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Introduction

The E.Z.N.A.® Blood DNA Kit provides a rapid and easy method for the isolation of genomic DNA from 1 µl-200µl fresh, frozen, and anticoagulated whole blood. The method can also be used for preparation of genomic DNA from buffy coat, serum, and plasma. The kit allows single or multiple, simultaneous processing of samples in less than 90 minutes. With modified protocol, up to 1 ml 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.® or the E-Z 96 ® Blood DNA method is ready for applications such as PCR*, Southern blotting, and restriction digestion.

Principle

E.Z.N.A.® Blood DNA Kits use 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 50 kb to bind to the matrix. Samples are first lysed under denaturing conditions and then applied to the HiBind® spin columns to which DNA binds, while cellular debris, hemoglobin, and other proteins are effectively washed away. High quality DNA is finally eluted in Elutioin Buffer provided, sterile deionized water or low salt buffer.

Storage and Stability

All components of the E.Z.N.A.® Blood DNA Kit, except the OB Protease should be stored at 22°C-25°C. Once reconstituted in water, OB Protease must 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.

Binding Capacity

Each HiBind® column can bind up to approximately 100 µg DNA. Use of more than 1 ml whole blood or 250 µl buffy coat is not recommended.

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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	D3482-00 D3392-00	D3492-01 D3392-01	D3492-02 D3492-02
Components			
HiBind® DNA columns	5	50	200
2 ml Collection Tubes	15	150	600
Buffer BL	5 ml	20 ml	60 ml
DNA Wash Buffer Concentrate	12 ml	24 ml	3 x 40 ml
HB Buffer	3 ml	30 ml	120 ml
Elution Buffer	2 ml	30 ml	100 ml
OB Protease	3 mg	30 mg	120 mg
RNase A	30µl	260µl	1.10 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 **D3492 & D3392**: Reconstitute OB Protease to each vial as follows and store vials of reconstituted protease at -20°C.:	
	D3492-00 /D3392-00:	Add 150µl Elution Buffer
	D3492-01/D3392-01	Add 1.5ml Elution Buffer
	D3492-02 D3392-02	Add 6.0 ml Elution Buffer
	2 Wash Buffer Concentrate must be diluted with absolute ethanol as follows and store at room temperature:	
	D3492-00 & D3392-00	Add 18 ml ethanol
	D3492-01 & D3392-01	Add 36 ml ethanol / bottle
	D3492-02 & D3392-02	Add 60 ml ethanol / bottle

All centrifugation steps must be carried out at room temperature.

A. Blood DNA Protocol

Materials Supplied by User
Have the following reagents and supplies ready:

- **Tabletop microcentrifuge and nuclease-free 1.5 ml tubes**
- **Water bath -** set to 70°C
- **Incubator or heat block** - preset to 60°C
- **Isopropanol -** approximately 0.3 ml per sample
- **Absolute Ethanol (96%-100%) -** (for buffy coat, cultured cells and leukocytes) approximately 0.3 ml per sample
- **10 mM Tris-HCl, pH 8.0**

NOTE: The procedure below has been optimized for use with FRESH or FROZEN blood samples 1 µl to 250 µl in volume. Anticoagulated blood, or Buffy Coat can also be used. Larger samples (up to 1 ml) can be processed according to the modification outlined on Page 4. In addition, $\leq 10^7$ leukocytes or cultured cells may be used with this procedure. For DNA extraction from tissue and mouse tail we suggest using the **E.Z.N.A. Tissue DNA Kit** (Product Number **D3396**). To isolate viral DNA from serum or other non-cellular body fluids use **the E.Z.N.A. Blood RNA Kit (R6814)**.

Bring samples and OB Protease solution to room temperature and have a water bath equilibrated to 60°C. Preheat an aliquot of Elution Buffer (approximately 0.5 ml per sample) at 60°C. **Carry out all centrifugation steps at room temperature**.

1. Add sample to a nuclease-free microcentrifuge tube and bring the volume up to 250 µl with 10 mM Tris-HCl, PBS, or Elution Buffer provided.

2. Add 25 µl OB Protease and mix throughly by vortexing. Add 250 µl of Buffer BL.. Vortex 10 sec to mix thoroughly.

3. Add 5 µl RNase A solution to each sample to remove RNA (if desired). Optionally, a solution of 5 µl RNase A per 250 µl Buffer BL (20 µl RNase A/1 ml Buffer BL) can be prepared in advance of Step 2 above to simplify delivery; in which case 255 µl Buffer BL/RNase A solution should be added to sample in Step 2.

4. Incubate sample at 60°C for 10 min.

5. Briefly vortex the tube once during incubation.

6. Add 260 µl of isopropanol to lysate and mix completely by vortexing. ***For buffy coat, isolated leukocytes and cultured cells, yields will improve if 260 µl absolute ethanol is used in place of isopropanol.***

7. Insert a HiBind® DNA spin column in a 2 ml collection tube (provided). Transfer the solution from Step 6 into the column and centrifuge at ≥8,000 x g for 1 min to bind DNA. **Discard the collection tube and flow-through liquid**.

Note: For buffy coat, leukocytes and cultured cells, centrifuge at maximum speed in step above to avoid clogging the spin column.

8. Insert the column into a **second 2 ml tube** and wash by adding 500µl HB Buffer. Centrifuge at 8,000 x g for 30 sec. Discard flow-through liquid; re-use the collection tube.

9. Insert the column into the **same 2 ml tube** from Step 8 and wash by adding 650 µl of DNA Wash Buffer diluted with ethanol. Centrifuge at 8,000 x g for 30 sec. 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 and Page 3. ***If refrigerated, the diluted wash buffer must be brought to room temperature before use.*** Refrigeration is NOT recommended.

10. Using the same 2ml collection tube, centrifuge empty column at maximum speed (>10,000 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.***

11. Place the column into a nuclease-free 1.5 ml microfuge tube and add 200 µl of preheated (70°C) Elution Buffer. Allow tubes to sit for 5 min at room temperature. (For higher yields, incubate 5 min at 60°C rather than at room temperature.)

12. To elute DNA from the column, centrifuge at 8,000 x g for 1 min. Retain flow-through containing the DNA. Place column into a second 1.5 ml tube and repeat elution step with another 200 µl of preheated Elution Buffer. Discard column.

   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 50 µl to 100 µl Elution Buffer. Volumes lower than 50 µl greatly reduce yields. ***Alternatively, use the first eluate to perform the second elution.***

If necessary the DNA can be concentrated. Add sodium chloride to a final concentration of 0.1 M followed by 2X volume of absolute (96%-100%) ethanol. Mix well and incubate at -20°C for 10 min. Centrifuge at 10,000 x g for 15 min and discard supernatant. Add 700 µl of 80% ethanol and centrifuge at 10,000 x g for 2 min. Discard supernatant, air dry the pellet (2 min) and resuspend DNA in 20 µl sterile deionized water or 10 mM Tris-HCl, pH 8. The expected yield from 250 µl blood is approximately 4-12 µg DNA.

B. Protocol for 1 ml of Blood

The following protocol is for rapid isolation of DNA from blood samples 250 µl to 1 ml in volume by increasing the volumes of OB Protease, Buffer BL, and isopropanol (or absolute ethanol) in proportion. However, this short protocol is not designed to obtain maximum DNA yield, please use E.Z.N.A®. Blood Midiprep Kit for 250 µl-1 ml blood sample if the maximum yield is desired.

1. Use up to 1 ml whole blood, plasma, serum, or body fluids (or 50 million leukocytes or cultured cells in 1 ml PBS). Adjust the total volume using PBS to a multiple of 250 µl.

2. Add 25 µl OB Protease stock solution per 250 µl of sample. Add 250 µl Buffer BL per 250 µl of sample. Mix thoroughly by vortexing.

3. **Optional:** Add 5 µl RNase A solution per 250 µl of sample to remove RNA. Optionally, a solution of 5 µl RNase A per 250 µl Buffer BL (20 µl RNase A/1 ml Buffer BL) can be prepared in advance of Step 2 above to simplify delivery; in which case 255 µl Buffer BL/RNase A solution should be added to sample in Step 2.

4 Incubate at 60°C for 10 min, mixing once by vortexing.

5. Add 260 µl isopropanol per 250 µl of initial blood volume and completely mix by vortexing ***For buffy coat, isolated leukocytes and cultured cells, yields will improve if 260 µl absolute ethanol is used in place of isopropanol.***.

6. Apply 650 µl of the lysate to a HiBind® DNA column inserted in a 2 ml collection tube. Centrifuge 1 min at 8,000 x g and discard the flow-through. Repeat until the entire lysate has been applied to the single spin column.

7. Follow the basic E.Z.N.A.® Blood DNA Protocol (Page 4) from Step 8.

Use of > 1 ml blood per column is not recommended as doing so can exceed the DNA binding capacity or clog the column, thus reducing yield and quality.

C. Blood Protocol for Dried Blood Samples

Dried blood samples on filter paper can be processed using the following method. You will require Buffer TL for this protocol. We recommend using OB Specimen Paper for spotting blood as this unique filter paper disintegrates when incubated in aqueous buffers and allows efficient recovery of DNA.

1. Cut or punch out the blood spot from the filter paper. (Up to 200 µl blood can be used for each spot.) Tear or cut filter into small pieces and place into a microfuge tube.

2. Add 250 µl Buffer TL and incubate at 95°C for 1-2 h. Vortex to mix every 20 min.

3. Add 25 µl OB Protease solution and mix. Incubate 30 min at 60°C with occasional mixing.

4. Centrifuge at 10,000 x g for 1 min at room temperature. Transfer the supernatant to a clean microfuge tube and add **ONE** volume of Buffer BL followed by **ONE** volume of isopropanol.

Vortex thoroughly to mix.

Tip: For example, if only 200 µl of clear supernatant is obtained, add 200 µl Buffer BL followed by 200 µl isopropanol.

5. Add the mixture to a HiBind® DNA mini column assembled in a 2 ml collection tube and proceed with the basic Blood DNA protocol from Step 8 (Page 4).

Blood spots from finger pricks usually contain no more than 50 µl blood and yield approximately 500 ng to 1 µg DNA. This is usually sufficient for PCR analysis. To obtain higher DNA concentrations, elute with 50 µl preheated Elution Buffer or TE and repeat with the first eluate.

D. 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.® 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.

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	If using more than 250 µl of blood, increase volumes of OB Protease/Proteinase K, Buffer BL, and isopropanol. Pass aliquots of lysate through one column successively.
	Sample too viscous	Divide sample into multiple tubes, adjust volume to 250 µl with 10 mM Tris-HCl.
Low DNA Yield	Clogged column	See above

Problem	Possible Cause	Suggestions
	Poor elution	Repeat elution or increase elution volume (see note on page 4). Incubation of column at 70°C for 5 min with Elution Buffer may increase yields.
	Improper washing	Wash Buffer Concentrate must be diluted with absolute (100%) ethanol as specified on page 5 before use.
	Buffy Coat used	With Buffy Coat samples, use absolute ethanol rather than isopropanol in step 6, page 4.
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 BL	Repeat the procedure, this time making sere to vortex the sample with Buffer BL immediately and completely.
	Hemoglobin remains on column	After application of sample to column, wash once with 300 µl Buffer BL.
No DNA Eluted	Poor cell lysis due to 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	Incomplete lysis due to improper mixing with Buffer BL.	Buffer BL is viscous and the sample must be vortexed thoroughly.
	No ethanol added to Wash Buffer Concentrate.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.
Eluted Material Has Red/Brown Color	Sample volume too large.	Reduce sample volume and follow directions
	Hemoglobin remains on column.	After applying sample, wash column once with 300 µl Buffer BL.