Contents

Revised January 2008

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 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 guandine 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 Protein Precipitation Reagent (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 DPR Buffer to precipitate DNA. RNA and DNA pellet are washed with 70% ethanol and dissolved with wateror 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 the 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 Cell Kit	2 x 10^7	2 x 10^8	8 x 10^8	6.7 x 10^9
Product No.	**R8043-00**	**R8043-01**	**R8043-03**	**R8043-04**
Number of cells processed per kit	2 x 10^7	2 x 10^8	8 x 10^8	6.7 x 10^9
Cell Lysis Buffer	3 ml	30 ml	120 ml	1000 ml
Protein Precipitation Reagent	1 ml	10 ml	40 ml	330 ml
DPR Buffer	2 ml	20 ml	80 ml	700 ml
DPD Buffer	2 ml	10 ml	40 ml	350 ml
DEPC Water	1.0 ml	5 ml	20 ml	150 ml
EB Buffer	1.0 ml	5 ml	20 ml	150 ml
User Manual	1	1	1	1

SQ DNA/RNA/Protein Tissue Kit

SQ DNA/RNA/Protein Tissue Kit	1 g	4.5 g	18 g	36 g	72 g
Product No.	**R8042-00**	**R8042-01**	**R8042-02**	**R8042-03**	**R8042-04**
Amount of tissue processed per kit	1 g	4.5 g	18 g	36 g	72 g
Cell Lysis Buffer	20 ml	90 ml	2x 160 ml	3 x 250 ml	6 x 250 ml
Protein Precipitation Reagent	8 ml	30 ml	120 ml	250 ml	2 x 250 ml
DPR Buffer	15 ml	60 ml	240 ml	2 x 250 ml	4 x 250 ml
DPD Buffer	15 ml	60 ml	240 ml	2 x 250 ml	4 x 250 ml
DEPC Water	5.0 ml	25 ml	100 ml	200 ml	2 x 200ml
EB Buffer	5.0 ml	25 ml	100 ml	200 ml	2 x 200 ml
User Manual	1	1	1	1	1

SQ DNA/RNA/Protein Blood Kit

SQ DNA/RNA/Protein Blood Kit	5 ml	50 ml	120 ml	240 ml
Product No.	**R8044-00**	**R8044-01**	**R8044-03**	**R8044-04**
Volume of blood processed per kit	5 ml	50 ml	120ml	240 ml
Cell Lysis Buffer	3 ml	30 ml	70 ml	140 ml
10 x ERL Lysis Buffer	5 ml	50 ml	120ml	240 ml
Protein Precipitation Reagent	1 ml	10 ml	25 ml	50 ml
DPR Buffer	2 ml	20 ml	50 ml	100 ml
DPD Buffer	2 ml	20 ml	50 ml	100 ml
DEPC Water	0.5 ml	25 ml	100 ml	200 ml
EB Buffer	0.5 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
Cell Lysis Buffer	75 µl	200 µl	300 µl	3 ml
Protein Precipitation Reagent	25 µl	67 µl	100 µl	1 ml
DPR Buffer	50 µl	133 µl	200 µl	2 ml
DPD Buffer	50 µl	133 µl	200 µl	2 ml

SQ DNA/RNA/Protein Blood Kit				
Volume of Blood	**100 µl**	**500 µl**	**1 ml**	**3 ml**
Tube size	0.6 ml	1.5 ml	2 ml	15 ml
ERL Buffer	300µl	1.5 ml	3 ml	9 ml
Cell Lysis Buffer	75 µl	150 µl	300 µl	3 ml
Protein Precipitation Reagent	25 µl	50 µl	100 µl	1 ml
DPR Buffer	50 µl	100 µl	200 µl	2 ml
DPD Buffer	50 µl	100 µl	200 µl	2 ml

SQ DNA/RNA/Protein Tissue Kit				
Amount of Tissue	**1-3 mg**	**3-10 mg**	**10-20 mg**	**200 mg**
Tube size	0.6 ml	1.5 ml	2 ml	15 ml
Cell Lysis Buffer	75 µl	150 µl	300 µl	3 ml
Protein Precipitation Reagent	25 µl	50 µl	100 µl	1 ml
DPR Reagent	50 µl	100 µl	200 µl	2 ml

Typical Yield Using SQ DNA/RNA/Protein Kit

(1 x 10^6 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 10^6 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 Cell Lysis Buffer 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

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 Cell Lysis Buffer 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 10^6 of cultured cells.

   B) Cells Grown in Monolayer

   Lyse cells directly in a culture dish by adding 300 µL of Cell Lysis Buffer per 2 x 10^6 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 µL of Protein Precipitation Reagent (1/3 volume of Cells lysis Buffer) to each sample, mix throughly by votexing at maximum speed for 30 seconds.

5. Incubate on ice for 10 minutes.

6. Centrifuge at maximum speed (≥13,000 x g) at 4°C for 10 minutes.

7. Transfer cleared supernatant (~400 µL) into new 1.5 ml centrifuge tube for RNA/DNA isolation. Remove any liquid from the original tube contains protein precipitate by invert the plate on a absorbent paper. Keep the tube contains protein pellet for protein isolation start 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 100µl DPD and 400µl or 4 ml of isopropanol to the sample. Mix throughly by vortexing for 30-60 seconds. Incubate the tube at room temperature for 5 minutes.

9. Centrifuge at maximum speed (≥13,000 x g) at 4°C for 10 minutes to precipitate Nucleic acid. Discord the supernatant.

10. Wash the nucleic acid pellet by adding 400 µL of 70% ethanol into the tube. Vortex the tube for 30 seconds .

11. Centrifuge at maximum speed (≥13,000 x 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 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 200 µL Buffer DPR (½ volume of supernatant) into the cleared supernatant from step 7, vortex to mix well.

14. Add equal volume (600 µL) of isopropanol. Mix throughly by vortexing. Incubate the tube

at room temperature for 5 minutes.

15. Centrifuge at maximum speed (≥13,000 x 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 µL of 70% ethanol into the tube. Vortex the tube for 30 seconds .

18. Centrifuge at maximum speed (≥13000 x 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 50-100µl 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 DPD Buffer (200 µL l) and mix by vortexing.

21. Centrifuge at maximum speed (≥13,000 x g) for 5 minutes at room temperature to collect precipitated DNA. Carefully discard supernatant.

22. Add 600 µL of 70% ethanol and mix throughly by vortexing for 30 seconds.

23. Centrifuge at maximum speed (≥13,000 x 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 100µl EB Buffer to the tube and incubate at 65°C for 30 minutes to re-hydrate the DNA.

Protein Isolation

26. Take the tube contains 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 µL 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 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 Cell Lysis Buffer 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 µm filter
- 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 15 ml centrifuge tube. Add 3 ml Cell lysis Buffer 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 Cell Lysis Buffer per 2-4 x 10^7 of cultured cells. .

B) Cells Grown in Monolayer

Lyse cells directly in a culture dish by adding 3 mL of Cell Lysis Buffer per 2 - 4 x 10^7 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 Protein Precipitation Reagent (PPR) to each sample, mix by vortexing at maximum speed for 30 seconds.

5. Incubate on ice for 10 minutes.

6. Centrifuge at maximum speed (≥4,000 x g) at 4°C for 10 minutes.

7. Transfer cleared supernatant (~4ml) into new 15 ml centrifuge tube for RNA/DNA isolation. Remove any liquid from the original deep-well plate contains protein precipitate by invert the plate on a absorbent paper. Keep the tube contains protein pellet for protein isolation start from step 26**.** For Total Nucleic acid isolation, proceed step 8-12. For RNA and DNA isolation, proceed step 13-25.

For Total Nucleic acid Isolation

8. Add 1 ml Buffer DPD and 4ml of isopropanol. Mix throughly by vortexing for 30-60 seconds. Incubate the tube at room temperature for 5 minutes.

9. Centrifuge at maximum speed (≥4,000 x g) at 4°C for 10 minutes to precipitate Nucleic acid. Discord 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 (≥4,000 x 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 DPR (½ volume of supernatant) into the cleared supernatant from step 7, vortex to mix well.

14. Add equal volume (6 ml) of isopropanol. Mix throughly by vortexing. Incubate the tube at room temperature for 5 minutes.

15. Centrifuge at maximum speed (≥4,000 x 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. Votex the tube for 30 seconds .

18. Centrifuge at maximum speed (≥4,000 x 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 200µl 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 DPD Buffer (2 ml) and mix by vortexing.

21. Centrifuge at maximum speed (≥4,000 x g) for 10 minutes at room temperature to collect precipitated DNA. Carefully discard supernatant.

22. Add 6 ml of 70% ethanol and mix throughly by vortexing for 30 seconds.

23. Centrifuge at maximum speed (≥4,000xg) 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 65C for 30 minutes to re-hydrate the DNA.

Protein Isolation

26. Take the tube contains 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 x 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 4C 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 Cell Lysis Buffer at 37°C water bath

Material and Equipments supplied by User

- Centrifuge with temperature control
- Table top centrifuge capable at least 4,000 x 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 Cell lysis Buffer 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 Cell Lysis Buffer 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 Protein Precipitation Reagent (PPR) to each sample, mix by vortexing at maximum speed for 30 seconds.

5. Incubate on ice for 10 minutes.

6. Centrifuge at maximum speed (≥4000 x g) at 4°C for 10 minutes.

7. Transfer cleared supernatant (~12 ml) into new 50ml centrifuge tube for RNA/DNA isolation. Remove any liquid from the original deep-well plate contains protein precipitate by invert the plate on a absorbent paper. Keep the tube contains protein pellet for protein isolation start 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 3 ml Buffer DPD to the supernatant, then add equal volume (12ml) of isopropanol. Mix throughly by vortexing for 30-60 seconds. Incubate the tube at room temperature for 5 minutes.

9. Centrifuge at maximum speed (≥4,000 x g) at 4°C for 10 minutes to precipitate Nucleic acid. Discord the supernatant.

10. Wash the nucleic acid pellet by adding 12 ml of 70% ethanol into the tube. Votex the tube for 30 seconds .

11. Centrifuge at maximum speed (≥4,000 x 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 6 ml Buffer DPR (½ volume of supernatant) into the cleared supernatant from step 7, vortex to mix well.

14. Add equal volume (18ml) of isopropanol. Mix throughly by vortexing. Incubate the tube at room temperature for 5 minutes.

15. Centrifuge at maximum speed (≥4,000 x 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. Votex the tube for 30 seconds .

18. Centrifuge at maximum speed (≥4,000 x 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 500µl 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 DPD Buffer (6ml) and mix by vortexing.

21. Centrifuge at maximum speed (≥4,000 x g) for 10 minutes at room temperature to collect precipitated DNA. Carefully discard supernatant.

22. Add 18 ml of 70% ethanol and mix throughly by vortexing for 30 seconds.

23. Centrifuge at maximum speed (≥4,000 x 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 500µl EB Buffer to the tube and incubate at 65°C for 30 minutes to re-hydrate the DNA.

Protein Isolation

26. Take the tube contains 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.

Protocol: DNA/ RNA/Protein Purification from whole blood or bone marrow using SQ DNA/RNA/Protein Blood Kit

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 Cell Lysis Buffer 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 15 ml Nuclease-Free centrifuge tubes
- 0.45 µm filter unit
- Absolute ethanol
- 70% ethanol
- 100% Isopropanol
- Ice

Starting Material

Please use table on page 5 as guideline to determine the volume of the reagent to be used on different starting volume of samples. The following protocol is for purification of DNA, RNA and Protein from 300µ or 3ml whole blood or bone marrow.

Procedure

1. Dispense 900µl or 15 ml of ERL into a 1.5 ml microcentrifuge or 15 ml centrifuge tube.

2. Add 300µl or 3ml whole blood or bone marrow and mix throughly by inverting 10-15 times.

3. Incubate 5 minutes on ice. Mix the sample 1-2 time by inverting tube 2 times during the incubation.

4. Centrifuge at 10,000 x g for 40 seconds or 2,000 x g for 5 minutes to pellet the white blood cells.

5. Carefully discard the supernatant by pipetting or pouring, leave approximately 10µl or 200 µl of residue liquid and white blood cell pellet. Vortex to resuspend the pellet.

6. Add 300 µl or 3 ml Cell Lysis Buffer and mix throughly by vortexing the tube for 30 seconds or pipetting up and down 20-30 times. Place the tube containing the homogenates on the bench at room temperature for 2 minutes.

7. Add 100ul or 1 ml of Protein Precipitation Reagent (PPR) to each sample, mix by vortexing at maximum speed for 30 seconds. Incubate on ice for 10 minutes.

8. Centrifuge at maximum speed (≥13,000 x g or ≥4,000 x g) at 4°C for 10 minutes.

9. Transfer cleared supernatant into new 1.5 or 15ml centrifuge tube for RNA/DNA isolation. Remove any liquid from the original deep-well plate contains protein precipitate by invert the plate on a absorbent paper. Keep the tube contains protein pellet for protein isolation start from step 28**.** For Total Nucleic acid isolation, proceed step10 -14. For RNA and DNA isolation, proceed step 15-27.

Total Nucleic acid Isolation

10. Add 100µl or 1 ml Buffer DPD to the supernatant, then add 400µl or 4 ml of isopropanol to the sample. Mix throughly by vortexing for 30-60 seconds. Incubate the tube at room temperature for 5 minutes.

11. Centrifuge at maximum speed (≥13000 x g or ≥4,000 x g) at 4°C for 10 minutes to precipitate Nucleic acid. Discord the supernatant.

12. Wash the nucleic acid pellet by adding 1 ml or 4 ml of 70% ethanol into the tube. Votex the tube for 30 seconds.

13. Centrifuge at maximum speed (≥13000 x g or ≥4,000 x 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.

14. Add 50-100 µl or 100-200 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

15. Add 200ul or 2 ml Buffer DPR (½ volume of supernatant) into the cleared supernatant from step 7, vortex to mix well.

16. Add equal volume (600 µl or 6 ml) of isopropanol. Mix throughly by vortexing. Incubate the tube at room temperature for 5 minutes.

17. Centrifuge at maximum speed (≥13000 x g or ≥4,000 x g) at 4°C for 10 minutes to precipitate RNA. A bi-phase will be formed in the supernatant after the centrifugation.

18. Transfer entire supernatant to a new 1.5 ml or 15 ml tube and keep the tube for DNA isolation starting at step 22.
    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.

19. Wash the RNA pellet by adding 1 ml or 4 ml of 70% ethanol into the tube. Votex the tube for 30 seconds .

20. Centrifuge at maximum speed (≥13000 x g or ≥4,000 x 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

21. Add 50-100µl or 100-200 ul 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

22. Add 1/6 volume of DPD Buffer (200ul or 2 ml) and mix by vortexing.

23. Centrifuge at maximum speed (≥13000 x g or ≥4,000 x g) for 10 minutes at room temperature to collect precipitated DNA. Carefully discard supernatant.

24. Add 1 ml or 4 ml of 70% ethanol and mix throughly by vortexing for 30 seconds.

25. Centrifuge at maximum speed (≥13000 x g or ≥4,000 x g) for 5 minutes at room temperature Carefully discard supernatant.

26. Air dry the DNA pellet by inverting the tube on a absorbent paper for 5-10 minutes.

27. Add 100µl or 200ul EB Buffer to the tube and incubate at 65°C for 30 minutes to re-hydrate the DNA.

Protein Isolation

28. Take the tube contains protein pellet from step 7.

29. Add 1ml of 95% ethanol and vortex the tube for 30 seconds.

30. 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.

31. Add 100-200µ 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 Solid Tissue using SQ DNA/RNA/Protein Tissue Kit

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 Cell Lysis Buffer. 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 10-15mg or 100-150mg frozen ground tissue into a 1.5 ml or 15 ml centrifuge tube contains 300µl or 3 ml Cell Lysis Buffer.

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 100µl or 1 ml of Protein Precipitation Reagent (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 x g or 4000 x g at 4°C for 10 minutes.

7. Transfer cleared supernatant (~400ul or 4ml) into new 1.5 or 15ml centrifuge tube for RNA/DNA isolation. Remove any liquid from the original deep-well plate contains protein precipitate by invert the plate on a absorbent paper. Keep the tube contains protein pellet for protein isolation start 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 100µl or 1 ml Buffer DPD to the supernatant, then add 400µl or 4 ml of isopropanol to the sample. Mix throughly by vortexing for 30-60 seconds. Incubate the tube at room temperature for 5 minutes.

9. Centrifuge at maximum speed (≥13,000 x g or 4,000 x g) at 4°C for 10 minutes to precipitate Nucleic acid. Discord the supernatant.

10. Wash the nucleic acid pellet by adding 400u or 4 ml 70% ethanol into the tube. Votex the tube for 30 seconds .

11. Centrifuge at maximum speed (≥13,000 x g or 4,000 x 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 DPR (½ volume of supernatant) into the cleared supernatant from step 7, vortex to mix well.

14. Add equal volume (600ul or 6ml) of isopropanol. Mix throughly by vortexing. Incubate the tube at room temperature for 5 minutes.

15. Centrifuge at maximum speed (≥13,000 x g or 4,000 x 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 (≥13,000 x g or 4,000 x 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 DPD Buffer (200ul or 2ml) and mix by vortexing.

21. Centrifuge at maximum speed (≥13,000 x g or 4,000 x 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 throughly by vortexing for 30 seconds.

23. Centrifuge at maximum speed (≥13,000 x g or 4,000 x 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 500µl EB Buffer to the tube and incubate at 65°C for 30 minutes to re-hydrate the DNA.

Protein Isolation

26. Take the tube contains 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.