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Introduction

SQ DNA/RNA/Protein Isolation Kit is designed for rapid and reliable isolation of total proteins, DNA and RNA from single biological sample. The SQ DNA/RNA/Protein Isolation Kit uses a proprietary buffer system to provide highly efficient method for simultaneously isolation of total proteins, genomic DNA and RNA from small amount animal tissue and culture cells. There is no toxic substance such as phenol/chloroform or guanidine salts involved in this system. The system can be easily scaled up or down, allowing for the purification from various amounts of starting materials.

Principle

If using the SQ DNA/RNA/Protein for the first time, please read this booklet to become familiar with the procedure and its various modifications. Samples are first lysed in a specially formulated buffer. The protein is precipitated by adding SQ2 (PPR). After removal of the protein, the supernatant is mixed with 1 volume of isopropanol to precipitate the RNA. After the centrifugation to pellet the RNA, the supernatant is transfer to a new tube and mixed with SQ3 Buffer to precipitate DNA. RNA and DNA pellet are washed with 70% ethanol and dissolved with water or low ionic strength buffer. Purified DNA, RNA and protein can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the SQ DNA/RNA/Protein Kit are stable for at least 12 months from date of purchase when stored at 8°C-25°C. During shipment, or storage in cool ambient conditions, precipitates may form in some of the buffers. Dissolve such deposits by warming the solution at 37°C.

Kit Contents

SQ DNA/RNA/Protein Cell Kit

SQ DNA/RNA/Protein Tissue Kit

SQ DNA/RNA/Protein Tissue Kit				
Product No.	R804 2-00	R 8042-01	R8042-02	R804 2-03
Amount of tissue processed per kit	1 g 2.2 x 10 ⁸	4.5 gram 1 x 10 ⁹	18 gram 4 x 10 ⁹	36 gram 8 x 10 ⁹
SQ1	20 ml	90 ml	360 ml	750 ml
SQ2	8 ml	30 ml	120 ml	250 ml
SQ3 Buffer	15 ml	60 ml	240 ml	500 ml
SQ4 Buffer	15 ml	60 ml	240 ml	500 ml
DEPC Water	5.0 ml	25 ml	100 ml	200 ml
EB Buffer	5.0 ml	25 ml	100 ml	200 ml
User Manual	1	1	1	1

Before Starting

Please take a few minutes to read this booklet thoroughly and become familiar with the protocol. Prepare all materials required before starting in order to minimize RNA degradation. *Wear gloves/protective goggles and take great care when working with chemicals.*

- Whenever working with RNA, always wear latex gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.
- During the procedure work carefully but quickly.

- Under cool ambient conditions, crystals may form in some of the buffers. This is normal and the bottle should be warmed to re-dissolve the salt.

Starting Material

The SQ DNA/RNA/Protein Purification Kits are flexible on the starting material. Use the following table as guideline for the usage of starting material and reagents:

SQ DNA/RNA/Protein Cell Kit				
Number of cells	100-50,000	0.5-1 x 10 ⁶	1-2 x 10 ⁶	1-2 x 10 ⁷
Tube size	0.6 ml	1.5 ml	2 ml	15 ml
SQ1	75 µl	200 µl	300 µl	3 ml
SQ2	25 µl	67 µl	100 µl	1 ml
SQ3 Buffer	50 µl	133 µl	200 µl	2 ml
SQ4 Buffer	50 µl	133 µl	200 µl	2 ml

Typical Yield Using SQ DNA/RNA/Protein Kit
(1 x 10⁶ cells or 10mg of tissue)

Sample	RNA yield (µg)	DNA yield (µg)	Protein yield (µg)
Liver	20-30	10-20	800-1000
Kidney	10-20	10-20	1000-1200
Lung	10-15	10-20	600-800
Heart	15-20	10-20	800-1000
Brain	10-25	15-25	800-1000
Muscle	5-20	10-20	800-1000
Adipose tissue	5-16	5-15	500-800
Hela Cell	10-15	6-10	50-60
NIH/3T3	8-10	6-10	50-60
Blood (300µl)	0.5-2	2.5-3	50-60
Blood (1 ml)	3-4	25-30	150-200
Blood (2 ml)	3-4	50-60	300-400

Protocol: DNA/RNA/Protein Purification from Cultured Cells
Using SQ DNA/RNA/Protein Cell Kit (1-2 x 10⁶ cells)

Before starting:

- Fresh or flash-frozen cultured cells can be used in this protocol. Collect suspended cells and place on ice until use. Determine the cell number by using a hemacytometer or other cell counter.
- Preheat the water bath to 65°C
- water bath or heating block preset at 37°C
- Pre-set the centrifuge at 4C for RNA isolation.
- Frozen cells should be thawed quickly using a 37°C water bath with gently agitation and place on ice until use.
- Warm up the SQ1 at 37°C water bath

Material and Equipments supplied by User

Have the following reagents and supplies ready before starting procedure:

- Centrifuge with temperature control
- Table top centrifuge capable at least 13,000 x g
- 1.5 or 2 ml Nuclease-Free centrifuge tubes
- 0.45 µm filter unit
- Absolute ethanol
- 70% ethanol
- 100% Isopropanol
- Ice Bath

Procedure

1. Harvest and lyse the cell:

A) Cells Grown in Suspension

Pellet cells by centrifugation in a 1.5 ml tube. Lyse cells in SQ1 by repetitive pipetting. Alternately, vortex the tube at maximum speed for 1 minute to lysis the cell. Use 300 µL of the reagent per 2 x 10⁶ of cultured cells.

B) Cells Grown in Monolayer

Lyse cells directly in a culture dish by adding 300 µL of SQ1 per 2 x 10⁶ of cultured cells directly into each well of multiwell cell culture plate or flask, and lyse the cell by passing the cell lysate several times through a blue pipette tip. Alternately, shake the plate on a orbital shaker at maximum speed for 1 minute to lysis the cells.

2. Homogenize the samples:

Cultured cells can be effectively homogenized by pipetting up and down or vortexing

after addition of Cell lysis buffer.

3. Place the tube containing the homogenates on the bench at room temperature for 2 minutes.
4. Add 100 μ L of SQ2 (1/3 volume of Cells lysis Buffer) to each sample, mix thoroughly by vortexing at maximum speed for 30 seconds.
5. Incubate sample in an ice bath for 10 minutes.
6. Centrifuge at maximum speed ($\geq 13,000 \times g$) at 4°C for 10 minutes.
7. Transfer cleared supernatant (~400 μ L) into new 1.5 ml centrifuge tube for RNA/DNA isolation. Remove any liquid from the original tube containing the protein precipitate by inverting the tube on a absorbent paper. Keep the tube with the protein pellet for protein isolation starting from step 26. For Total Nucleic acid isolation, proceed step 8-12. For RNA and DNA isolation, proceed step 13-25.

Total Nucleic acid Isolation

8. Add equal volume (400 μ L) of isopropanol. Mix thoroughly by vortexing for 30 seconds. Incubate the tube at room temperature for 5 minutes.
9. Centrifuge at maximum speed ($\geq 13,000 \times g$) at 4°C for 10 minutes to precipitate Nucleic acid. Discard the supernatant.
10. Wash the nucleic acid pellet by adding 400 μ L of 70% ethanol into the tube. Vortex the tube for 30 seconds .
11. Centrifuge at maximum speed ($\geq 13,000 \times g$) at 4°C for 3 minutes. Aspirate the supernatant and air dry the pellet by inverting the tube on a absorbent paper for 5-10 minutes.
12. Add 50-100 μ l of nuclease free water into the tube to dissolve the Nucleic acid pellet. Vortex the tube for 30 seconds. Incubate the tube on ice or 4°C for 30-45 minutes to completely re-hydrate the nucleic acid.

For Total RNA Isolation

13. Add 200 μ L Buffer SQ3 (½ volume of supernatant) into the cleared supernatant from step 7, vortex to mix well.
14. Add equal volume (600 μ L) of isopropanol. Mix thoroughly by vortexing. Incubate the

tube at room temperature for 5 minutes.

15. Centrifuge at maximum speed ($\geq 13,000 \times g$) at 4°C for 5 minutes to precipitate RNA. A bi-phase will be formed in the supernatant after the centrifugation.
16. Transfer entire supernatant to a new 1.5 ml tube and keep the tube for DNA isolation starting at step 20.
Note: It is critical to remove any liquid drop at this step to minimize the DNA contamination. For best result, briefly spin and collect drop from the tube, remove any drop by pipettor.
17. Wash the RNA pellet by adding 600 μ L of 70% ethanol into the tube. Vortex the tube for 30 seconds .
18. Centrifuge at maximum speed ($\geq 13000 \times g$) at 4°C for 3 minutes. Aspirate the supernatant and air dry the RNA pellet by inverting the tube on a absorbent paper for 5-10 minutes
19. Add 50-100 μ l of DEPC water into the tube to dissolve the RNA pellet. Vortex the tube for 30 seconds. Incubate the tube on ice or 4°C for 30-45 minutes to completely re-hydrate the RNA.

DNA Isolation

20. Add 1/6 volume of SQ4 Buffer (200 μ L l) and mix by vortexing.
21. Centrifuge at maximum speed ($\geq 13,000 \times g$) for 5 minutes at room temperature to collect precipitated DNA. Carefully discard supernatant.
22. Add 600 μ L of 70% ethanol and mix thoroughly by vortexing for 30 seconds.
23. Centrifuge at maximum speed ($\geq 13,000 \times g$) for 3 minutes at room temperature Carefully discard supernatant.
24. Air dry the DNA pellet by inverting the tube on a absorbent paper for 5-10 minutes.
25. Add 100 μ l EB Buffer to the tube and incubate at 65C for 30 minutes to re-hydrate the DNA.

Protein Isolation

26. Using the centrifuge tube which contains the protein pellet from step 7.

27. Add 1ml of 95% ethanol and vortex the tube for 30 seconds.
28. Centrifuge at maximum speed for 2 minutes. Discard the supernatant. Air dry the protein pellet by inverting the tube on a absorbent paper for 5-10 minutes.
29. Add 100-200 μ L protein loading dye (Laemmli Loading dye) to the tube to dissolve the protein pellet. If the protein will not be analyzed by SDS-PAGE, dissolve the protein in a buffer that compatible with downstream applications.

Protocol: DNA/ RNA/Protein Purification from Cultured Cells Using SQ DNA/RNA/Protein Cell Kit (1-2 x 10⁷ cells)

Before starting:

1. Fresh or flash-frozen cultured cells can be used in this protocol. Collect suspended cells and place on ice until use. Determine the cell number by using a hemacytometer or other cell counter.
2. Preheat the water bath to 65°C
3. water bath or heating block preset at 37°C
4. Pre-set the centrifuge at 4°C for RNA isolation.
5. Frozen cells should be thawed quickly using a 37°C water bath with gently agitation and place on ice until use.
6. Warm up the SQ1 at 37°C water bath

Material and Equipments supplied by User

Have the following reagents and supplies ready before starting procedure:

- Centrifuge with temperature control
- Table top centrifuge capable at least 4,000 x g
- 15 ml Nuclease-Free centrifuge tubes
- 0.45 μ m filter
- Absolute ethanol
- 70% ethanol
- 100% Isopropanol
- Ice Bath

Procedure

1. **Harvest and lyse the cell:**
 - A) **Cells Grown in Suspension**

Pellet cells by centrifugation in a 15 ml centrifuge tube. Add 3 ml SQ1 and lyse the cells by repetitive pipetting. Alternately, shake the plate on a orbital shaker at maximum speed for 1 minute to lysis the cell. Use 3 mL of the SQ1 per 2- 4 x 10⁷ of cultured cells. .

B) Cells Grown in Monolayer

Lyse cells directly in a culture dish by adding 3 mL of SQ1 per 2 - 4 x 10⁷ of cultured cells directly into the flask, and lyse the cell by passing the cell lysate several times through a blue pipette tip. Alternately, shake the plate on a orbital shaker at maximum speed for 1 minute to lysis the cells. Transfer the cell lysate into a 15 ml tube.

2. Homogenize the samples:

Cultured cells can be effectively homogenized by pipetting up and down or vortexing after addition of Cell lysis buffer.

3. Place the tube containing the homogenates on the bench at room temperature for 2 minutes.
4. Add 1 ml of SQ2 (PPR) to each sample, mix by vortexing at maximum speed for 30 seconds.
5. Incubate sample in an ice bath for 10 minutes.
6. Centrifuge at maximum speed (\geq 4,000 x g) at 4°C for 10 minutes.
7. Transfer cleared supernatant into new 15 ml centrifuge tube for RNA/DNA isolation. Remove any liquid from the original tube containing the protein precipitate by inverting the tube on an absorbent paper. Keep the tube with the protein pellet for protein isolation starting from 26. For Total Nucleic acid isolation, proceed step 8-12. For RNA and DNA isolation, proceed step 13-25.

Total Nucleic acid Isolation

8. Add equal volume (4ml) of isopropanol. Mix thoroughly by vortexing for 30 seconds. Incubate the tube at room temperature for 5 minutes.
9. Centrifuge at maximum speed (\geq 4,000 x g) at 4°C for 10 minutes to precipitate Nucleic acid. Discard the supernatant.
10. Wash the nucleic acid pellet by adding 4 ml of 70% ethanol into the tube. Vortex the tube for 30 seconds .

11. Centrifuge at maximum speed ($\geq 4,000 \times g$) at 4°C for 5 minutes. Aspirate the supernatant and air dry the pellet by inverting the tube on a absorbent paper for 5-10 minutes.
12. Add 200 μl of nuclease free water into the tube to dissolve the Nucleic acid pellet. Vortex the tube for 30 seconds. Incubate the tube on ice or 4°C for 30-45 minutes to completely re-hydrate the nucleic acid.

For Total RNA Isolation

13. Add 2 ml Buffer SQ3 ($\frac{1}{2}$ volume of supernatant) into the cleared supernatant from step 7, vortex to mix well.
14. Add equal volume (6 ml) of isopropanol. Mix thoroughly by vortexing. Incubate the tube at room temperature for 5 minutes.
15. Centrifuge at maximum speed ($\geq 4,000 \times g$) at 4°C for 10 minutes to precipitate RNA. A bi-phase will be formed in the supernatant after the centrifugation.
16. Transfer entire supernatant to a new 15 ml tube and keep the tube for DNA isolation starting at step 20.
Note: It is critical to remove any liquid drop at this step to minimize the DNA contamination. For best result, briefly spin and collect drop from the tube, remove any drop by pipettor.
17. Wash the RNA pellet by adding 6 ml of 70% ethanol into the tube. Vortex the tube for 30 seconds .
18. Centrifuge at maximum speed ($\geq 4,000 \times g$) at 4°C for 5 minutes. Aspirate the supernatant and air dry the RNA pellet by inverting the tube on a absorbent paper for 5-10 minutes
19. Add 200 μl of DEPC water into the tube to dissolve the RNA pellet. Vortex the tube for 30 seconds. Incubate the tube on ice or 4°C for 30-45 minutes to completely re-hydrate the RNA.

DNA Isolation

20. Add $\frac{1}{6}$ volume of SQ4 Buffer (2 ml) and mix by vortexing.
21. Centrifuge at maximum speed ($\geq 4,000 \times g$) for 10 minutes at room temperature to collect precipitated DNA. Carefully discard supernatant.
22. Add 6 ml of 70% ethanol and mix thoroughly by vortexing for 30 seconds.

23. Centrifuge at maximum speed ($\geq 4,000 \times g$) for 5 minutes at room temperature Carefully discard supernatant.
24. Air dry the DNA pellet by inverting the tube on a absorbent paper for 5-10 minutes.
25. Add 200 μl EB Buffer to the tube and incubate at 65°C for 30 minutes to re-hydrate the DNA.

Protein Isolation

26. Using the centrifuge tube which contains the protein pellet from step 7.
27. Add 5ml of 95% ethanol and vortex the tube for 30 seconds.
28. Centrifuge at maximum speed for 2 minutes. Discard the supernatant. Air dry the protein pellet by inverting the tube on a absorbent paper for 5-10 minutes.
29. Add proper amount of protein loading dye (Laemmli Loading dye) to the tube to dissolve the protein pellet. If the protein will not be analyzed by SDS-PAGE, dissolve the protein in a buffer that compatible with downstream applications.

Protocol: DNA/ RNA/Protein Purification from Cultured Cells Using SQ DNA/RNA/Protein Cell Kit ($3-6 \times 10^7$ cells)

Before starting:

1. Fresh or flash-frozen cultured cells can be used in this protocol. Collect suspended cells and place on ice until use. Determine the cell number by using a hemacytometer or other cell counter.
2. Preheat the water bath to 65°C
3. water bath or heating block preset at 37°C
4. Pre-set the centrifuge at 4°C for RNA isolation.
5. Frozen cells should be thawed quickly using a 37°C water bath with gently agitation and place on ice until use.
6. Warm up the SQ1 at 37°C water bath

Material and Equipments supplied by User

- Centrifuge with temperature control
- Table top centrifuge capable at least $4,000 \times g$
- 50 ml Nuclease-Free centrifuge tubes
- Absolute ethanol

- 70% ethanol
- 100% Isopropanol
- Ice

Procedure

1. Harvest and lyse the cell:

A) Cells Grown in Suspension

Pellet cells by centrifugation in a 50 ml centrifuge tube. Add 9 ml SQ1 and lyse the cells by repetitive pipetting. Alternately, vortex the tube at maximum speed for 30 seconds to lysis the cell.

B) Cells Grown in Monolayer

Lyse cells directly in a culture dish by adding 9 mL of SQ1 per directly into the flask, and lyse the cell by passing the cell lysate several times through a blue pipette tip. Transfer the cell lysate into a 50 ml tube and vortex the tube at maximum speed for 1-2 minutes to lysis the cells.

2. Homogenize the samples:

Cultured cells can be effectively homogenized by pipetting up and down or vortexing after addition of Cell lysis buffer.

3. Place the tube containing the homogenates on the bench at room temperature for 2 minutes.
4. Add 3 ml of SQ2 (PPR) to each sample, mix by vortexing at maximum speed for 30 seconds.
5. Incubate sample in an ice bath for 10 minutes..
6. Centrifuge at maximum speed ($\geq 4000 \times g$) at 4°C for 10 minutes.
7. Transfer cleared supernatant into new 50 ml centrifuge tube for RNA/DNA isolation. Remove any liquid from the original tube containing the protein precipitate by inverting the tube on an absorbent paper. Keep the tube with the protein pellet for protein isolation starting from 26. For Total Nucleic acid isolation, proceed step 8-12. For RNA and DNA isolation, proceed step 13-25.

Total Nucleic acid Isolation

8. Add equal volume (12ml) of isopropanol. Mix thoroughly by vortexing for 30 seconds. Incubate the tube at room temperature for 5 minutes.

9. Centrifuge at maximum speed ($\geq 4,000 \times g$) at 4°C for 10 minutes to precipitate Nucleic acid. Discard the supernatant.
10. Wash the nucleic acid pellet by adding 12 ml of 70% ethanol into the tube. Vortex the tube for 30 seconds .
11. Centrifuge at maximum speed ($\geq 4,000 \times g$) at 4°C for 5 minutes. Aspirate the supernatant and air dry the pellet by inverting the tube on a absorbent paper for 5-10 minutes.
12. Add 500 μ l of nuclease free water into the tube to dissolve the Nucleic acid pellet. Vortex the tube for 30 seconds. Incubate the tube on ice or 4°C for 30-45 minutes to completely re-hydrate the nucleic acid.

For Total RNA Isolation

13. Add 6 ml Buffer SQ3 ($\frac{1}{2}$ volume of supernatant) into the cleared supernatant from step 7, vortex to mix well.
14. Add equal volume (18ml) of isopropanol. Mix thoroughly by vortexing. Incubate the tube at room temperature for 5 minutes.
15. Centrifuge at maximum speed ($\geq 4,000 \times g$) at 4°C for 10 minutes to precipitate RNA. A bi-phase will be formed in the supernatant after the centrifugation.
16. Transfer entire supernatant to a new 50 ml tube and keep the tube for DNA isolation starting at step 20.
Note: It is critical to remove any liquid drop at this step to minimize the DNA contamination. For best result, briefly spin and collect drop from the tube, remove any drop by pipettor.
17. Wash the RNA pellet by adding 18 ml of 70% ethanol into the tube. Vortex the tube for 30 seconds .
18. Centrifuge at maximum speed ($\geq 4,000 \times g$) at 4°C for 5 minutes. Aspirate the supernatant and air dry the RNA pellet by inverting the tube on a absorbent paper for 5-10 minutes
19. Add 500 μ l of DEPC water into the tube to dissolve the RNA pellet. Vortex the tube for 30 seconds. Incubate the tube on ice or 4°C for 30-45 minutes to completely re-hydrate the RNA.

Protocol: DNA/ RNA/Protein Purification from Solid Tissue using SQ DNA/RNA/Protein Tissue Kit

DNA Isolation

20. Add 1/6 volume of SQ4 Buffer (6ml) and mix by vortexing.
21. Centrifuge at maximum speed ($\geq 4,000 \times g$) for 10 minutes at room temperature to collect precipitated DNA. Carefully discard supernatant.
22. Add 18 ml of 70% ethanol and mix thoroughly by vortexing for 30 seconds.
23. Centrifuge at maximum speed ($\geq 4,000 \times g$) for 5 minutes at room temperature Carefully discard supernatant.
24. Air dry the DNA pellet by inverting the tube on a absorbent paper for 5-10 minutes.
25. Add 500 μ l EB Buffer to the tube and incubate at 65C for 30 minutes to re-hydrate the DNA.

Protein Isolation

26. Using the centrifuge tube which contains the protein pellet from step 7.
27. Add 10ml of 95% ethanol and vortex the tube for 30 seconds.
28. Centrifuge at maximum speed for 2 minutes. Discard the supernatant. Air dry the protein pellet by inverting the tube on a absorbent paper for 5-10 minutes.
29. Add proper amount of protein loading dye (Laemmli Loading dye) to the tube to dissolve the protein pellet. If the protein will not be analyzed by SDS-PAGE, dissolve the protein in a buffer that compatible with downstream applications.

Starting Material

Please use table on page 5-6 as guideline to determine the volume of the reagent to be used according to different amount of starting materials. The following protocol is for purification of DNA, RNA and Protein from 10-20mg or 200 mg solid tissue.

1. Homogenize the samples:

A) Homogenize the sample using a conventional rotor-stator homogenizer until the sample is uniformly homogeneous. Go to step 3.

Rotor-stator homogenizers can effectively simultaneously disrupt and homogenize most samples. The process usually takes less than a minute depending on the tissue. Many Rotor-stator homogenizers operate with differently sized probes or generators that allow processing of small volumes in microfuge tubes.

B) Homogenize using Bead Mill. Add one stainless steel bead to each well of the deep well plate contains tissue and SQ1. Homogenize the sample by vigorously shaking or vortexing.

Note: By using bead milling, cells and tissues can be disrupted and homogenized by rapid agitation in the present of beads and lysis buffer. The optimal beads to use for RNA isolation are 0.5mm glass beads for yeast and unicellular cells, and 4-8 mm beads for animal tissue samples.

2. Add 10-20mg or 100-200 mg frozen ground tissue into a 1.5 ml or 15 ml centrifuge tube contains 300 μ l or 3 ml SQ1.
3. Homogenize the sample quickly by vortexing the tube for 30-60 seconds at maximum speed. Place the tube containing the homogenates on the bench at room temperature for 2 minutes.
4. Add 100 μ l or 1 ml of SQ2 (PPR) to each sample, mix by vortexing at maximum speed for 30 seconds.
5. Incubate on ice bath for 10 minutes.
6. Centrifuge at 13000 $\times g$ or 4000 $\times g$ at 4°C for 10 minutes.
7. Transfer cleared supernatant into new 1.5 or 15 ml centrifuge tube for RNA/DNA isolation. Remove any liquid from the original tube containing the protein precipitate

by inverting the tube on a absorbent paper. Keep the tube with the protein pellet for protein isolation starting from step 26. For Total Nucleic acid isolation, proceed step 8-12. For RNA and DNA isolation, proceed step 13-25.

Total Nucleic acid Isolation

8. Add equal volume (~400ul or 4 ml) of isopropanol. Mix thoroughly by vortexing for 30 seconds. Incubate the tube at room temperature for 5 minutes.
9. Centrifuge at maximum speed ($\geq 13,000 \times g$ or $4,000 \times g$) at 4°C for 10 minutes to precipitate Nucleic acid. Discard the supernatant.
10. Wash the nucleic acid pellet by adding 400ul or 4 ml 70% ethanol into the tube. Vortex the tube for 30 seconds .
11. Centrifuge at maximum speed ($\geq 13,000 \times g$ or $4,000 \times g$) at 4°C for 5 minutes. Aspirate the supernatant and air dry the pellet by inverting the tube on a absorbent paper for 5-10 minutes.
12. Add 500 μl of nuclease free water into the tube to dissolve the Nucleic acid pellet. Vortex the tube for 30 seconds. Incubate the tube on ice or 4°C for 30-45 minutes to completely re-hydrate the nucleic acid.

For Total RNA Isolation

13. Add 200ul or 2 ml of Buffer SQ3 ($\frac{1}{2}$ volume of supernatant) into the cleared supernatant from step 7, vortex to mix well.
14. Add equal volume (600ul or 6ml) of isopropanol. Mix thoroughly by vortexing. Incubate the tube at room temperature for 5 minutes.
15. Centrifuge at maximum speed ($\geq 13,000 \times g$ or $4,000 \times g$) at 4°C for 10 minutes to precipitate RNA. A bi-phase will be formed in the supernatant after the centrifugation.
16. Transfer entire supernatant to a new 1.5 or 15 ml tube and keep the tube for DNA isolation starting at step 20.
Note: It is critical to remove any liquid drop at this step to minimize the DNA contamination. For best result, briefly spin and collect drop from the tube, remove any drop by pipettor.
17. Wash the RNA pellet by adding 600ul or 6 ml of 70% ethanol into the tube. Vortex the

tube for 30 seconds .

18. Centrifuge at maximum speed ($\geq 13,000 \times g$ or $4,000 \times g$) at 4°C for 5 minutes. Aspirate the supernatant and air dry the RNA pellet by inverting the tube on a absorbent paper for 5-10 minutes
19. Add 100 or 500ul of DEPC water into the tube to dissolve the RNA pellet. Vortex the tube for 30 seconds. Incubate the tube on ice or 4°C for 30-45 minutes to completely re-hydrate the RNA.

DNA Isolation

20. Add 1/6 volume of SQ4 Buffer (200ul or 2ml) and mix by vortexing.
21. Centrifuge at maximum speed ($\geq 13,000 \times g$ or $4,000 \times g$) for 10 minutes at room temperature to collect precipitated DNA. Carefully discard supernatant.
22. Add 600ul or 6 ml of 70% ethanol and mix thoroughly by vortexing for 30 seconds.
23. Centrifuge at maximum speed ($\geq 13,000 \times g$ or $4,000 \times g$) for 5 minutes at room temperature Carefully discard supernatant.
24. Air dry the DNA pellet by inverting the tube on a absorbent paper for 5-10 minutes.
25. Add 100ul or 500 μl EB Buffer to the tube and incubate at 65°C for 30 minutes to re-hydrate the DNA.

Protein Isolation

26. Using the centrifuge tube which contains the protein pellet from step 7.
27. Add 10ml of 95% ethanol and vortex the tube for 30 seconds.
28. Centrifuge at maximum speed for 2 minutes. Discard the supernatant. Air dry the protein pellet by inverting the tube on a absorbent paper for 5-10 minutes.
29. Add proper amount of protein loading dye (Laemmli Loading dye) to the tube to dissolve the protein pellet. If the protein will not be analyzed by SDS-PAGE, dissolve the protein in a buffer that compatible with downstream applications.

Trouble Shooting

Problem	Likely Cause	Suggestions
Low nucleic acid yield	Incomplete disruption and homogenization of	See cell lysis and homogenization instruction. If the lysate is too viscous, a mechanic homogenizer may be needed.
	RNA or DNA degraded during sample storage	Make sure the sample is properly stored and make sure the samples are processed immediately after collection or removal from storage.
	Loss of DNA or RNA pellet during operation	Be careful not to lose the DNA or RNA pellet during the operation
	Ethanol carryover	Make sure the magnetic beads are completely removed before DNA or RNA elution
Carryover of the magnetic beads in the elution	Carryover from the magnetic beads in the eluted RNA or DNA will not effect downstream applications.	To remove the carryover magnetic beads from eluted RNA, simply magnetize the magnetic beads and carefully transfer to a new tube or plate.