from Step 5.

7. Invert the tube 30-40 times to gently mix.

8. Centrifuge at maximum speed for 10 minutes at room temperature. Remove the supernatant and drain the tube briefly on clean absorbent paper.

9. Add 70% ethanol according to the table below. Invert the tube a few times to wash the RNA pellet.

Amount of Cells	70% Ethanol
100-10,000 cells	100 µL
2×10^5 - 5×10^5	150 µL
3×10^6 - 5×10^6	600 µL

10. Centrifuge at maximum speed for 3 minutes at room temperature. Carefully remove the ethanol. The pellet may be very loose at this point so pour slowly and watch the pellet.

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

12. Add Nuclease-free Water according to the table below. Vortex for 1 minute to mix.

Amount of Cells	Nuclease-free Water
100-10,000 cells	10 µL
2×10^5 - 5×10^5	15 µL
3×10^6 - 5×10^6	100 µL

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

14. Store RNA at -70°C.

E.Z.N.A.® SQ Total RNA Kit - 1 x 10^7 to 5 x 10^7 Cultured Cells

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 3,000*g*
- Nuclease-free 15 mL centrifuge tubes
- Ice bucket
- 70% ethanol
- 100% isopropanol
- Vortexer

Before Starting:

- Prepare an ice bucket.

1. Collect cells by one of the methods below.

   A. Cells grown in suspension
      1. Pellet the cells by centrifugation.
      2. Discard the media leaving 0.1-0.2 mL media remaining.
      3. Resuspend cells.
      4. Add RCL Buffer according to the table below.
      5. Pipet up and down 3-5 times to lyse cells.
      6. Proceed to Step 2.

   B. Cells grown in monolayer
      1. Add RCL Buffer directly to the culture plate or flask according to the table below.
      2. Shake or vortex the culture plate or tube to lyse cells.
      3. Transfer lysate to a new 15 mL centrifuge tube (not provided).
      4. Proceed to Step 2.

Amount of Cells	RCL Buffer
1 x 10^7 - 2 x 10^7	3 mL
3 x 10^7- 5 x 10^7	6 mL

2. Add PNP Buffer according to the table below. Invert the tube 10 times to gently mix.

Amount of Cells	PNP Buffer
1 x 10^7 - 2 x 10^7	1 mL
3 x 10^7- 5 x 10^7	2 mL

3. Let sit on ice for 5 minutes.

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

5. Add 100% isopropanol to a 15 mL centrifuge tube according to the table below.

Amount of Cells	100% Isopropanol
1 x 10^7 - 2 x 10^7	3 mL
3 x 10^7- 5 x 10^7	6 mL

6. Transfer the supernatant from Step 4 to the tube of 100% isopropanol prepared in Step 5.

7. Invert the tube 30-40 times to gently mix.

8. Centrifuge at 3,000-5,000*g* for 15 minutes at room temperature. Remove the supernatant and drain the tube briefly on clean absorbent paper.

9. Add 70% ethanol according to the table below. Invert the tube a few times to wash the RNA pellet.

Amount of Cells	70% Ethanol
1 x 10^7 - 2 x 10^7	3 mL
3 x 10^7- 5 x 10^7	6 mL

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

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

12. Add Nuclease-free Water according to the table below. Vortex for 1 minute to mix.

Amount of Cells	Nuclease-free Water
1 x 10^7 - 2 x 10^7	250 µL
3 x 10^7- 5 x 10^7	500 µL

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

14. Store RNA at -70°C.

Notes:

For more purification solutions, visit **www.omegabiotek.com**

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

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