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Revised April 2007

Introduction

DNA isolation from fish or avian blood sample could be difficult because they comitans nucleated red blood cells. E.Z.N.A.™ SQ NRBC Blood DNA Kit is designed for isolating high molecular weight genomic DNA from fresh, frozen blood samples contains nucleated red blood cells. The kit allows single or multiple, simultaneous processing of samples in under 60 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 NRBC Blood DNA method is ready for applications such as PCR*, Southern blotting, and restriction digestion.

Principle

E.Z.N.A.® SQ NRBC 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 fish or avian whole blood samples. Blood samples are first resuspend with RS1 Buffer, and then immediately lysed with RL1 buffer in the presence of proteinase K. Proteins and other contaminates are precipitated with PCP buffer 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 NRBC Blood DNA Kit should be stored at 22ºC-25ºC. Under cool ambient conditions, a precipitate may form in the Buffer RS1. In case of such an event, heat the bottle at 55ºC to dissolve.

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

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

DNA Yields From Various Starting Materials

Species and Material	Amount of Starting material	Typical Yield
Fish Blood	2 µL	5-15 µg
	5 µL	7.5-37.5 µg
	10 µL	25-75 µg
	40 µL	100-300 µg
Avian Blood	2 µL	5-15 µg
	5 µL	7.5-37.5 µg
	10 µL	25-75 µg
	40 µL	100-300 µg

Kit Contents

Product	D0713-05	D0713-10
Total Blood Volume	0.5 mL	10 mL
Buffer RS1	40 mL	650 mL
Buffer RL1	40 mL	650 mL
PCP Buffer	25 mL	450 mL
EB Buffer	30 mL	500 mL
RNase A (25mg/ml)	120 µL	2 mL
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A. DNA Purification Protocol for 2 µL whole blood with Nucleated Red Blood Cells

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 and 65°C
- Isopropanol
- 70% ethanol

1. **Collect blood with syringe contains 100mM EDTA at a final concentration of 20µl/ml of blood.**
2. **Add 2µL whole blood to a nuclease-free 1.5 mL microcentrifuge tube containing 150 µL Buffer RS1.** Mix throughly by vortexing for 20 seconds.
3. **Immediately add 150 µl of Buffer RL1 and 0.4 µl RNase A solution, mix throughly by pipetting up and down for 3-5 times to mix the sample**. Incubate at 37°C for 10 minutes to digest RNA.
4. **Optional:** add 2ul Proteinase K (20mg/ml) into the lysate, incubate at 55°C for 30-60 minutes to digest proteins.
5. Cool the sample down to room temperature. **Add 100 µl of PCP Buffer to the sample. Mix the sample throughly by vortexing at maxi speed for 20 seconds.**
6. **Incubate on ice for 10 minutes.**
7. **Centrifuge at ≥14,000 x g for 3 minutes at 4°C.** The precipitated protein will form a tight pellet. If the pellet is not tight or visible, incubate the tube in ice for 10 minutes and repeat Step 7.
8. **Transfer the supernatant to a new nuclease-free 1.5 mL centrifuge tube containing 300 µL of 100% isopropanol.**
9. Gently mix the solution by inverting the tube 30-40 times.
10. Centrifuge at ≥14,000 x g for 3 minute. 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 300µL of 70% ethanol and invert the tube few times to wash the DNA pellet.**

12. Centrifuge at 10,000 x g for 3 minutes. 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 absorbent paper towel and air dry the pellet for 10 minutes.

14. **Add 50-100 µL of Buffer EB.** Incubate sample at 65ºC for 30-60 min to dissolve DNA Pellet. Some samples may need to incubate at room temperature for overnight rehydrate DNA.

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

B. DNA Purification Protocol for 5 µL whole blood with Nucleated Red Blood Cells

Materials to be supplied by user

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water Bath preset at 65˚C
- Isopropanol
- 70% ethanol

1. **Collect blood with syringe contains 100mM EDTA at a final concentration of 20µl/ml of blood.**

2. **Add 5µL whole blood to a nuclease-free 1.5 mL microcentrifuge tube containing 300 µL Buffer RS1.** Mix throughly by vortexing for 20 seconds.

3. **Immediately add 300 µl of Buffer RL1 and 1ul Rnase Solution, mix throughly by pipetting up and down for 3-5 times to mix the sample.** Incubate at 37˚C for 10 minutes to digest RNA.

4. **Optional:** add 5ul Proteinase K (20mg/ml) into the lysate, incubate at 55˚C for 30-60 minutes to digest proteins.

5. Cool the sample down to room temperature. **Add 200µl of PCP Buffer to the sample. Mix the sample throughly by vortexing at maxi speed for 20 seconds.**

6. Incubate on ice for 10 minutes.

7. **Centrifuge at ≥14,000 x g for 3 minutes at 4˚C.** The precipitated protein will form a tight pellet. If the pellet is not tight or visible, incubate the tube in ice for 10 minutes and repeat Step 7.

8. **Transfer the supernatant to a new nuclease-free 1.5 mL centrifuge tube containing 600 µL of 100% isopropanol.**

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

10. **Centrifuge at ≥14,000 x g for 3 minute.** 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 600µL of 70% ethanol and invert the tube few times to wash the DNA pellet.**

12. Centrifuge at 10,000 x g for 3 minutes. 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 absorbent paper towel and air dry the pellet for 10-15 minutes.

14. **Add 50-100 µL of Buffer EB. Incubate sample at 65ºC for 30-60 min to dissolve the DNA Pellet.** Some samples may need to incubate at room temperature for overnight rehydrate DNA.

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

C. DNA Purification Protocol for 10 µL whole blood with Nucleated Red Blood Cells

Materials to be supplied by user

- Microcentrifuge capable of 3000 x g
- Nuclease-free 15 mL microcentrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

1. **Collect blood with syringe contains 100mM EDTA at a final concentration of 20µl /ml of blood.**

2. **Add 10µL whole blood to a nuclease-free 15 mL microcentrifuge tube containing 600µL RS1 Buffer**. Mix by vortexing the tube for 20 seconds.

3. **Immediately add 600 µl of Buffer RL1 and 2 ul Rnase Solution, mix throughly by pipetting up and down for 3-5 times to mix the sample.** Incubate at 37°C for 10 minutes to digest RNA.

4. **Optional:** add 10ul Proteinase K (20mg/ml) into the lysate, incubate at 55°C for 30-60 minutes to digest proteins.

5. Cool the sample down to room temperature. **Add 400µl of PCP Buffer to the sample. Mix the sample throughly by vortexing at maxi speed for 20 seconds.**

6. **Incubate on ice for 10 minutes.**

7. **Centrifuge at ≥2,000 x g for 10 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 in ice for 5 minutes and repeat Step 7.

8. **Transfer the supernatant to a new nuclease-free 15 mL centrifuge tube containing 1.2 ml 100% isopropanol.**

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

10. Centrifuge at ≥2,000 x g for 5 minute. 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 1.2 mL of 70% ethanol and invert the tube few times to wash the DNA pellet.

12. Centrifuge at ≥2,000 x g for 2 minutes. 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 absorbent paper towel and air dry the pellet for 10-15 minutes.

14. Add 100-400 µL of Buffer EB. Incubate sample at 65ºC for 30-60min to dissolve the DNA Pellet. Some samples may need to incubate at room temperature for overnight rehydrate DNA.

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

D. DNA Purification Protocol for 20 µL whole blood with Nucleated Red Blood Cells

Materials to be supplied by user

- Microcentrifuge capable of 3000 x g
- Nuclease-free 15 mL microcentrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

1. **Collect blood with syringe contains 100mM EDTA at a final concentration of 20µl /ml of blood.**

2. **Add 20µL whole blood to a nuclease-free 15 mL microcentrifuge tube containing 1.2 mL RS1 Buffer**. Mix by vortexing the tube for 20 seconds.

3. **Immediately add 1.2 ml of Buffer RL1 and 4 ul Rnase Solution, mix**

throughly by pipetting up and down for 3-5 times to mix the sample. Incubate at 37°C for 10 minutes to digest RNA.

4. **Optional:** add 20ul Proteinase K (20mg/ml) into the lysate, incubate at 55°C for 30-60 minutes to digest proteins.

5. **Cool the sample down to room temperature. Add 800µl of PCP Buffer to the sample. Mix the sample throughly by vortexing for 20 seconds.**

6. **Incubate on ice for 10 minutes.**

7. **Centrifuge at ≥2,000 x g for 10 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 in ice for 5 minutes and repeat Step 6.

8. **Transfer the supernatant to a new nuclease-free 15 mL centrifuge tube containing 2.4 ml 100% isopropanol.**

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

10. **Centrifuge at ≥2,000 x g for 10 minute.** 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 2.4 mL of 70% ethanol and invert the tube few times to wash the DNA pellet.**

12. Centrifuge at ≥2,000 x g for 5 minute. 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 absorbent paper towel and air dry the pellet for 10-15 minutes.

14. **Add 200-400 µL of Buffer EB.** Incubate sample at 65ºC for 30-60 min to dissolve the DNA Pellet. Some samples may need to incubate at room temperature for overnight rehydrate DNA.

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

E. DNA Purification Protocol for 40 µL whole blood with Nucleated Red Blood Cells

Materials to be supplied by user

- Microcentrifuge capable of 3000 x g
- Nuclease-free 15 mL microcentrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

1. **Collect blood with syringe contains 100mM EDTA at a final concentration of 20µl /ml of blood.**

2. **Add 40µL whole blood to a nuclease-free 15 mL microcentrifuge tube containing 2.4 mL RS1 Buffer.** Mix by vortexing the tube for 20 seconds.

3. **Immediately add 2.4 mL of Buffer RL1 and 10 ul Rnase Solution mix throughly by pipetting up and down for 3-5 times to mix the sample.** Incubate at 37°C for 10 minutes to digest RNA.

4. **Optional:** add 40ul Proteinase K (20mg/ml) into the lysate, incubate at 55°C for 30-60 minutes to digest proteins.

5. **Cool the sample down to room temperature. Add 1.6 mL of PCP Buffer to the sample.** Mix the sample throughly by vortexing at maxi speed for 20 seconds.

6. **Centrifuge at ≥2,000 x g for 10 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 in ice for 5 minutes and repeat Step 6.

7. **Transfer the supernatant to a new nuclease-free 15 mL centrifuge tube containing 4.8 ml 100% isopropanol.**

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

9. **Centrifuge at ≥2,000 x g for 5 minute.** 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 4.8 mL of 70% ethanol and invert the tube few times**

to wash the DNA pellet.

11. Centrifuge at ≥2,000 x g for 5 minutes. 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 500 µL of Buffer EB. Incubate sample at 65ºC for 30-60 min to dissolve the DNA Pellet. Some samples may need to incubate at room temperature for overnight rehydrate DNA.

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

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl buffer, or Elution Buffer as blank. Dilute the DNA in TE buffer and calculate concentration as:

[DNA] = (Absorbance$_{260}$) x (0.05 µg/µL*) x (Dilution factor)*

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio of (A_{260}/A_{280}) of 1.7-1.9 corresponds to 85%-95% purity.

Expected yields range from 4 µg to 12 µg DNA per 250 µL whole blood, depending on source of sample, its age, and the method of storage. Yields are generally 5-fold higher with Buffy Coat samples.

Troubleshooting Guide

Problem	Possible Cause	Suggestions
Low DNA yield	Blood sample is too old.	Try to use fresh blood if possible.
	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.
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 RL1	Repeat the procedure, this time making sure to vortex the sample with RS1 Bufefr and add Buffer RL1 immediately and mix throughly.
	PCP Buffer was not mixed with sample 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.