E.Z.N.A.® SQ Blood DNA Kit II

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OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

The E.Z.N.A.® SQ Blood DNA Kit II is designed for isolating high molecular weight genomic DNA from fresh, frozen, and anti-coagulated whole blood. The method can also be used for preparation of genomic DNA from buffy coat, bone marrow or cultured cells. The procedure can be easily scaled up and down, allowing purification from different amount of starting material. The whole procedure can be performed in single tube so it can reduce the waste the chance for potential cross contamination. This 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 DNA Kit II method is ready for applications such as PCR, Southern blotting, and restriction digestion.

Overview

E.Z.N.A.® SQ Blood DNA Kit II 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. Plasma membranes are lysed with NL Buffer. Cell nuclei and mitochondria are pelleted by centrifugation. The pellet is resuspended and lysed in XL Buffer which contains chaotropic salt and proteinase. This step effectively removes most contaminate such as proteins. High-quality genomic DNA is then purified by isopropanol precipitation.

New in this Edition: This manual has been edited for content and redesigned to enhance user readability.

- Proteinase K is now supplied in a liquid form thereby eliminating the resuspension step prior to use.
- Proteinase K Solution can be stored at room temperature for 12 months.

Kit Contents

Product	D0714-05	D0714-50	D0714-250
Blood Volume Processed per Kit	5 mL	50 mL	250 mL
NL Buffer	14 mL	140 mL	700 mL
XL Buffer	3 mL	30 mL	150 mL
EB Buffer (hydration buffer)	5 mL	50 mL	250 mL
Proteinase K Solution	3 mg	6 mg	35 mg
User Manual	✓	✓	✓

Storage and Stability

All components of the E.Z.N.A.® SQ Blood DNA Kit II should be stored at room temperature. Proteinase K Solution can be stored at room temperature for 12 months. For long-term storage (>12 months), store Proteinase K Solution at 2-8°C. Under cool ambient conditions, a precipitate may form in the XL Buffer. If a precipitate is present, heat the bottle at 55°C to dissolve. All the components are guaranteed for at least 12 months from date of purchase.

Blood Storage and DNA Yield

Storage of Blood Samples

The E.Z.N.A.® SQ Blood DNA Kit II can use either fresh or frozen whole blood treated with EDTA, heparin, or citrate. Fresher blood yields better results. For short-term storage (for up to 2 weeks), it is recommended to collect blood in a vacutainer containing EDTA as the anticoagulant. For long-term storage, sample should be collected in a vacutainer containing EDTA as the anticoagulant and stored at -70°C.

DNA Yields From Various Starting Materials

Species and Material	Amount of Starting material	Typical Yield
Human Whole Blood Yield will vary depending on the quantity of white blood cells present	50 µL	0.3-0.6 µg
	100 µL	1-5 µg
	200 µL	3-10 µg
	300 µL	5-15 µg
	500 µL	7-23 µg
	600 µL	10-30 µg
Mouse Whole Blood	50 µL	0.2-0.6 µg
	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

- Prepare XL Buffer with Proteinase K Solution. Add 5 µL Proteinase K Solution per 500 µL XL Buffer for each 1 mL whole blood.

Blood Volume	Proteinase K Solution	XL Buffer
1 mL	5 µL	500 µL
5 mL	25 µL	2.5 mL
10 mL	50 µL	5 mL
50 mL	250 µL	25 mL
100 mL	500 µL	50 mL
250 mL	1.25 mL	125 mL

E.Z.N.A.® SQ Blood DNA Kit Protocol - 100-500 µL Whole Blood

Note: The buffer volumes indicated in the following protocol are for 200 µL whole blood samples. This procedures can be scaled up and down for use with FRESH or FROZEN blood samples 100 µL to 500 µL in volume by adjusting the buffer volumes up or down in proportion to the volume of sample used. (Except the EB Buffer volume for 100 µL blood). Frozen blood should be thawed quickly in a 37°C water bath with gentle agitation and stored on ice before starting the procedure.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 14,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water baths, incubators, or heat blocks capable of 37°C and 65°C
- 100% Isopropanol
- 70% ethanol

Before Starting:

- Prepare XL Buffer according to the instructions in the "Preparing Reagents" section on Page 5.
- Set the water baths and/or incubators to 30°C and 65°C.
- Preheat the EB Buffer to 65°C.

Buffer Volumes Required for Processing 100-500 µL Whole Blood

Buffer	Blood Volume				
	100 µL	200 µL	300 µL	400 µL	500 µL
NL Buffer	250 µL	500 µL	750 µL	1000 µL	1250 µL
XL Buffer/Proteinase K	50 µL	100 µL	150 µL	200 µL	250 µL
100% isopropanol	50 µL	100 µL	150 µL	200 µL	250 µL
70% ethanol	50 µL	100 µL	150 µL	200 µL	250 µL
EB Buffer	100 µL	200 µL	200 µL	200 µL	200 µL

1. Add 500 µL NL Buffer to a nuclease-free 1.5 mL microcentrifuge tube.

2. Add 200 µL whole blood. Invert the tube 5 times.

3. Centrifuge at 10,000 x *g* for 30 seconds at room temperature.

4. Aspirate and discard supernatant.

5. Invert the tube on an absorbent paper towel for 2-3 minutes. Make sure the pellet remains in the tube.

6. Add 100 µL XL Buffer containing Proteinase K Solution (see Page 5 for instructions). Vortex immediately for 10-30 seconds or until the pellet is completely resuspended.

   Important: When processing multiple samples, vortex each tube immediately after addition of XL Buffer and Proteinase K Solution.

7. Centrifuge at 10,000 x *g* for 5 seconds to bring down any liquid droplets from the tube lid.

8. Incubate at 65°C for 5 minutes.

   Note: The sample should change color from red to olive green during proteinase digestion.

9. Add 100 µL isopropanol.

10. Gently mix the solution by inverting the tube 20-30 times or until the DNA precipitate become visible as threads or clumps.

11. Centrifuge at 14,000 x *g* for 5 minute at room temperature. DNA will be visible as a small white pellet.

12. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel.

13. Add 100 µL 70% ethanol. Vortex for 10 seconds.

14. Centrifuge at 14,000 x *g* for 2 minutes at room temperature.

15. Carefully pour off the ethanol. Pellet may be very loose at this point, so pour slowly and watch the pellet.

16. Invert the tube on a clean absorbent paper towel and air dry the pellet for 5-10 minutes.

17. Add 200 µL EB Buffer. Vortex for 1 minute.

18. Incubate at 65°C for 10 minutes. Some samples may need to incubate at 65°C for 1 hour to rehydrate the DNA.

19. Store DNA at -20°C.

E.Z.N.A.® SQ Blood DNA Kit Protocol - 1-3 mL Whole Blood

Note: The buffer volumes indicated in the following protocol are for 2 mL whole blood samples. This procedures can be scaled up and down for use with FRESH or FROZEN blood samples 1 mL to 3 mL in volume by adjusting the buffer volumes up or down in proportion to the volume of sample used. (Except the EB Buffer volume for 3 mL blood). Frozen blood should be thawed quickly in a 37°C water bath with gentle agitation and stored on ice before starting the procedure.

Materials and Equipment to be Supplied by User:

- Centrifuge capable of 2,000 x *g*
- Nuclease-free 15 mL centrifuge tubes
- Water baths, incubators, or heat blocks capable of 37°C and 65°C
- 100% Isopropanol
- 70% ethanol

Before Starting:

- Prepare XL Buffer according to the instructions in the "Preparing Reagents" section on Page 5.
- Set the water baths and/or incubators to 30°C and 65°C.
- Preheat the EB Buffer to 65°C.

Buffer Volumes Required for Processing 100-500 µL Whole Blood

Buffer	Blood Volume		
	1 mL	2 mL	3 mL
NL Buffer	2.5 mL	5 mL	7.5 mL
XL Buffer/Proteinase K	500 µL	1 mL	1.5 mL
100% isopropanol	500 µL	1 mL	1.5 mL
70% ethanol	500 µL	1 mL	1.5 mL
EB Buffer	200 µL	200 µL	300 µL

1. Add 5 mL NL Buffer to a nuclease-free 15 mL centrifuge tube.

2. Add 2 mL whole blood. Invert the tube 5 times.

3. Centrifuge at 2,000 x *g* for 5 minutes at room temperature.

4. Aspirate and discard supernatant.

5. Invert the tube on an absorbent paper towel for 2-3 minutes. Make sure the pellet remains in the tube.

6. Add 1 mL XL Buffer containing Proteinase K Solution (see Page 5 for instructions). Vortex immediately for 10-30 seconds or until the pellet is completely resuspended.

   Important: When processing multiple samples, vortex each tube immediately after addition of XL Buffer and Proteinase K Solution.

7. Centrifuge at 2,000 x *g* for 5 seconds to bring down any liquid droplets from the tube lid.

8. Incubate at 65°C for 5 minutes.

   Note: The sample should change color from red to olive green during proteinase digestion.

9. Add 1 mL isopropanol.

10. Gently mix the solution by inverting the tube 20-30 times or until the DNA precipitate become visible as threads or clumps.

11. Centrifuge at 2,000 x *g* for 5 minute at room temperature. DNA will be visible as a small white pellet.

12. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel.

13. Add 1 mL 70% ethanol. Vortex for 10 seconds.

14. Centrifuge at 2,000 x *g* for 3 minutes at room temperature.

15. Carefully pour off the ethanol. Pellet may be very loose at this point, so pour slowly and watch the pellet.

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

17. Add 200 µL EB Buffer. Vortex for 1 minute.

18. Incubate at 65°C for 10 minutes. Some samples may need to incubate at 65°C for 1 hour to rehydrate the DNA.

19. Store DNA at -20°C.

E.Z.N.A.® SQ Blood DNA Kit Protocol - 4-14 mL Whole Blood

Note: The buffer volumes indicated in the following protocol are for 12 mL whole blood samples. This procedures can be scaled up and down for use with FRESH or FROZEN blood samples 4 mL to 14 mL in volume by adjusting the buffer volumes up or down in proportion to the volume of sample used. (Except the EB Buffer volume for 3 mL blood). Frozen blood should be thawed quickly in a 37°C water bath with gentle agitation and stored on ice before starting the procedure.

Materials and Equipment to be Supplied by User:

- Centrifuge capable of 2,000 x *g*
- Nuclease-free 50 mL centrifuge tubes
- Water baths, incubators, or heat blocks capable of 37°C and 65°C
- 100% Isopropanol
- 70% ethanol

Before Starting:

- Prepare XL Buffer according to the instructions in the "Preparing Reagents" section on Page 5.
- Set the water baths and/or incubators to 30°C and 65°C.
- Preheat the EB Buffer to 65°C.

Buffer Volumes Required for Processing 4-8 mL Whole Blood

Buffer	Blood Volume				
	4 mL	5 mL	6 mL	7 mL	8 mL
NL Buffer	10 mL	12.5 mL	15 mL	17.5 mL	20 mL
XL Buffer/Proteinase K	2 mL	2.5 mL	3 mL	3.5 mL	4 mL
100% isopropanol	2 mL	2.5 mL	3 mL	3.5 mL	4 mL
70% ethanol	2 mL	2.5 mL	3 mL	3.5 mL	4 mL
EB Buffer	400 µL	200 µL	200 µL	200 µL	200 µL

Buffer Volumes Required for Processing 9-14 mL Whole Blood

Buffer	Blood Volume					
	9 mL	10 mL	11 mL	12 mL	13 mL	14 mL
NL Buffer	22.5 mL	25 mL	27.5 mL	30 mL	32.5 mL	35 mL
XL Buffer/Proteinase K	4.5 mL	5 mL	5 mL	5 mL	5 mL	5 mL
100% isopropanol	4.5 mL	5 mL	5 mL	5 mL	5 mL	5 mL
70% ethanol	4.5 mL	5 mL	5 mL	5 mL	5 mL	5 mL
EB Buffer	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL

1. Add 30 mL NL Buffer to a nuclease-free 50 mL centrifuge tube.

2. Add 2 mL whole blood. Invert the tube 5 times.

3. Centrifuge at 2,000 x *g* for 5 minutes at room temperature.

4. Aspirate and discard supernatant.

5. Invert the tube on an absorbent paper towel for 2-3 minutes. Make sure the pellet remains in the tube.

6. Add 5 mL XL Buffer containing Proteinase K Solution (see Page 5 for instructions). Vortex immediately for 10-30 seconds or until the pellet is completely resuspended.

   Important: When processing multiple samples, vortex each tube immediately after addition of XL Buffer and Proteinase K Solution.

7. Centrifuge at 2,000 x *g* for 5 seconds to bring down any liquid droplets from the tube lid.

8. Incubate at 65°C for 5 minutes.

   Note: The sample should change color from red to olive green during proteinase digestion.

9. Add 5 mL isopropanol.

10. Gently mix the solution by inverting the tube 20-30 times or until the DNA precipitate become visible as threads or clumps.

11. Centrifuge at 2,000 x *g* for 5 minute at room temperature. DNA will be visible as a small white pellet.

12. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel.

13. Add 5 mL 70% ethanol. Vortex for 10 seconds.

14. Centrifuge at 2,000 x *g* for 3 minutes at room temperature.

15. Carefully pour off the ethanol. Pellet may be very loose at this point, so pour slowly and watch the pellet.

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

17. Add 1 mL EB Buffer. Vortex for 1 minute.

18. Incubate at 65°C for 10 minutes. Some samples may need to incubate at 65°C overnight to rehydrate the DNA.

19. Store DNA at -20°C.

E.Z.N.A.® SQ Blood DNA Kit Protocol - 20 mL Whole Blood

Materials and Equipment to be Supplied by User:

- Centrifuge capable of 2,000 x *g*
- Nuclease-free 50 mL centrifuge tubes
- Water baths, incubators, or heat blocks capable of 37°C and 65°C
- 100% Isopropanol
- 70% ethanol

Before Starting:

- Prepare XL Buffer according to the instructions in the "Preparing Reagents" section on Page 5.
- Set the water baths and/or incubators to 30°C and 65°C.
- Preheat the EB Buffer to 65°C.

1. Add 25 mL NL Buffer to a nuclease-free 50 mL centrifuge tube.

2. Add 10 mL whole blood. Invert the tube 5 times.

3. Centrifuge at 2,000 x *g* for 5 minutes at room temperature.

4. Aspirate and discard supernatant.

5. Using the same 50 mL centrifuge tube, repeat Steps 1-4 for the remaining 10 mL blood sample.

6. Invert the tube on an absorbent paper towel for 2-3 minutes. Make sure the pellet remains in the tube.

7. Add 5 mL XL Buffer containing Proteinase K Solution (see Page 5 for instructions). Vortex immediately for 10-30 seconds or until the pellet is completely resuspended.

   Important: When processing multiple samples, vortex each tube immediately after addition of XL Buffer and Proteinase K Solution.

8. Centrifuge at 2,000 x *g* for 5 seconds to bring down any liquid droplets from the tube lid.

9. Incubate at 65°C for 5 minutes.

   Note: The sample should change color from red to olive green during proteinase digestion.

10. Add 5 mL isopropanol.

11. Gently mix the solution by inverting the tube 20-30 times or until the DNA precipitate become visible as threads or clumps.

12. Centrifuge at 2,000 x *g* for 5 minute at room temperature. DNA will be visible as a small white pellet.

13. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel.

14. Add 5 mL 70% ethanol. Vortex for 10 seconds.

15. Centrifuge at 2,000 x *g* for 3 minutes at room temperature.

16. Carefully pour off the ethanol. Pellet may be very loose at this point, so pour slowly and watch the pellet.

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

18. Add 1 mL EB Buffer. Vortex for 1 minute.

19. Incubate at 65°C for 10 minutes. Some samples may need to incubate at 65°C overnight to rehydrate the DNA.

20. Store DNA at -20°C.

E.Z.N.A.® SQ Blood DNA Kit Protocol - 100-500 µL Buffy Coat

Note: The buffer volumes indicated in the following protocol are for 200 µL whole blood samples. This procedures can be scaled up and down for use with FRESH or FROZEN blood samples 100 µL to 500 µL in volume by adjusting the buffer volumes up or down in proportion to the volume of sample used. (Except the EB Buffer volume for 100 µL blood). Frozen blood should be thawed quickly in a 37°C water bath with gentle agitation and stored on ice before starting the procedure.

The buffy coat fraction of whole blood is enriched with leukocytes and usually gives at least 5-fold more DNA than the same volume of blood. To prepare the buffy coat from fresh whole blood, simply centrifuge the sample at 3,000-4,000 x *g* for 10 minutes at room temperature. Three layers should form; a plasma upper layer, a buffy coat middle layer, and a erythrocyte 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 Kit II or frozen at -70°C.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 14,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water baths, incubators, or heat blocks capable of 37°C and 65°C
- 100% Isopropanol
- 70% ethanol

Before Starting:

- Prepare XL Buffer according to the instructions in the "Preparing Reagents" section on Page 5.
- Set the water baths and/or incubators to 30°C and 65°C.
- Preheat the EB Buffer to 65°C.

Buffer Volumes Required for Processing 100-500 µL Buffy Coat

Buffer	Blood Volume				
	100 µL	200 µL	300 µL	400 µL	500 µL
NL Buffer	250 µL	500 µL	750 µL	1000 µL	1250 µL
XL Buffer/Proteinase K	100 µL	200 µL	300 µL	400 µL	500 µL
100% isopropanol	100 µL	200 µL	300 µL	400 µL	500 µL
70% ethanol	100 µL	200 µL	300 µL	400 µL	500 µL
EB Buffer	200 µL	200 µL	200 µL	200 µL	200 µL

1. Add 500 µL NL Buffer to a nuclease-free 1.5 mL microcentrifuge tube.

2. Add 200 µL whole blood. Invert the tube 5 times.

3. Centrifuge at 14,000 x *g* for 30 seconds at room temperature.

4. Aspirate and discard supernatant.

5. Invert the tube on an absorbent paper towel for 2-3 minutes. Make sure the pellet remains in the tube.

6. Add 200 µL XL Buffer containing Proteinase K Solution (see Page 5 for instructions). Vortex immediately for 10-30 seconds or until the pellet is completely resuspended.

   Important: When processing multiple samples, vortex each tube immediately after addition of XL Buffer and Proteinase K Solution.

7. Centrifuge at 10,000 x *g* for 5 seconds to bring down any liquid droplets from the tube lid.

8. Incubate at 65°C for 5 minutes.

   Note: The sample should change color from red to olive green during proteinase digestion.

9. Add 200 µL isopropanol.

10. Gently mix the solution by inverting the tube 20-30 times or until the DNA precipitate become visible as threads or clumps.

11. Centrifuge at 14,000 x *g* for 5 minute at room temperature. DNA will be visible as a small white pellet.

12. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel.

13. Add 200 µL 70% ethanol. Vortex for 10 seconds.

14. Centrifuge at 14,000 x *g* for 2 minutes at room temperature.

15. Carefully pour off the ethanol. Pellet may be very loose at this point, so pour slowly and watch the pellet.

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

17. Add 200 µL EB Buffer. Vortex for 1 minute.

18. Incubate at 65°C for 10 minutes. Some samples may need to incubate at 65°C overnight to rehydrate the DNA.

19. Store DNA at -20°C.

E.Z.N.A.® SQ Blood DNA Kit Protocol - 1-2 x 10^6 Cultured Cells

This protocol is designed for isolating genomic DNA from 1-2 million cultured cells.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 14,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water baths, incubators, or heat blocks capable of 37°C and 65°C
- 100% Isopropanol
- 70% ethanol
- PBS

Before Starting:

- Prepare XL Buffer according to the instructions in the "Preparing Reagents" section on Page 5.
- Set the water baths and/or incubators to 30°C and 65°C.
- Preheat the EB Buffer to 65°C.

1. Transfer 1-2 x 10^6 cells from a cell culture to a 1.5 mL microcentrifuge tube. For adherent cells, trypsinize the cells before harvesting.

2. Centrifuge at 300 x *g* for 5 minutes.

3. Aspirate and discard cell culture media.

4. Resuspend cells in PBS.

5. Centrifuge at 300 x *g* for 5 minutes to pellet the cells.

6. Aspirate and discard the supernatant.

7. Leave the tube inverted on an absorbent paper towel for 2-3 minutes. Make sure the pellet remains in the tube.

8. Add 300 µL NL Buffer. Pipet up and down until cells are completely resuspended.

9. Add 300 µL XL Buffer containing Proteinase K Solution (see Page 5 for instructions). Vortex immediately for 10-30 seconds or until the pellet is completely resuspended.

   Important: When processing multiple samples, vortex each tube immediately after addition of XL Buffer and Proteinase K Solution.

10. Centrifuge at 10,000 x *g* for 5 seconds to bring down any liquid droplets from the tube lid.

11. Incubate at 65°C for 5 minutes.

    Note: The sample should change color from red to olive green during proteinase digestion.

12. Add 600 µL isopropanol.

13. Gently mix the solution by inverting the tube 20-30 times or until the DNA precipitate become visible as threads or clumps.

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

15. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel.

16. Add 600 µL 70% ethanol. Vortex for 10 seconds.

17. Centrifuge at 14,000 x *g* for 2 minutes at room temperature.

18. Carefully pour off the ethanol. Pellet may be very loose at this point, so pour slowly and watch the pellet.

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

20. Add 200 µL EB Buffer. Vortex for 1 minute.

21. Incubate at 65°C for 10 minutes. Some samples may need to incubate at 65°C overnight to rehydrate the DNA.

22. Store DNA at -20°C.

E.Z.N.A.® SQ Blood DNA Kit Protocol - 10 mL Clotted Blood

Materials and Equipment to be Supplied by User:

- Centrifuge with a swing bucket rotor capable of 2,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water baths, incubators, or heat blocks capable of 37°C and 65°C
- 100% Isopropanol
- 70% ethanol
- PBS

Before Starting:

- Prepare XL Buffer according to the instructions in the "Preparing Reagents" section on Page 5.
- Set the water baths and/or incubators to 30°C and 65°C.
- Preheat the EB Buffer to 65°C.

1. Transfer up to 10 mL clotted blood to a 50 mL centrifuge tube.

2. Homogenize the sample with a rotor-stator homogenizer until the sample is homogenous.

3. Add 25 µL NL Buffer. Invert tube 5-7 times.

4. Centrifuge at 2,000 x *g* for 5 minutes.

5. Aspirate and discard supernatant.

6. Invert the tube on an absorbent paper towel for 2-3 minutes. Make sure the pellet remains in the tube.

7. Add 5 mL XL Buffer containing Proteinase K Solution (see Page 5 for instructions). Vortex immediately for 10-30 seconds or until the pellet is completely resuspended.

   Important: When processing multiple samples, vortex each tube immediately after addition of XL Buffer and Proteinase K Solution.

8. Centrifuge at 10,000 x *g* for 5 seconds to bring down any liquid droplets from the tube lid.

9. Incubate at 65°C for 30 minutes.

   Note: The sample should change color from red to olive green during proteinase digestion.

10. Add 5 mL isopropanol.

11. Gently mix the solution by inverting the tube 20-30 times or until the DNA precipitate become visible as threads or clumps.

12. Centrifuge at 2,000 x *g* for 5 minutes at room temperature. DNA will be visible as a small white pellet.

13. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel.

14. Add 5 mL 70% ethanol. Vortex for 10 seconds.

15. Centrifuge at 2,000 x *g* for 5 minutes at room temperature.

16. Carefully pour off the ethanol. Pellet may be very loose at this point, so pour slowly and watch the pellet.

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

18. Add 1 mL EB Buffer. Vortex for 1 minute.

19. Incubate at 65°C for 10 minutes. Some samples may need to incubate at 65°C overnight to rehydrate the DNA.

20. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Low DNA yield	Blood sample contains too few white blood cells	Draw a new blood sample.
	Blood sample is too old.	Try to use fresh blood if possible.
	XL Buffer and Proteinase K Solution were not prepared correctly	Ensure that the XL Buffer and Proteinase K Solution is prepared fresh and according to the instructions on Page 5.
	Incomplete sample lysis	Mix the sample thoroughly after the addition of NL Buffer.
	DNA pellet was lost during isopropanol precipitation	Be very careful not to lose the DNA when removing isopropanol or ethanol during the precipitation and wash steps.
Low A_{260}/A_{280} ratio	Proteinase digestion was not complete	Ensure that the XL Buffer and Proteinase K Solution is prepared fresh and according to the instructions on Page 5.
	Poor cell lysis due to incomplete mixing with NL Buffer	Make sure to immediately vortex the sample after the addition of NL Buffer.
	Hemoglobin remains	Make sure to use the correct volume of NL Buffer.
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 the rehydration step	Shake a few times during the rehydration step.
Gel-like traces of pellet remaining after resuspension of pellet in XL/ Proteinase mixture	After addition of the XL/ Proteinase K, the sample was left too long before the vortexing	Immediately mix the sample after the addition of XLBuffer and Proteinase K Solution.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
NL Buffer, 100 mL	PD071
XL Buffer, 100 mL	PD072
EB Buffer, 500 mL	PD089
1.5 mL DNase/RNase-free Microcentrifuge Tubes, 500/pk, 10 pk/cs	SSI-1210-00

Notes: