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## Introduction

The E.Z.N.A.® SQ Blood DNA Kit is designed for isolating high molecular weight genomic DNA from fresh, frozen or anticoagulated whole blood. The method can also be used for preparation of genomic DNA from buffy coat, bone marrow or cultured cells. The kit allows single or multiple, simultaneous processing of samples in under 90 minutes. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation are eliminated. DNA purified using the E.Z.N.A.® SQ Blood DNA method is ready for applications such as PCR*, Southern blotting, and restriction digestion.

The E.Z.N.A.® SQ Blood DNA Kit uses a highly efficient solution based system to provide a convenient, fast, reliable and non-toxic method to isolate high molecular weight genomic DNA from whole blood or buffy coat. Red blood cells are first lysed with ERL buffer, followed by lysis of the white blood cells and their nuclei in the WTL Buffer. Cellular proteins are removed by precipitation and high molecular weight genomic DNA will remain in solution. High quality genomic DNA is then purified by isopropanol precipitation.

## Storage and Stability

All components of the E.Z.N.A.® SQ Blood DNA Kit should be stored at 22°C-25°C. Under cool ambient conditions, a precipitate may form in the Buffer WTL. In case of such an event, heat the bottle at 55°C to dissolve.

**Expiration Date:** All E.Z.N.A.® SQ Blood DNA Kit components are guaranteed for at least 24 months from the date of purchase when stored at 22-25°C

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

## Kit Contents

| Product | D5032-00 | D5032-01 | D5032-02 | D5032-03 |
|---|---|---|---|---|
| Total Blood Volume | 10 ml | 50 ml | 150 ml | 300 ml |
| ERL Buffer (10 x) | 5 ml | 25 ml | 80 ml | 160 ml |
| Buffer WTL | 10 ml | 50 ml | 150 ml | 2 x 150 ml |
| Buffer PCP | 4 ml | 20 ml | 60 ml | 120 ml |
| Buffer EB | 5 ml | 20 ml | 60 ml | 125 ml |
| RNase A | 50 µl | 250 µl | 750 µl | 1.5 ml |
| User Manual | 1 | 1 | 1 | 1 |

## DNA Yields From Various Starting Materials

| Species and Material | Amount of Starting material | Typical Yield |
|---|---|---|
| **Human Whole Blood** (Yield varies depending on the quantity of white blood cells present) | 100 µl | 1 - 5 µg |
| | 200 µl | 3 - 10 µg |
| | 300 µl | 5 - 15 µg |
| **Mouse Whole Blood** | 100 µl | 0.5 - 1.0 µg |
| | 200 µl | 2 - 5 µg |
| | 300 µl | 4 - 7 µg |
| **Cultured Cells** | 2 x $10^6$ cells | 10 - 15 µg |

## Before Starting

| **IMPORTANT** | ERL Buffer Concentrate must be diluted with $ddH_2O$ as follows before use: | |
|---|---|---|
| | **D5302-00** | Add 45 ml $ddH_2O$ to a new bottle |
| | **D5032-01** | Add 225 ml $ddH_2O$ to a new bottle |
| | **D5032-02** | Add 720 ml $ddH_2O$ to a new bottle |
| | **D5032-03** | Add 1440 ml $ddH_2O$ to a new bottle |



## A. SQ Blood DNA Mini Protocol for 100-400 µl whole blood

**Materials to be supplied by user**

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 1.5 ml microcentrifuge tubes for 100-200 µl Blood
- Nuclease-free 2.0 ml microcentrifuge tubes for 300-400 µl Blood
- Water Bath preset at 37°C
- Isopropanol
- 70% ethanol
- Using different volume of Solution according blood volume as following:

| Blood Volume | 100 µl | 200 µl | 300 µl | 400 µl |
|---|---|---|---|---|
| Need tubes | 1.5 ml | 1.5 ml | 1.5 ml | 2.0 ml |
| Buffer ERL(1x) | 300 µl | 600 µl | 900 µl | 1.2 ml |
| Buffer WTL | 100 µl | 200 µl | 300 µl | 400 µl |
| Buffer PCP | 33 µl | 67 µl | 100 µl | 133 µl |
| RNase A | 0.5 µl | 1 µl | 1.5 µl | 2.0 µl |
| Isopropanol | 100 µl | 200 µl | 300µl | 400 µl |
| 70% ethanol | 100 µl | 200 µl | 300 µl | 400 µl |
| Buffer EB | 35 µl | 67 µl | 100 µl | 133 µl |

1. Add one volume of whole blood (or bone marrow) to a nuclease-free 1.5 or 2 ml microcentrifuge tube containing 3 volume of ERL Buffer. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during the incubation.

   **NOTE:** ERL Buffer is supplied as a 10x concentrate and must be diluted with $ddH_2O$ according to bottle label or Page 3 above before use.

2. Centrifuge at 14,000 x g for 30 seconds at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 0.1 volume of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.

   **Note:** If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

3. Vortex the tube vigorously until the white blood cells are completely resuspended.

4. Add one volume of Buffer WTL to the tube containing the resuspended cells. Pipet up and down or vortex at maxi speed for 30 seconds to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

5. **(Optional)** Add correct volume of RNase A (see page 4) solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for 10 minutes.

6. Cool the sample to room temperature. Add 1/3 volume of PCP Buffer to the cell lysate. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate on ice for 5 minutes.

7. Centrifuge at max speed (>14,000 x g) for 3 minutes at 4°C. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, incubate the tube on ice for 5 minutes and repeat Step 7.

8. Transfer the supernatant to a new nuclease-free 1.5 ml centrifuge tube containing one volume of 100% isopropanol. Gently mix the solution by inverting the tube 30-40 times.

9. Centrifuge at 14,000 x g for 2 minute at room temperature. DNA will be visible as a small white pellet.

10. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add one volume of 70% ethanol and invert the tube a few times to wash the DNA pellet.

11. Centrifuge at 14,000 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.**

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

13. Add 1/3 volume of Buffer EB and vortex for 1 minute to mix.

14. Incubate sample at 65°C for 10 min. Some samples may need to incubate at 65°C for 1 hour to rehydrate DNA. Store DNA at 2-8°C. For long-term storage, store at -20°C.



## B. SQ Blood DNA Midi Protocol for 500µl-3ml whole blood

**Materials to be supplied by user**

- Centrifuge capable of 2,000 x g
- Nuclease-free 15 ml microcentrifuge tubes
- Water baths preset at 37°C
- Paper towels
- Isopropanol (100%)
- 70% ethanol
- Using different volume of Solution according blood volume as following:

| Blood Volume | 500 µl | 1 ml | 2 ml | 3 ml |
|---|---|---|---|---|
| Tubes | 15 ml | 15 ml | 15 ml | 15 ml |
| Buffer ERL (1 x ) | 1.5 ml | 3 ml | 6 ml | 9 ml |
| Buffer WTL | 500 µl | 1 ml | 2 ml | 3 ml |
| Buffer PCP | 167 µl | 333 µl | 667µl | 1 ml |
| RNase A | 2.5 µl | 5 µl | 10 µl | 15 µl |
| Isopropanol | 500 µl | 1 ml | 2 ml | 3 ml |
| 70% ethanol | 500 µl | 1 ml | 2 ml | 3 ml |
| Buffer EB | 167 µl | 333 µl | 667 µl | 1 ml |

1. Add one volume of whole blood (or bone marrow) to a nuclease-free 15 ml centrifuge tube containing 3 volume of Buffer ERL. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during incubation.

   **NOTE:** ERL Buffer is supplied as a 10x concentrate and must be diluted with $ddH_2O$ according to bottle label or Page 3 before use.

2. Centrifuge at 2,000 x g for 5 minutes at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 0.1 volume of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.
   **Note:** If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

3. Vortex the tube vigorously until the white blood cells are completely resuspended.

4. Add one volume of WTL Buffer to the tube containing the resuspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If cell clumps are

visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

5. **(Optional)** Add correct volume of RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for approximately 10 minutes.

6. Cool the sample to room temperature. Add1/ 3 Volume of Buffer PCP to the cell lysate. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing.

7. Centrifuge at 2,000 x g or higher for 5 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, incubate the tube on ice for 5 minutes, and then repeat Step 7.

8. Transfer the supernatant to a new nuclease-free 15 ml centrifuge tube containing one volume of 100% isopropanol. Do not transfer the protein pellet.

9. Gently mix the solution by inverting the tube 40-50 times.

10. Centrifuge at 2,000 x g for 3 minutes at room temperature. DNA will be visible as a small white pellet.

11. Pour out the supernatant and drain the tube briefly on an absorbent paper towel. Add one volume of 70% ethanol and invert the tube a few times to wash the DNA pellet.

12. Centrifuge at 2,000 x g for 5 minutes at room temperature. Carefully pour out the ethanol. **Pellet may be very loose at this point, so pour slowly and be careful not to pour out the pellet.**

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

14. Add 0.1 volume of Buffer EB and vortex for 1 minute to mix.

15. Incubate sample at 65°C for 1 hour to rehydrate DNA. Gently shake the tube several times during incubation to disperse DNA. Some samples may need to incubate at 65°C overnight to rehydrate DNA. Store DNA at 2-8°C or -20°C.



## C. SQ Blood DNA Maxi Protocol for 4-10 ml whole blood

**Materials to be supplied by user**

- Centrifuge capable of 2,000 x g
- Nuclease-free 50 ml microcentrifuge tubes
- Water baths preset at 37°C
- Paper towels
- Isopropanol (100%)
- 70% ethanol
- Using different volume of Solution according blood volume as following:

| Blood Volume | 4 ml | 5 ml | 8 ml | 10 ml |
|---|---|---|---|---|
| Tubes | 50 ml | 50 ml | 50 ml | 50 ml |
| Buffer ERL (1 x ) | 12 ml | 15 ml | 24 ml | 30 ml |
| Buffer WTL | 4 ml | 5 ml | 8 ml | 10 ml |
| Buffer PCP | 1.33 ml | 1.67 ml | 2.67 ml | 3.33 ml |
| RNase A | 20 µl | 25 µl | 40 µl | 50 µl |
| Isopropanol | 4 ml | 5 ml | 8 ml | 10 ml |
| 70% ethanol | 4 ml | 5 ml | 8 ml | 10 ml |
| Buffer EB | 1.33 ml | 1.67 ml | 2.67 µl | 3.33 ml |

1. Add one volume of whole blood (or bone marrow) to a nuclease-free 50 ml centrifuge tube containing 3 volume of Buffer ERL. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during incubation.

   **NOTE:** ERL Buffer is supplied as a 10x concentrate and must be diluted with $ddH_2O$ according to bottle label or Page 3 before use.

2. Centrifuge at 2,000 x g for 5 minutes at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 0.1 volume of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.

   **Note:** If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

3. Vortex the tube vigorously until the white blood cells are completely resuspended.

4. Add one volume of Buffer WTL to the tube containing the resuspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

5. **(Optional)** Add correct volume of RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for approximately 10 minutes.

6. Cool the sample to room temperature. Add 1/3 Volume of Buffer PCP to the cell lysate. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing.

7. Centrifuge at 2,000 x g or higher for 5 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, incubate the tube on ice for 5 minutes, and then repeat Step 7.

8. Transfer the supernatant to a new nuclease-free 15 ml centrifuge tube containing one volume of 100% isopropanol. Do not transfer the protein pellet.

9. Gently mix the solution by inverting the tube 40-50 times.

10. Centrifuge at 2,000 x g for 3 minutes at room temperature. DNA will be visible as a small white pellet.

11. Pour out the supernatant and drain the tube briefly on an absorbent paper towel. Add one volume of 70% ethanol and invert the tube a few times to wash the DNA pellet.

12. Centrifuge at 2,000 x g for 2 minutes at room temperature. Carefully pour out the ethanol. **Pellet may be very loose at this point, so pour slowly and be careful not to pour out the pellet.**

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

14. Add 0.1 volume of Buffer EB and vortex for 1 minute to mix.

15. Incubate sample at 65°C for 1 hour to rehydrate DNA. Gently shake tube several times during incubation to disperse DNA. Some samples may need to incubate at 65°C overnight to rehydrate DNA. Store DNA at 2-8°C or -20°C.



## D. SQ Blood DNA Mini Protocol for Buffy Coat (Prepared from 1-1.5 ml whole blood)

**Materials to be supplied by user**

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 5.0 ml microcentrifuge tubes
- Water Bath preset at 37°C
- Isopropanol
- 70% ethanol

The buffy coat fraction of whole blood is enriched with WBC, and usually gives at least 5-fold more DNA than the same volume of blood. To prepare buffy coat from fresh whole blood, simply centrifuge the sample at 3,000-4,000 x g for 10 min at room temperature. Three layers should be obtained, with plasma in the upper layer, leucocytes in the middle layer (buffy coat), and erythrocytes in bottom layer. Carefully aspirate the plasma, making sure not to disturb the layer of concentrated leukocytes. The buffy coat can be drawn off with a pipette and used directly in the E.Z.N.A.® SQ Blood DNA Protocol, or frozen at -70°C for storage.

1. Add 75-120 µl buffy coat preparation (prepared from 1.5 ml whole blood) to a nuclease-free 2 ml microcentrifuge tube containing 400µl ERL Buffer. Mix by inverting the tube a few times. Incubate 5 minutes at room temperature. Invert the tube several times during incubation.

2. Centrifuge at 14,000 x g for 30 seconds at room temperature. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 15µl of residue liquid in the tube. If the blood sample has been frozen, repeat Steps 1-2 until the pellet is white.

   **Note:** If some red blood cells or cell debris are still visible along with the white blood cell pellet, resuspend the white blood cell pellet and mix with 2 volumes ERL Buffer. Incubate 2 min at room temperature. Then pellet the white blood cells by repeating Step 2.

3. Vortex the tube vigorously until the white blood cells are completely resuspended. Add 1.3 ml WTL Buffer to the tube containing the resuspended cells. Pipet up and down to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.
4. **(Optional)** Add 5 µl RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for 5-10 minutes.

5. Cool the sample to room temperature. Add 433µl PCP Buffer to the cell lysate.

6. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

7. Centrifuge at max speed for 3 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat Step 7.

8. Transfer the supernatant to a new nuclease-free 2.0 ml centrifuge tube containing 1.3 ml of 100% isopropanol.

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

10. Centrifuge at 14,000 x g for 1 minute at room temperature. DNA will be visible as a small white pellet.

11. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 1.3 ml of 70% ethanol and invert the tube a few times to wash the DNA pellet.

12. Centrifuge at 14,000 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.**

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

14. Add 500 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minute to mix.

15. Incubate sample at 65°C for 10 min. Some samples may need to incubate at 65°C for 1 hour to rehydrate DNA.

16. Store DNA at 2-8ºC. For long-term storage, store at -20°C.

## E. SQ Blood Mini Protocol for Cultured Cells

**Materials to be supplied by user**

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 2.0 ml microcentrifuge tubes
- Water Bath preset at 37°C
- Isopropanol
- 70% ethanol

1. This protocol is designed for isolating genomic DNA from 0.5-1 million cultured cells. For larger or smaller amounts of starting cell numbers, please use protocol in the E.Z.N.A® SQ Tissue DNA Kit.

2. Harvest the cells and transfer them with salt balanced buffer (such as PBS) to a 2.0 ml microcentrifuge tube. For adherent cells, trypsinize the cells before harvesting.

3. Centrifuge at 14,000 x g for 10 seconds to pellet the cells. Remove the cells and leave behind about 10µl residue liquid.



4. Vortex the cells to resuspend the cells in the residue liquid. Make no cell clumps visible at this point.

5. Add 150µl of WTL Buffer to the resuspended cells and mix by pipetting. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

6. **(Optional)** Add 1 µl RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for 5-10 minutes.

7. Cool the sample to room temperature. Add 50µl PCP Buffer to the cell lysate. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate on ice for 5 minutes.

8. Centrifuge at max speed for 3 minutes at room temperature. The precipitated protein will form a tight pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat Step 8.

9. Transfer the supernatant to a new nuclease-free 2.0 ml centrifuge tube containing 150µl of 100% isopropanol. Gently mix the solution by inverting the tube 30-40 times.

10. Centrifuge at 14,000 x g for 1 minute at room temperature. DNA will be visible as a small white pellet.

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

12. Centrifuge at 14,000 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.**

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

14. Add 50 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.

15. Incubate sample at 65ºC for 10 min. Some samples may need to incubate at 65ºC for 1 hour to rehydrate DNA. Store DNA at 2-8ºC. For long-term storage, store at -20°C.

## F. SQ Blood Mini Protocol for 50ul Clotted Blood

**Materials to be supplied by user**

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 1.5 ml microcentrifuge tubes
- Water Bath preset at 37°C
- Proteinase K (20mg/ml)
- Isopropanol
- 70% ethanol

1. Transfer the 50µl blood include any liquid residual into a 1.5 ml centrifuge tube.
2. Add 550µl WTL Buffer and pipet up an down a few times to mix.
3. Add 3 µl Proteinase K solution (20mg/ml) and mix by inverting 20 times.
4. Incubate at 55°C for 1 hour to overnight until clots has dissolved.
5. Place the tube on ice for 1 minute.
6. Add 3 µl RNase A to the cell lysate and invert 10 time to mix throughly. Incubate the tube at 37°C for 5 minutes.
7. Place the tube on ice for 1 minute. Add 200 µl PCP buffer to the cell lysate. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.
8. Centrifuge at max speed for 3 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat this step.
9. Transfer the supernatant to a new nuclease-free 2.0 ml centrifuge tube containing 600µl of 100% isopropanol. If the DNA yield is expected to be lower than 2µg, add 2µl of glycogen (20mg/ml) per sample.
10. Gently mix the solution by inverting the tube 30-40 times. Centrifuge at 14,000 x g for 1 minute at room temperature. DNA will be visible as a small white pellet.
11. Pour of the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 600µl of 70% ethanol and invert the tube a few times to wash the DNA pellet.
12. Centrifuge at 14,000 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.**

13. Invert the tube on a clean adsorbent paper towel and air dry the pellet for 10-15 minutes.
14. Add 20 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minute to mix.
15. Incubate sample at 65°C for 10 min. Some samples may need to incubate at 65°C for 1 hour to rehydrate DNA.
16. Store DNA at 2-8°C. For long-term storage, store at -20°C.

## G. SQ Blood Mini Protocol for 1ml Clotted Blood

**Materials to be supplied by user**

- Microcentrifuge capable of 2,000 x g
- Nuclease-free 50 ml microcentrifuge tubes
- Water Bath preset at 37°C
- Proteinase K (20mg/ml)
- Isopropanol
- 70% ethanol

1. Transfer 1 ml clotted blood including any liquid residual into a 50 ml centrifuge tube.
2. Add 11 ml WTL Buffer and pipet up an down a few times to mix.
3. Add 50 µl Proteinase K solution (20mg/ml) and mix by inverting 20 times.
4. Incubate at 55°C for 3 hour to overnight until clots has dissolved.
5. Place the tube on ice for 1-2 minute.
6. Add 50 µl RNase A to the cell lysate and invert 10 time to mix throughly. Incubate the tube at 37°C for 5 minutes.
7. Place the tube on ice for 1-2 minute.
8. Add 4 ml PCP buffer to the cell lysate.
9. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 10 minutes.
10. Centrifuge at 2000 x g for 10 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat this step.
11. Transfer the supernatant to a new 50 ml centrifuge tube containing 12 ml of 100%

isopropanol. Add 20µl of glycogen (20mg/ml) per sample.

12. Gently mix the solution by inverting the tube 30-40 times. Centrifuge at 2000 x g for 5 minute at room temperature. DNA will be visible as a small white pellet.
13. Pour of the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 12ml of 70% ethanol and invert the tube a few times to wash the DNA pellet.
14. Centrifuge at 2000 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.**
15. Invert the tube on a clean adsorbent paper towel and air dry the pellet for 10-15 minutes.
16. Add 400 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minute to mix.
17. Incubate sample at 65°C for 1 hour. Some samples may need to incubate at room temperature for overnight to rehydrate DNA. Store DNA at 2-8°C. For long-term storage, store at -20°C.

## Troubleshooting Guide

| Problem | Possible Cause | Suggestions |
|---|---|---|
| Low DNA yield | Blood Sample contains too few white blood cells | Draw new blood samples |
| | Blood sample is too old. | Try to use fresh blood if possible. |
| | Incompletely resuspended white blood cell pellet before adding WTL buffer. | Vortex vigorously to completely resuspend white blood cell. |
| | DNA pellet was lost during isopropanol precipitation | Be very careful not to lose the DNA when removing isopropanol or ethanol during precipitation and wash steps. |



| Problem | Possible Cause | Suggestions |
|---|---|---|
| Low $A_{260}/A_{280}$ ratio | The sample was not cooled to room temperature before adding PCP buffer | Cool the sample to room temperature or chill on ice for at least 5 minutes before adding PCP buffer. |
| | Poor cell lysis due to incomplete mixing with Buffer WTL | Repeat the procedure, this time making sure to vortex the sample with Buffer WTL immediately and completely. |
| | Hemoglobin remains | Repeat the procedure, this time making sure enough volume of ERL is used and white blood cell pellet is white in color . |
| | PCP Buffer was not mixed with WTL buffer throughly. | Make sure that PCP buffer and cell lysate is mixed throughly. |
| No DNA | DNA pellet was lost during isopropanol precipitation | Be very careful not to lose the DNA when removing isopropanol or ethanol during precipitation and wash steps. |
| DNA Pellet is difficult to dissolve | DNA pellet was over dried | Rehydrate the DNA by incubating the DNA pellet with EB Buffer at 65°C for 1 hour and then leave the sample at room temperature or 4°C for overnight. |
| | DNA pellet was not mixed well during rehydration step. | Shake a few times during the rehydration step. |