EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.002_Appendix 0_Omega E.Z.N.A SQ II DNA Blood

Standard Operating Procedure Staff Review Page:

I have read and understand the procedure listed above.

Employee name	Date SOP review complete

Standard Operating Procedure Approval Page:

Date Implemented:	23 January 2008	
Updated:	Ashima Amin **Name**	15 June 2020 **Date**
Supersedes:	Ashima Amin **Name**	11 April 2016 **Date**

Annual Review and Approval

Michael Zwick, PhD
Laboratory Director

Changes Made:
061520: Review, Protocols split out into appendixes. Renumbered SOPs. Previously EIGC.003.

Changes to previous procedures:
041116: Review, LIMS steps updated.

Note: No laboratory personnel should use this piece of equipment without proper training by the laboratory supervisor or other designated person.

Introduction:

The E.Z.N.A.™ SQ 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 amounts of starting material. The whole procedure can be performed in a single tube so it can reduce 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.

Principle:

E.Z.N.A.™ SQ 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 membrane are first lysed with NL Buffer, cell nuclei and mitochondria are then pelleted by centrifugation. The pellet is resuspended and lysed by 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.

Storage and Stability:

All components of the E.Z.N.A.™ SQ DNA Kit II should be stored at 22-25 °C. Under cool ambient conditions, a precipitate may form in the NL Buffer. In case of such an event, heat the bottle at 55 °C to dissolve. All E.Z.N.A.™ SQ DNA Kit II components are guaranteed for one year from the date of receipt when stored at 22-25 °C.

Yield:

DNA yield depends on the number of nucleated cell numbers presented in the sample. Yields from whole blood may vary widely. The following table shows the typical yields obtained from different samples. The purified DNA size can be up to 200kb.

Species and Material	Amount of Starting material	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
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

Kit Contents:

Product	D0714-05	D0714-50	D0714-250
Volume of Blood can be 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
Elution Buffer	5 ml	50 ml	250 ml
Proteinase K Solution	30 µl	300 µl	1.4 ml
User Manual	1	1	1

Safety: All employees are required to follow universal safety protocols when working with potentially infectious biohazardous materials. Safety procedures are outlined in the Emory University Bloodborne Pathogen Exposure Control Plan (see Employee Safety Notebook).

Extraction Recording Keeping and LIMS: The extraction date is recorded in the LIMS system as the date DNA is received. Extraction kit lot numbers are recorded in the QC report that is passed on to the client.

Procedural Steps:

Before Starting

Important	Prepare the **XL Buffer/Proteinase K** mixture For each 1 ml whole blood, mix 500µl XL Buffer with 5µl of Proteinase K Solution.

Storage of Blood Samples

The procedure can use whole blood treated with EDTA, heparin, or citrate with either fresh or frozen condition. Fresh blood yield better results. For short term storage (for up to 2 weeks), it is recommended to collect blood in a tube containing EDTA as an anticoagulant. For long term storage, sample should be collected in a tube containing EDTA as an anticoagulant and stored at -70°C.

A. DNA Purification Protocol for 100-500 µl whole blood

NOTE: The buffer volume of the following protocol is for isolating 200µl whole blood sample. This procedure can be scaled up and down for use with fresh or frozen blood samples 100 µl to 500 µl in volume by scaling reagent volume up and down in proportion to the volume of sample used. (Except the Elution 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.

Reagent volumes required for processing 100-500 µl whole blood samples

Reagent	**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 Solution	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
Elution Buffer	100 µl	200 µl	200 µl	200 µl	200 µl

Materials to be supplied by user

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 1.5 ml or 2 ml microcentrifuge tubes
- Water bath preset at 37 °C and 65 °C
- 100% isopropanol
- 70% ethanol

1. **Add 200 µl whole blood (or bone marrow) to a nuclease-free 1.5 ml microcentrifuge tube containing 500 µl NL Buffer. Mix by inverting the tube 5 times.**
2. **Centrifuge at 10,000 x g for 30 seconds at room temperature. Remove and discard supernatant. Leave the tube inverted on an absorbent paper for 2-3 minutes. Make sure the pellet remains in the tube.**
3. **Add 100 µl XL Buffer/Proteinase K mixture to the tube containing the nuclei pellet. Vortex immediately for 10-30 seconds or until the pellet is completely homogenized. Important: When processing multiple samples, vortex each tube immediately after addition of XL/Proteinase K mixture**
4. **Centrifuge at 10,000 x for 5 seconds to bring down any liquid drops from tube lid.**
5. **Incubate at 65 °C for 5 minutes in a water bath or heating block.**

   Note: The sample should change color from red to olive green during proteinase digestion.
6. **Add 100 µl isopropanol to the lysate.**
7. **Gently mix the solution by inverting the tube 20-30 times or until the DNA precipitate become visible as threads or clumps.**
8. **Centrifuge at 14,000 x g for 5 minutes at room temperature. DNA will be visible as a small white pellet.**
9. **Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 100 µl of 70% ethanol and vortex the tube for 10 seconds to wash the DNA pellet.**
10. **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.**
11. **Invert the tube on a clean absorbent paper towel and air dry the pellet for 5-10 minutes.**
12. **Add 200 µl of DNA rehydration solution (Elution Buffer) and vortex for 1 minute to mix.**
13. **Incubate sample at 65 °C for 10 min. Some samples may need to incubate at 65 °C for 1 hour to rehydrate DNA.**
14. **Store DNA at 2-8 °C. For long-term storage, store at -20 °C**.

B. DNA Purification Protocol for 1-3 ml whole blood

NOTE: The buffer volume of the following protocol is for isolating 2 ml whole blood sample. This procedure can be scaled up and down for use with fresh or frozen blood samples 1 ml to 3 ml in volume by scaling reagent volume up and down in proportion to the volume of sample used. (Except the Elution 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.

Reagent volumes required for processing 1-3 ml whole blood samples

Reagent	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
Elution Buffer	200 µl	200 µl	300 µl

Materials to be supplied by user

- Microcentrifuge capable of 2,000 x g
- Nuclease-free 15 ml centrifuge tubes
- Water Bath preset at 37°C and 65 °C
- 100% isopropanol
- 70% ethanol

1. **Add 2 ml whole blood (or bone marrow) to a nuclease-free 15 ml centrifuge tube containing 5 ml NL Buffer. Mix by inverting the tube 5 times.**

2. **Centrifuge at 2000 x g for 5 minutes at room temperature. Remove and discard supernatant. Leave the tube inverted on an absorbent paper for 2-3 minutes. Make sure the pellet remains in the tube.**

3. **Add 1 ml XL Buffer/Proteinase K mixture to the tube containing the nuclei pellet. Vortex immediately for 10 seconds or until the pellet is completely homogenized. Important: When processing multiple samples, vortex each tube immediately after addition of XL/Proteinase K mixture.**

4. **Incubate at 65 °C for 5 minutes in a water bath or heating block.**

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

5. **Add 1 ml isopropanol and gently mix the solution by inverting the tube 20-30 times or until the DNA precipitate become visible as threads or clumps.**
6. **Centrifuge at 2000 x g for 5 minutes at room temperature. DNA will be visible as a small white pellet.**
7. **Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel.**
8. **Add 1ml of 70% ethanol and vortex the tube for 10 seconds to wash the DNA pellet.**
9. **Centrifuge at 2000 x g for 3 minutes at room temperature. Carefully pour off the ethanol. Pellet may be very loose at this point, so pour slowly and watch the pellet.**
10. **Invert the tube on a clean absorbent paper towel and air dry the pellet for 10-15 minutes.**
11. **Add 200 µl of DNA rehydration solution (Elution Buffer) and vortex for 20 seconds to mix.**
12. **Incubate sample at 65 °C for 10 mins. Some samples may need to incubate at 65 °C for 1 hour to rehydrate DNA.**
13. **Store DNA at 2-8 °C. For long-term storage, store at -20 °C.**

C. DNA Purification Protocol for 4 -14 ml whole blood

NOTE: The buffer volume of the following protocol is for isolating 12 ml whole blood sample. This procedure can be scaled up and down for use with fresh or frozen blood samples 4 ml to 14 ml in volume by scaling reagent volume up and down in proportion to the volume of sample used. (Except the Elution 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.

Reagent volumes required for processing 4-8 ml whole blood samples

Reagent	**Blood Volume**				
	4 ml	5 ml	6 ml	7 ml	8 ml
NL Buffer	10 ml	12.5ml	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
Elution Buffer	400 uL	500 µl	600 µl	700 µl	800 µl

Reagent volumes required for processing 9-14 ml whole blood samples

Reagent	**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
Elution Buffer	900 uL	1 ml	1 ml	1 ml	1 ml	1 ml

Materials to be supplied by user

- Microcentrifuge capable of 2000 x g
- Nuclease-free 50 ml centrifuge tubes
- Water Bath preset at 37 °C and 65 °C
- 100% isopropanol
- 70% ethanol

1. Add 12 ml whole blood (or bone marrow) to a nuclease-free 50 ml centrifuge tube containing 30 ml NL Buffer. Mix by inverting the tube 5 times.
2. Centrifuge at 2000 x g for 5 minutes at room temperature. Remove and discard supernatant. Leave the tube inverted on a absorbent paper for 2-3 minutes. Make sure the pellet remains in the tube.
3. Add 5 ml XL Buffer/Proteinase K mixture to the tube containing the nuclei pellet. Vortex immediately for 10 seconds or until the pellet is completely homogenized.

   Important: When process multiple samples, vortex each tube immediately after addition of XL/Proteinase K mixture.
4. Incubate at 65 °C for 5 minutes in a water bath or heating block.

   Note: The sample should change color from red to olive green during proteinase digestion
5. Add 5 ml isopropanol and gently mix the solution by inverting the tube 20-30 times or until the DNA precipitate become visible as threads or clumps.
6. Centrifuge at 2000 x g for 5 minutes at room temperature. DNA will be visible as a small white pellet.
7. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel.
8. Add 5 ml of 70% ethanol and vortex the tube for 10 seconds to wash the DNA pellet.
9. Centrifuge at 2000 x g for 3 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point, so pour slowly and watch the pellet.**
10. Invert the tube on a clean absorbent paper towel and air dry the pellet for 10-15 minutes.
11. Add 1 ml of DNA rehydration solution (Elution Buffer) and vortex for 1 minute to mix.
12. Incubate sample at 65°C for 1 hour. Some sample may need to incubate at 65 °C for overnight to rehydrate DNA.
13. Store DNA at 2-8 °C. For long-term storage, store at -20 °C.

D. DNA Purification Protocol for 20 ml whole blood

Materials to be supplied by user

- Microcentrifuge capable of 2000 x g
- Nuclease-free 50 ml centrifuge tubes
- Water Bath preset at 37 °C and 65 °C
- 100% isopropanol
- 70% ethanol

1. Add 10 ml whole blood (or bone marrow) to a nuclease-free 50 ml centrifuge tube containing 25 ml NL Buffer. Mix by inverting the tube 5 times.
2. Centrifuge at 2000 x g for 5 minutes at room temperature. Remove and discard supernatant. Make sure the pellet remains in the tube.
3. Add another 10 ml whole blood (or bone marrow) to a nuclease-free 50 ml centrifuge tube containing 25 ml NL Buffer. Mix by inverting the tube 5 times.
4. Centrifuge at 2000 x g for 5 minutes at room temperature. Remove and discard supernatant. Leave the tube inverted on an absorbent paper for 2-3 minutes. Make sure the pellet remains in the tube.
5. Add 5 ml XL Buffer/Proteinase K mixture to the tube containing the nuclei pellet. Vortex immediately for 10 seconds or until the pellet is completely homogenized. **Important**: When process multiple samples, vortex each tube immediately after addition of XL/Proteinase K mixture.
6. Incubate at 65 °C for 5 minutes in a water bath or heating block.

   NOTE: The sample should change color from red to olive green during proteinase digestion
7. Add 5 ml isopropanol to the lysate and gently mix the solution by inverting the tube 20-30 times or until the DNA precipitate become visible as threads or clumps.
8. Centrifuge at 2000 x g for 5 minutes at room temperature. DNA will be visible as a small white pellet.
9. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel.
10. Add 5 ml of 70% ethanol and vortex the tube for 10 seconds to wash the DNA pellet.
11. Centrifuge at 2000 x g for 3 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 ml of DNA rehydration solution (Elution Buffer) and vortex for 1 minute to mix.
14. Incubate sample at 65 °C for 1 hour. Some sample may need to incubate at 65 °C for overnight to rehydrate DNA.
15. 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.