Contents

Introduction 2

Storage and Stability 2

Applications 2

Kit Contents 3

Before Starting 3

A. SE Blood DNA Kit Protocol (for up to 300µl whole Blood) 4

B. SE Blood DNA Kit Protocol (for 0.3-1 ml whole Blood) 6

C. Buffy Coat 7

Determination of DNA Yield and Quality 7

Troubleshooting Guide 8

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Introduction

The E.Z.N.A.® SE Blood DNA Kit provides a rapid and easy method for the isolation of genomic DNA from 1 µl-1ml fresh, frozen, and anticoagulated whole blood. The kit allows single or multiple, simultaneous processing of samples in less than 30 minutes. Normally, up to 300 µl of whole blood can be used in a single experiment. There is no need for phenol-chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation, and precipitation with isopropanol or ethanol, are eliminated. DNA purified using the E.Z.N.A.® SE Blood DNA method is ready for applications such as PCR*, Southern blotting, and restriction digestion.

The E.Z.N.A.® SE Blood DNA Kit uses the reversible nucleic acid-binding properties of Omega Bio-Tek's HiBind® matrix, combined with the speed of mini-column spin technology. A specially formulated buffer system allows genomic DNA up to 60 kb to bind to the matrix. Samples are first lysed under denaturing conditions and then applied to the HiBind® DNA spin columns to which DNA binds, while cellular debris, hemoglobin, and other proteins are effectively washed away. High quality DNA is finally eluted in sterile deionized water or low salt buffer.

Storage and Stability

All components of the E.Z.N.A.® SE Blood DNA Kit should be stored at 22°C-25°C, expect that RNase A should be stored at -20°C. Under these conditions, DNA has successfully been purified and used for PCR after 24 months of storage. Under cool ambient conditions, a precipitate may form in Buffer BL. In case of such an event, heat the bottle at 37°C to dissolve.

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

Applications

The E.Z.N.A.® SE Blood DNA Kit don't require Proteinase K or OB Protease to digest samples. This kits is suitable for isolation of genomic DNA from whole blood, purified leukocytes and some animal cells. Some samples may need to add Proteinase K to increase the yield. At this conditions, you may choose E.Z.N.A.® Blood DNA Kit (D3392). For recovery of DNA from dried blood spots use the E.Z.N.A.® Forensic DNA Kits (D3591) or order Buffer TL (TL-100, 100 ml) to use with the Blood DNA Kit. For isolation of DNA or Viral DNA from blood or other body fluids, use the E.Z.N.A.® Blood DNA Kit.

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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.

Kit Contents

Product Number	D3471-00	D3471-01	D3471-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind® DNA columns	5	50	200
2 ml Collection Tubes*	5	50	200
Buffer ERL (10x)	3 ml	25 ml	70 ml
Buffer WTL	2 ml	20 ml	60 ml
Buffer BL	2 ml	20 ml	60 ml
DNA Wash Buffer Concentrate	2 ml	20 ml	3 x 20 ml
RNase A	12 µl	120 µl	420 µl
Buffer HB	3 ml	30 ml	110 ml
Elution Buffer	2 ml	15 ml	60 ml
User Manual	1	1	1

Buffer BL contains a chaotropic salt. Use gloves and protective eyeware when handling this solution.

Before Starting

IMPORTANT

1 **Dilute 10 x Buffer ERL** as follows and store diluted 1 x Buffer ERL at room temperature.

D3471-00 Add 27 ml ddH_2O

D3471-01 Add 225 ml ddH_2O in a new bottle

D3471-02 Dilute with 630 ml ddH_2O in a new bottle

2 **DNA Wash Buffer Concentrate** must be diluted with absolute ethanol as follows and store at room temperature:

D3471-00 Add 8 ml absolute ethanol (96-100%)

D3471-01 Add 80 ml absolute ethanol to bottle

D3471-02 Add 80 ml absolute ethanol to each bottle

All centrifugation steps must be carried out at room temperature.

A. SE Blood DNA Kit Protocol (Up to 300ul whole blood)

Materials to be Provided by User

- **Tabletop microcentrifuge and nuclease-free 2 ml tubes**
- **Water bath -** set to 65°C
- **Incubator or heat block** - preset to 37°C
- **Absolute Ethanol (96%-100%)**
- **Optional: Proteinase K (20mg/ml)**

1. Transfer the blood sample to a 2 ml nuclease-free micro-centrifuge tube. **Add 3 volume of 1 x Buffer ERL and mix by inverting the tube a few times.** Incubate 5 minutes at room temperature. Invert the tube several times during the incubation.
   NOTE: Buffer ERL is supplied as a 10 x concentrate and must be diluted with ddH_2O according to bottle label or 'before use' on Page 3.

2. **Centrifuge at 14,000 x g for 30 seconds at room temperature.** Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 10µl of residue liquid in the tube.
   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 1 x Buffer ERL. Incubate at room temperature for 2 minutes. Then pellet the white blood cells by repeating Step 2.

3. **Vortex to resuspend cells in residue liquid and add 240 µl Buffer WTL to the tube.** Pipet up and down or vortex at maxi speed for 30 seconds to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

4. **(Optional for maximum yield) Add 3 µl Proteinase K (20mg/ml) to the cell lysate and vortex to mix well.** Incubate at 55°C for 10 -30 minutes.

5. **Add 2 µl RNase A solution to the cell lysate and mix well**. Incubate the mixture at room temperature for 10 minutes.

6. **Add 250µl Buffer BL and mix throughly by vortexing, Incubate at 65°C for 10 min.** Vortex to mix twice during incubation.

7. **Add 250 µl absolute ethanol to the lysate and mix completely by vortexing.** If precipitation can be seen at this point, break the precipitation by pipetting up and down 10 times.

8. Insert a HiBind® DNA column in a 2 ml collection tube (provided). **Load the lysate from Step 7 (including any precipitation) into the column and centrifuge at ≥8,000 x g for 1 min to bind DNA.** Discard flow-through and re-use the collection tube.

9. Insert the column back into the 2 ml collection tube and **wash by adding 500µl HB Buffer. Centrifuge at ≥8,000 x g for 1 min.** Discard flow-through liquid and re-use the 2 ml collection tube.

10. Insert the column back into the 2 ml collection tube **and wash by adding 650 µl of DNA Wash Buffer diluted with ethanol.** Centrifuge at ≥8,000 x g for 1 min. Discard flow-through liquid and re-use the collection tube in the next step.

    Note: DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle label or Page 3. ***If refrigerated, the diluted wash buffer must be brought to room temperature before use.*** Refrigeration is NOT recommended.

11. Insert the column back into the 2 ml collection tube and **wash by adding another 650 µl of DNA Wash Buffer diluted with ethanol.** Centrifuge at ≥8,000 x g for 1min. Discard flow-through liquid and re-use the collection tube in the next step.

12. Insert the column back into the empty 2 ml collection tube, **centrifuge the empty column at maximum speed (≥13,000 x g) for 2 min to dry the column membrane.**

    This step is critical for removal of trace ethanol that might otherwise interfere with downstream applications.

13. **Place the column into a nuclease-free 2 ml microfuge tube and add 50-100 µl of preheated (70°C) Elution Buffer.** Allow column to set for 5 min at room temperature. (For higher yields, incubate 5 min at 60°C rather than at room temperature.)

14. **To elute DNA from the column, centrifuge at 10,000 x g for 1 min**. Retain flow-through containing the DNA.

15. **Place column into a second 1.5 ml tube and repeat elution step with another 50-100 µl of preheated Elution Buffer.**
    Note: Each elution typically yields 60%-70% of the DNA bound to the column. Thus two elutions generally yield >90%. However, increasing elution volume reduces the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using two of 50 µl Elution Buffer. Volumes lower than 50 µl greatly reduce yields. ***Alternatively, use the first eluate to perform the second elution.***

B. SE Blood DNA Kit Protocol for 400µl -1 ml whole Blood

Materials Supplied by User

- Microcentrifuge capable of at least 13,000 x g
- Table top centrifuge capable of 2000 x g with rotor for 15 ml tubes
- Water bath or heat block preset at 37°C
- Nuclease-free 1.5 ml microcentrifuge tube
- Optional: Proteinase K (20mg/ml)

1. Add up to 1 ml whole blood sample into a 15 ml tube. **Add 3 volume of 1 X Buffer ERL and mix throughly by inverting the tube a few times**. Incubate 5 minutes at room temperature. Invert the tube several times during the incubation.
   NOTE: Buffer ERL is supplied as a 10 x concentrate and must be diluted with ddH_2O according to bottle label or Page 3 before use.

2. **Centrifuge at 2,000 x g for 5 minutes at room temperature.** Remove and discard as much supernatant as possible without disturbing the visible white pellet. Leave about 20µl of residue liquid in the tube.
   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 1 x Buffer ERL. Incubate at room temperature for 2 minutes. Then pellet the white blood cells by repeating Step 2.

3. **Vortex to resuspend cells in residue liquid and then add 230 µl Buffer WTL.** Pipet up and down or vortex at maxi speed for 30 seconds to lyse the cells. The solution should become very viscous. If cell clumps are visible after mixing, incubate the solution at 37°C until the clumps cannot be seen.

4. **Optional for maximum yield: Add 3 µl Proteinase K (20mg/ml) to the cell lysate and vortex to mix well.** Incubate at 55°C for 30 minutes.

5. **Add 2µl RNase A solution to the cell lysate and mix well.** Incubate the mixture at room temperature for 10 minutes.

6. **Add 250µl Buffer BL and mix throughly by vortexing.** Incubate at 65°C for 10 min. Vortex to mix twice during incubation.

7. **Add 250 µl absolute ethanol to the lysate and mix immediately by vortexing.** If precipitation can be seen at this point, break the precipitation by pipetting up and down 10 times.

8. Following Protocol A step 8-15 on page 5.

C. Buffy Coat

The buffy coat fraction of whole blood is enriched with WBC, and usually gives at least 5-fold more DNA than the same volume of blood. To prepare buffy coat from fresh whole blood, simply centrifuge the sample at 3,000-4,000 x g for 10 min at room temperature. Three layers should be obtained with plasma in the upper layer, leukocytes in the middle layer (buffy coat), and erythrocytes in the 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.® SE Blood DNA Protocol or frozen at -70°C for storage.

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.

Blood is a complex mixture of cells, proteins, metabolites and many other substances. About 50% of human blood volume is comprised of cells, more than 99% of which are erythrocytes. Therefore, these cells are unsuitable for isolation of genomic DNA because they don't contain nuclei. Only 0.3% leukocytes contain nuclei. Normally, healthy blood, for example contains fewer than 10^7 leukocytes per 1ml, while blood from an infected donor may have a tenfold higher leukocyte concentration. 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
Clogged Column	Incomplete lysis	Add the correct volume of Buffer BL and incubate for specified time at 70°C. It may be necessary to extend incubation time by 10 min.
	Sample too large	Reduce the sample volume.
	Sample too viscous	Increase the volume of all buffer proportionally
Low DNA Yield	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume
	Improper washing	DNA Wash Buffer Concentrate must be diluted with absolute ethanol
Low A_{260}/A_{280} ratio	Extended centrifugation during elution step.	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation — it will not interfere with PCR or restriction digests.
	Poor cell lysis due to incomplete mixing with Buffer WTL	Repeat the procedure, this time making sure to vortex the WBC pellet with ERL residue in the tube before mix Buffer WTL
	Hemoglobin remains	Repeat the RBC lysis step with step 1-3
No DNA Eluted	improper mixing with Buffer BL.	Mix thoroughly with Buffer BL prior to loading HiBind™ column.
	Absolute ethanol not added to Buffer BL.	Before applying sample to column, an aliquot of Buffer BL/ethanol must be added. See protocol above.
	No ethanol added to Wash Buffer Concentrate.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.
Washing Leaves Colored Residue In Column	improper mixing with Buffer BL.	Buffer BL is viscous and the sample must be vortexed thoroughly.
	No ethanol added to Wash Buffer	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.
Eluted Material Has Red/Brown Color	Sample too large.	Reduce sample volume and follow directions