omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® SQ Blood DNA Kit

D5032-00	10 mL
D5032-03	300 mL

Manual Date: February 2020
Revision Number: v3.0

For Research Use Only

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E.Z.N.A.® SQ Blood DNA Kit

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Introduction and Overview

The E.Z.N.A.® SQ Blood DNA Kit is designed to isolate high molecular weight genomic DNA from fresh, frozen, and clotted 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. The E.Z.N.A.® SQ Blood DNA system is a solution-based system and the protocols can be easily modified based on the sample starting amount.

DNA purified using the E.Z.N.A.® SQ Blood DNA method is ready for applications such as PCR, Southern blotting, and restriction digestion.

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 lysed with ERL Buffer, followed by white blood cell lysis in WTL Buffer. Cellular proteins are removed by precipitation as the high molecular weight genomic DNA remains in solution. High-quality genomic DNA is then purified by isopropanol precipitation.

New in this Edition:

February 2020:

- This manual has been edited for content and redesigned to enhance user readability.
- EB Buffer has been renamed Elution Buffer. This is a name change only. The formulation has not changed.

Kit Contents

Product	D5032-00	D5032-03
Total Amount of Blood that can be Processed	10 mL	300 mL
ERL Buffer (10X)	5 mL	2 x 80 mL
WTL Buffer	10 mL	300 mL
PCP Buffer	4 mL	120 mL
Elution Buffer	15 mL	125 mL
RNase A	50 µL	1.5 mL
User Manual	✓	✓

Storage and Stability

All E.Z.N.A.® SQ Blood DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. RNase A should be stored at 2-8°C. Store all other components at room temperature. Check buffers for precipitates before use. Redissolve any precipitates by warming to 37˚C.

Preparing Reagents

- Dilute ERL Buffer (10X) with sterile water as follows and store at room temperature.

Kit	Sterile Water to be Added
D5032-00	45 mL
D5032-03	720 mL per bottle

Expected Yields

DNA Yields from Various Samples

Species and Sample	Amount of Sample	Typical Yield
Human Whole Blood *Yield varies 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
	800 µL	12-35 µg
	1 mL	15-48 µg
	2 mL	30-90 µg
	3 mL	50-150 µg
	4 mL	65-200 µg
	5 mL	100-300 µg
	10 mL	150-600 µg
	12 mL	200-700 µg
Buffy Coat	from 300 µL blood	5-15 µg
	from 2 mL blood	25-75 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

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

Note: The procedure below has been optimized for use with FRESH or FROZEN blood samples. Additional sample types can be processed using the E.Z.N.A.® SQ Blood DNA Kit. Please refer to the Buffy Coat Protocol on Pages 18-20, Cultured Cells Protocols on Pages 21-26, or the Clotted Blood Protocols on Pages 27-35 for procedures optimized for those sample types.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000*g*
- Water baths capable of at least 65°C
- Vortexer
- Ice bucket
- Nuclease-free 2 mL microcentrifuge tubes
- 100% isopropanol
- 70% ethanol
- Sterile water

Before Starting:

- Prepare ERL Buffer (10X) according to the directions in the "Preparing Reagents" section on Page 4.
- Set water baths to 37°C and 65°C.
- Prepare an ice bucket.

1. Add ERL Buffer to a 2 mL microcentrifuge tube (not provided) according to the table below based on the amount of whole blood (or bone marrow) to be used.

   Note: ERL Buffer (10X) must be diluted with sterile water before use. Please see Page 4 for instructions.

Amount of Blood	ERL Buffer
100 µL	300 µL
300 µL	900 µL
500 µL	1.5 mL

2. Add the whole blood sample (or bone marrow) to the tube of ERL Buffer prepared in Step 1. Invert several times to mix.

3. Let sit at room temperature for 5 minutes. Invert the tube several times during incubation.

4. Centrifuge at maximum speed (≥13,000*g*) for 30 seconds at room temperature. Remove supernatant without disturbing the visible white pellet. Leave ~15 µL residual liquid in the tube. If the blood sample has been frozen, repeat Steps 1, 3, and 4 (do not add more blood) 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. Let sit for 2 minutes at room temperature. Repeat Step 4 to pellet the white blood cells.

5. Vortex vigorously until the white blood cells are completely resuspended.

6. Add WTL Buffer according to the table below. Pipet up and down to lyse the cells. The solution should become viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

Amount of Blood	WTL Buffer
100 µL	100 µL
300 µL	300 µL
500 µL	500 µL

Optional: Add RNase A according to the table below. Invert the tube 20-25 times to mix. Incubate the mixture at 37°C for 5-10 minutes.

Amount of Blood	RNase A
100 µL	0.5 µL
300 µL	1.5 µL
500 µL	2.5 µL

7. Cool to room temperature.

8. Add PCP Buffer according to the table below. Vortex vigorously for 30 seconds to mix. Some protein clumps may be visible after vortexing.

Amount of Blood	PCP Buffer
100 µL	33 µL
300 µL	100 µL
500 µL	167 µL

9. Let sit on ice for 5 minutes.

10. Centrifuge at maximum 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, repeat Steps 9-10.

11. Add 100% isopropanol to a new 2 mL microcentrifuge tube according to the table below.

Amount of Blood	100% Isopropanol
100 µL	100 µL
300 µL	300 µL
500 µL	500 µL

12. Transfer the supernatant from Step 10 to the tube of 100% isopropanol prepared in Step 11.

13. Gently invert the tube 30-40 times to mix.

14. Centrifuge at maximum speed for 1 minute at room temperature. DNA will be visible as a small white pellet.

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

16. Add 70% ethanol according to the table below. Invert the tube few times to wash the DNA pellet.

Amount of Blood	70% Ethanol
100 µL	100 µL
300 µL	300 µL
500 µL	500 µL

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

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

19. Add Elution Buffer according to the table below. Vortex for 1 minute to mix.

Amount of Blood	Elution Buffer
100 µL	35 µL*
200 µL	65 µL*
300 µL	100 µL*
500 µL	100 µL

* For ≤300 µL blood volume, the volume of Elution Buffer used can be adjusted depending on the desired final concentration.

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

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

E.Z.N.A.® SQ Blood DNA Kit Protocol - 600 µL-3 mL Whole Blood

Note: The procedure below has been optimized for use with FRESH or FROZEN blood samples. Additional sample types can be processed using the E.Z.N.A.® SQ Blood DNA Kit. Please refer to the Buffy Coat Protocol on Pages 18-20, Cultured Cells Protocols on Pages 21-26, or the Clotted Blood Protocols on Pages 27-35 for procedures optimized for those sample types.

Materials and Equipment to be Supplied by User:

- Centrifuge with 15 mL centrifuge tube adaptor capable of 2,000*g*
- Water baths capable of at least 65°C
- Vortexer
- Ice bucket
- Nuclease-free 15 mL centrifuge tubes
- 100% isopropanol
- 70% ethanol
- Sterile water

Before Starting:

- Prepare ERL Buffer (10X) according to the directions in the "Preparing Reagents" section on Page 4.
- Set water baths to 37°C and 65°C.
- Prepare an ice bucket.

1. Add ERL Buffer to a 15 mL centrifuge tube (not provided) according to the table below based on the amount of whole blood (or bone marrow) to be used.

   Note: ERL Buffer (10X) must be diluted with sterile water before use. Please see Page 4 for instructions.

Amount of Blood	ERL Buffer
600 µL	1.8 mL
1 mL	3 mL
3 mL	9 mL

2. Add the whole blood sample (or bone marrow) to the tube of ERL Buffer prepared in Step 1. Invert several times to mix.

3. Let sit at room temperature for 5 minutes. Invert the tube several times during incubation.

4. Centrifuge at maximum speed (≥2,000*g*) for 5 minutes at room temperature. Remove supernatant without disturbing the visible white pellet. Leave residual liquid in the tube according to the table below. If the blood sample has been frozen, repeat Steps 1, 3, and 4 (do not add more blood) until the pellet is white.

Amount of Blood	Residual Liquid
600 µL	50 µL
1 mL	100 µL
3 mL	200 µL

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. Let sit for 2 minutes at room temperature. Repeat Step 4 to pellet the white blood cells.

5. Vortex vigorously until the white blood cells are completely resuspended.

6. Add WTL Buffer according to the table below. Pipet up and down to lyse the cells. The solution should become viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

Amount of Blood	WTL Buffer
600 µL	600 µL
1 mL	1 mL
3 mL	3 mL

Optional: Add RNase A according to the table below. Invert the tube 20-25 times to mix. Incubate the mixture at 37°C for 10 minutes.

Amount of Blood	RNase A
600 µL	3 µL
1 mL	5 µL
3 mL	15 µL

7. Cool to room temperature.

8. Add PCP Buffer according to the table below. Vortex vigorously for 30 seconds to mix. Some protein clumps may be visible after vortexing.

Amount of Blood	PCP Buffer
600 µL	200 µL
1 mL	335 µL
3 mL	1 mL

9. Let sit on ice for 5 minutes.

10. Centrifuge at maximum speed for 5 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, repeat Steps 9-10.

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

Amount of Blood	100% Isopropanol
600 µL	600 µL
1 mL	1 mL
3 mL	3 mL

12. Transfer the supernatant from Step 10 to the tube of 100% isopropanol prepared in Step 10.

13. Gently invert the tube 40-50 times to mix.

14. Centrifuge at maximum speed for 3 minutes at room temperature. DNA will be visible as a small white pellet.

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

16. Add 70% ethanol according to the table below. Invert the tube few times to wash the DNA pellet.

Amount of Blood	70% Ethanol
600 µL	600 µL
1 mL	1 mL
3 mL	3 mL

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

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

19. Add Elution Buffer according to the table below. Vortex for 1 minute to mix.

Amount of Blood	Elution Buffer
600 µL	100 µL
1 mL	100 µL
2 mL	200 µL
3 mL	250 µL

20. Incubate at 65°C for 1 hour. Gently mix several times during incubation. Some samples may need to sit overnight at room temperature to completely rehydrate the DNA pellet.

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

E.Z.N.A.® SQ Blood DNA Kit Protocol - 4 mL-10 mL Whole Blood

Note: The procedure below has been optimized for use with FRESH or FROZEN blood samples. Additional sample types can be processed using the E.Z.N.A.® SQ Blood DNA Kit. Please refer to the Buffy Coat Protocol on Pages 18-20, Cultured Cells Protocols on Pages 21-26, or the Clotted Blood Protocols on Pages 27-35 for procedures optimized for those sample types.

Materials and Equipment to be Supplied by User:

- Centrifuge with 50 mL centrifuge tube adaptor capable of 2,000*g*
- Water baths capable of at least 65°C
- Vortexer
- Ice bucket
- Nuclease-free 50 mL centrifuge tubes
- 100% isopropanol
- 70% ethanol
- Sterile water

Before Starting:

- Prepare ERL Buffer (10X) according to the directions in the "Preparing Reagents" section on Page 4.
- Set water baths to 37°C and 65°C.
- Prepare an ice bucket.

1. Add ERL Buffer to a 50 mL centrifuge tube (not provided) according to the table below based on the whole blood sample volume used.

   Note: ERL Buffer (10X) must be diluted with sterile water before use. Please see Page 4 for instructions.

Amount of Blood	ERL Buffer
4 mL	12 mL
6 mL	18 mL
10 mL	30 mL

2. Add the whole blood sample (or bone marrow) to the tube of ERL Buffer prepared in Step 1. Invert several times to mix.

3. Let sit at room temperature for 5 minutes. Invert the tube several times during the incubation.

4. Centrifuge at maximum speed (≥2,000*g*) for 2 minutes at room temperature. Remove supernatant without disturbing the visible white pellet. Leave ~350 µL residual liquid in the tube. If the blood sample has been frozen, repeat Steps 1, 3, and 4 (do not add more blood) 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. Let sit for 2 minutes at room temperature. Repeat Step 4 to pellet the white blood cells.

5. Vortex vigorously until the white blood cells are completely resuspended.

6. Add WTL Buffer according to the table below. 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.

Amount of Blood	WTL Buffer
4 mL	4 mL
6 mL	6 mL
10 mL	10 mL

Optional: Add RNase A according to the table below. Invert the tube 20-25 times to mix. Incubate the mixture at 37°C for 10 minutes.

Amount of Blood	RNase A
4 mL	20 µL
6 mL	30 µL
10 mL	50 µL

7. Cool to room temperature.

8. Add PCP Buffer according to the table below. Vortex vigorously for 30 seconds to mix. Some protein clumps may be visible after vortexing.

Amount of Blood	PCP Buffer
4 mL	1.3 mL
6 mL	2 mL
10 mL	3.3 mL

9. Let sit on ice for 5 minutes.

10. Centrifuge at maximum speed for 5 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, repeat Steps 9-10.

11. Add 100% isopropanol to a new 50 mL centrifuge tube according to the table below.

Amount of Blood	100% Isopropanol
4 mL	4 mL
6 mL	6 mL
10 mL	10 mL

12. Transfer the supernatant from Step 10 to the tube of 100% isopropanol prepared in Step 10.

13. Gently invert the tube 40-50 times to mix.

14. Centrifuge at maximum speed for 3 minutes at room temperature. DNA will be visible as a small white pellet.

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

16. Add 70% ethanol according to the table below. Invert the tube few times to wash the DNA pellet.

Amount of Blood	70% Ethanol
4 mL	4 mL
6 mL	6 mL
10 mL	10 mL

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

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

19. Add Elution Buffer according to the table below. Vortex for 1 minute to mix.

Amount of Blood	Elution Buffer
4 mL	400 µL
6 mL	600 µL
10 mL	1 mL

20. Incubate at 65°C for 1 hour. Gently mix several times during incubation. Some samples may need to sit overnight at room temperature to completely rehydrate the DNA pellet.

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

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

Note: The buffy coat fraction of whole blood is enriched with leucocytes (white blood cells) 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*g* for 10 minutes 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 Kit II , or frozen at -70°C for storage.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000*g*
- Water baths capable of at least 65°C
- Vortexer
- Ice bucket
- Nuclease-free 2 mL microcentrifuge tubes
- 100% isopropanol
- 70% ethanol
- Sterile water

Before Starting:

- Prepare ERL Buffer (10X) according to the directions in the "Preparing Reagents" section on Page 4.
- Set water baths to 37°C and 65°C.
- Prepare an ice bucket.

1. Add 400 µL ERL Buffer to a 2 mL microcentrifuge tube (not provided).

   Note: ERL Buffer (10X) must be diluted with sterile water before use. Please see Page 4 for instructions.

2. Isolate the buffy coat from a 1.5 mL whole blood sample and add to the tube of ERL Buffer prepared in Step 1. Invert several times to mix.

3. Let sit at room temperature for 5 minutes. Invert the tube several times during the incubation.

4. Centrifuge at maximum speed (≥14,000*g*) for 30 seconds at room temperature. Remove supernatant without disturbing the visible white pellet. Leave ~15 µL residual liquid in the tube. If the blood sample has been frozen, repeat Steps 1, 3, and 4 (do not add more blood) 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. Let sit for 2 minutes at room temperature. Repeat Step 4 to pellet the white blood cells.

5. Vortex vigorously until the white blood cells are completely resuspended.

6. Add 1.3 mL WTL Buffer. 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.

Optional: Add 5 µL RNase A. Invert the tube 20-25 times to mix. Incubate the mixture at 37°C for 5-10 minutes.

7. Cool to room temperature.

8. Add 433 µL PCP Buffer. Vortex vigorously for 30 seconds to mix. Some protein clumps may be visible after vortexing.

9. Let sit on ice for 5 minutes.

10. Centrifuge at maximum 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, repeat Steps 9-10.

11. Add 1.3 mL 100% isopropanol to a new 2 mL microcentrifuge tube.

12. Transfer the supernatant from Step 10 to the tube of 100% isopropanol prepared in Step 11.

13. Gently invert the tube 30-40 times to mix.

14. Centrifuge at maximum speed for 1 minute at room temperature. DNA will be visible as a small white pellet.

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

16. Add 1.3 mL 70% ethanol. Invert the tube few times to wash the DNA pellet.

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

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

19. Add 500 µL Elution Buffer. Vortex for 1 minute to mix.

20. Incubate at 65°C for 1 hour. Gently mix several times during incubation. Some samples may need to sit overnight at room temperature to completely rehydrate the DNA pellet.

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

E.Z.N.A.® SQ Blood DNA Kit Protocol - 0.5-5 x 10^6 Cultured Cells

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000*g*
- Water baths capable of at least 65°C
- Vortexer
- Ice bucket
- Nuclease-free 2 mL microcentrifuge tubes
- 100% isopropanol
- 70% ethanol
- PBS
- Optional: Trypsin

Before Starting:

- Set water baths to 37°C and 65°C.
- Prepare an ice bucket.

1. Harvest cells, resuspend in PBS, and transfer to a 2 mL microcentrifuge tube (not provided). For adherent cells, trypsinize before harvesting.

2. Centrifuge at maximum speed (≥14,000*g*) for 10 seconds at room temperature. Remove supernatant without disturbing the cell pellet. Leave ~25 µL residual liquid in the tube.

3. Vortex until the cells are completely resuspended.

4. Add WTL Buffer according to the table below based on the amount of cells used. Pipet up and down to lyse the cells. The solution should become viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

Amount of Cultured Cells	WTL Buffer
0.5-1 x 10^6	150 µL
3-5 x 10^6	600 µL

Optional: Add RNase A according to the table below. Invert the tube 20-25 times to mix. Incubate the mixture at 37°C for 5-10 minutes.

Amount of Cultured Cells	RNase A
0.5-1 x 10^6	1 µL
3-5 x 10^6	3 µL

5. Cool to room temperature.

6. Add PCP Buffer according to the table below. Vortex vigorously for 30 seconds to mix. Some protein clumps may be visible after vortexing.

Amount of Cultured Cells	PCP Buffer
0.5-1 x 10^6	50 µL
3-5 x 10^6	200 µL

7. Let sit on ice for 5 minutes.

8. Centrifuge at maximum 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, repeat Steps 7-8.

9. Add 100% isopropanol to a new 2 mL microcentrifuge tube according to the table below.

Amount of Cultured Cells	100% Isopropanol
0.5-1 x 10^6	150 µL
3-5 x 10^6	600 µL

10. Transfer the supernatant from Step 8 to the tube of 100% isopropanol prepared in Step 9.

11. Gently invert the tube 30-40 times to mix.

12. Centrifuge at maximum speed for 1 minute at room temperature. DNA will be visible as a small white pellet.

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

14. Add 70% ethanol according to the table below. Invert the tube few times to wash the DNA pellet.

Amount of Cultured Cells	70% Ethanol
0.5-1 x 10^6	150 µL
3-5 x 10^6	600 µL

15. Centrifuge at maximum speed for 2 minutes at room temperature. Carefully remove the ethanol. The pellet may be very loose at this point, so pour or pipette 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 Elution Buffer according to the table below. Vortex for 1 minute to mix.

Amount of Cultured Cells	Elution Buffer
0.5-1 x 10^6	25 µL
3-5 x 10^6	100 µL

18. Incubate at 65°C for 1 hour. Gently mix several times during incubation. Some samples may need to sit overnight at room temperature to completely rehydrate the DNA pellet.

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

E.Z.N.A.® SQ Blood DNA Kit Protocol - 3-5 x 10^7 Cultured Cells

Materials and Equipment to be Supplied by User:

- Centrifuge with 15 mL centrifuge tube adaptor capable of 2,000*g*
- Water baths capable of at least 65°C
- Vortexer
- Ice bucket
- Nuclease-free 15 mL centrifuge tubes
- 100% isopropanol
- 70% ethanol
- PBS
- Optional: Trypsin

Before Starting:

- Set water baths to 37°C and 65°C.
- Prepare an ice bucket.

1. Harvest cells, resuspend in PBS, and transfer to a 15 mL centrifuge tube (not provided). For adherent cells, trypsinize before harvesting.

2. Centrifuge at 500*g* for 3 minutes at room temperature. Remove supernatant without disturbing the cell pellet. Leave ~2.5 mL residual liquid in the tube.

3. Vortex until the cells are completely resuspended.

4. Add 6 mL WTL Buffer. 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.

Optional: Add 30 µL RNase A. Invert the tube 20-25 times to mix. Incubate the mixture at 37°C for 5-10 minutes.

5. Cool to room temperature.

6. Add 2 mL PCP Buffer. Vortex vigorously for 30 seconds to mix. Some protein clumps may be visible after vortexing.

7. Let sit on ice for 5 minutes.

8. Centrifuge at maximum speed (≥2,000*g*) for 10 minutes at room temperature. The precipitated protein will form a tight pellet. If the pellet is not tight or visible, repeat Steps 7-8.

9. Add 6 mL 100% isopropanol to a new 15 mL centrifuge tube.

10. Transfer the supernatant from Step 8 to the tube of 100% isopropanol prepared in Step 9.

11. Gently invert the tube 30-40 times to mix.

12. Centrifuge at maximum speed for 10 minutes at room temperature. DNA will be visible as a small white pellet.

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

14. Add 6 mL 70% ethanol. Invert the tube few times to wash the DNA pellet.

15. Centrifuge at maximum speed for 10 minutes at room temperature. Carefully remove the ethanol. The pellet may be very loose at this point, so pour or pipette 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 Elution Buffer. Vortex for 2 minutes to mix.

18. Incubate sample at 65°C for 1 hour. Gently mix several times during incubation. Some samples may need to sit overnight at room temperature to completely rehydrate the DNA pellet.

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

E.Z.N.A.® SQ Blood DNA Kit Protocol - 50 µL Clotted Blood

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 14,000*g*
- Water baths capable of at least 65°C
- Vortexer
- Ice bucket
- Nuclease-free 2 mL microcentrifuge tubes
- 100% isopropanol
- 70% ethanol
- Proteinase K solution (25 mg/mL)
- Optional: Glycogen (20 mg/mL) if DNA yields are expected to be <2 µg

Before Starting:

- Set water baths to 37°C, 55°C, and 65°C.
- Prepare an ice bucket.

1. Transfer 50 µL clotted blood including any residual liquid to a 2 mL microcentrifuge tube (not provided).

2. Add 550 µL WTL Buffer. Pipet up and down to mix.

3. Add 3 µL Proteinase K solution (not provided). Invert the tube 20 times to mix.

4. Incubate at 55°C for 1 hour or until clots have completely dissolved. An overnight incubation may be required.

5. Let sit on ice for 1 minute.

6. Add 3 µL RNase A. Invert the tube 10 times to mix. Incubate the mixture at 37°C for 5 minutes.

7. Let sit on ice for 1 minute.

8. Add 200 µL PCP Buffer. Vortex vigorously for 30 seconds to mix. Some protein clumps may be visible after vortexing.

9. Let sit on ice for 5 minutes.

10. Centrifuge at maximum speed (≥14,000*g*) for 3 minutes at room temperature. The precipitated protein will form a tight, dark brown pellet. If the pellet is not tight or visible, repeat Steps 9-10.

11. Add 600 µL 100% isopropanol to a new 2 mL microcentrifuge tube.

12. Transfer the supernatant from Step 10 to the tube of 100% isopropanol prepared in Step 11.

Optional: If DNA yields are expected to be <2 µg, add 2 µL glycogen (20 mg/mL).

13. Gently invert the tube 30-40 times to mix.

14. Centrifuge at maximum speed for 10 minutes at room temperature. DNA will be visible as a small white pellet.

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

16. Add 600 µL 70% ethanol. Invert the tube few times to wash the DNA pellet.

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

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

19. Add 20 µL Elution Buffer. Vortex for 1 minute to mix.

20. Incubate sample at 65°C for 1 hour. Gently mix several times during incubation. Some samples may need to sit overnight at room temperature to completely rehydrate the DNA pellet.

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

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

Materials and Equipment to be Supplied by User:

- Centrifuge with 50 mL centrifuge tube adaptor capable of 2,000*g*
- Water baths capable of at least 65°C
- Vortexer
- Ice bucket
- Nuclease-free 50 mL centrifuge tubes
- 100% isopropanol
- 70% ethanol
- Proteinase K solution (25 mg/mL)
- Glycogen (20 mg/mL)

Before Starting:

- Set water baths to 37°C, 55°C, and 65°C.
- Prepare an ice bucket.

1. Transfer 1 mL clotted blood including any residual liquid to a 50 mL centrifuge tube (not provided).

2. Add 11 mL WTL Buffer. Pipet up and down to mix.

3. Add 50 µL Proteinase K solution (not provided). Invert the tube 20 times to mix.

4. Incubate at 55°C for 3 hours or until clots have completely dissolved. An overnight incubation may be required.

5. Let sit on ice for 1-2 minutes.

6. Add 50 µL RNase A. Invert the tube 10 times to mix. Incubate the mixture at 37°C for 5 minutes.

7. Let sit on ice for 1-2 minutes.

8. Add 4 mL PCP Buffer. Vortex vigorously for 30 seconds to mix. Some protein clumps may be visible after vortexing.

9. Let sit on ice for 10 minutes.

10. Centrifuge at maximum speed (≥2,000*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, repeat Steps 9-10.

11. Add 12 mL 100% isopropanol to a new 50 mL centrifuge tube.

12. Transfer the supernatant from Step 10 to the tube of 100% isopropanol prepared in Step 11.

13. Add 20 µL glycogen (20 mg/mL) (not provided).

14. Gently invert the tube 30-40 times to mix.

15. Centrifuge at maximum speed for 5 minutes at room temperature. DNA will be visible as a small white pellet.

16. Remove the supernatant and drain the tube briefly on a clean absorbent paper towel.

17. Add 12 mL 70% ethanol. Invert the tube few times to wash the DNA pellet.

18. Centrifuge at maximum speed for 2 minutes at room temperature. Carefully remove the ethanol. The pellet may be very loose at this point, so pour or pipette 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 400 µL Elution Buffer. Vortex for 1 minute to mix.

21. Incubate sample at 65°C for 1 hour. Gently mix several times during incubation. Some samples may need to sit overnight at room temperature to completely rehydrate the DNA pellet.

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

E.Z.N.A.® SQ Blood DNA Kit Protocol - ≥1 mL Clotted Blood

Materials and Equipment to be Supplied by User:

- Rotor-stator homogenizer
- Centrifuge with 50 mL centrifuge tube adaptor capable of 2,000*g*
- Water baths capable of at least 65°C
- Vortexer
- Ice bucket
- Nuclease-free 50 mL centrifuge tubes
- 100% isopropanol
- 70% ethanol
- Proteinase K solution (25 mg/mL)

Before Starting:

- Set water baths to 37°C and 65°C.
- Prepare an ice bucket.

1. Transfer the clotted blood including any residual liquid to a 50 mL centrifuge tube (not provided).

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

3. Add 3 volumes ERL Buffer. Invert the tube 5-7 times to mix.

4. Let sit for 5 minutes at room temperature.

5. Centrifuge at maximum speed (≥2,000*g*) for 5 minutes.

6. Remove the supernatant and drain the tube for 2 minutes on a clean absorbent paper towel.

7. Add 5 mL WTL Buffer and 50 µL Proteinase K solution (not provided). Vortex immediately.

   Important: When processing multiple samples, vortex each tube immediately after addition of WTL Buffer. Do not wait until buffer has been added to all samples before vortexing. The pellet can be easily homogenized with a few pulses of high-speed vortexing, however, traces of pellet with a jelly-like consistency (often barely visible) may remain. If seen, vortex sample for another 30 seconds.

8. Incubate at 65°C for 30 minutes.

9. Cool to room temperature.

10. Add 1.7 mL PCP Buffer. Vortex vigorously for 15 seconds to mix.

11. Let sit on ice for 10 minutes.

12. Centrifuge at maximum speed (≥2,000*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, repeat Steps 11-12.

13. Add 4.75 mL 100% isopropanol to a new 50 mL centrifuge tube.

14. Transfer the supernatant from Step 12 to the tube of 100% isopropanol prepared in Step 13.

15. Gently invert the tube 20-30 times to mix.

16. Centrifuge at maximum speed for 10 minutes at room temperature. DNA will be visible as a small white pellet.

17. Remove the supernatant and drain the tube briefly on a clean absorbent paper towel.

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

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

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

21. Add 1 mL Elution Buffer. Vortex for 1 minute to mix.

22. Incubate sample at 65°C for 1 hour. Gently mix several times during incubation. Some samples may need to sit overnight at room temperature to completely rehydrate the DNA pellet.

23. Store DNA at 2-8°C. For long-term storage, store 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 new blood samples.
	Blood sample is too old	Try to use fresh blood if possible.
	White blood cell pellet not completely resuspended before adding WTL Buffer	Vortex vigorously to completely resuspend white blood cell pellet.
Problem	Cause	Solution
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 WTL Buffer	Repeat the procedure, vortex the sample immediately after addition of WTL Buffer.
	Hemoglobin remains	Repeat the procedure, verify and add enough ERL Buffer. The cell pellet should be white in color.
	PCP Buffer was not mixed with WTL Buffer thoroughly.	Make sure that the PCP Buffer and the cell lysate is mixed thoroughly.
Problem	Cause	Solution
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.
Problem	Cause	Solution
DNA Pellet is difficult to dissolve	DNA pellet was over dried	Rehydrate the DNA by incubating the DNA pellet with Elution 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.

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