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Introduction

E.Z.N.A.™ Blood DNA Kit provides a rapid and easy method for the isolation of genomic DNA from 1 µl-250 µl fresh, frozen, and anticoagulated whole blood. The method can also be used for preparation of genomic DNA from buffy coat, serum, plasma, saliva, Buccal Swab and other body fluids. The kit allows single or multiple, simultaneous processing of samples in under 30 minutes. Normally, 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.® Blood DNA method is ready for applications such as PCR^{*}, Southern blotting, and restriction digestion.

E.Z.N.A.® Blood DNA Kit uses the reversible nucleic acid-binding properties of HiBind® matrix, combined with the speed of mini-column spin technology. A specifically formulated buffer system allows genomic DNA 30-60 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.® Blood DNA Kit should be stored at 22°C-25°C. Proteinase K should be stored at 15°C-25°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 the Buffer BL. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffer BL at room temperature.

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

Binding Capacity

Each HiBind™ column can bind approximately 30 µg DNA. Using greater than 1 ml whole blood or 250 µl buffy coat is not recommended.

^{*}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 No.	D3392-00	D3392-01	D3392-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind™ DNA Mini columns	5	50	200
2 ml Collection Tubes	15	150	600
Buffer BL	5 ml	20 ml	60 ml
Buffer HB	5 ml	30 ml	110 ml
DNA Wash Buffer	5 ml	20 ml	3 x 20 ml
Elution Buffer	5 ml	40 ml	160 ml
OB Protease	140 µl	1.4 ml	4 x 1.4 ml
User Manual	1	1	1

CAUTION!

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

Before Starting

IMPORTANT	DNA Wash Buffer must be diluted with absolute ethanol (96-100%) as follows:
D3392-00	Add 20 ml absolute ethanol
D3392-01	Add 80 ml absolute ethanol
D3392-02	Add 80 ml absolute ethanol per bottle
Store diluted DNA Wash Buffer at room temperature	

All centrifugation steps must be carried out at room temperature.

A. Blood & Body Fluid DNA Spin Protocol

Materials and equipments Supplied by User

- **Tabletop microcentrifuge and sterile 1.5 ml tubes.**
- **Water bath** - set to 65°C.
- **Ethanol** - approximately 0.3 ml per sample.
- **RNase A** - Prepare a stock solution of RNase A at 50 mg/ml.

NOTE: The procedure below has been optimized for use with FRESH or FROZEN blood samples 1 to 250 µl in volume. Anticoagulated blood, Saliva, Serum, Buffy Coat or other Body Fluid can also be used. 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 **D3495** and **D3496**). To isolate viral RNA from serum or other non-cellular body fluids use **E.Z.N.A.® Viral RNA Kit**.

Bring samples and OB Protease solution to room temperature and have a water bath equilibrated to 65°C. Preheat an aliquot of Elution Buffer (approximately 0.5 ml per sample) at 65°C. **Carry out all centrifugation steps at room temperature.**

1. Add sample to a sterile 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 250 µl of Buffer BL. Vortex at maxi speed for 15s to mix thoroughly. If RNA-free genomic DNA is required, add 5µl RNase A (50mg/ml) to each sample.
3. Incubate sample at 65°C for 10 min.
4. Briefly vortex the tube once during incubation.
5. Add 260 µl of absolute ethanol (room temperature, 96-100%) to lysate and vortex at maxi speed for 20s to mix thoroughly. Briefly centrifuge the tube to collect any drops from the inside of the lid.
6. Assemble an HiBind® DNA Mini column in a 2 ml collection tube (provided). Transfer the lysate from step 5 into the column and centrifuge at 8,000 x g for 1 min to bind DNA. Discard the collection tube and flow-through liquid.
7. Place the column into a **second 2 ml tube** (provided) and wash by pipetting 500 µl of Buffer HB. Centrifuge at 8,000 x g for 1 min. Again, Discard flow-through liquid and reuse the collection tube for next step.

- Place the column into a **same 2 ml tube** from step 7 and wash by pipetting 700 μ l of DNA Wash Buffer diluted with ethanol. Centrifuge at 8,000 x g for 1 min. Again, dispose of collection tube and flow-through liquid.

Note that DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle or page 3. If refrigerated, the diluted wash buffer must be brought to room temperature before use.

- Using a **new collection tube**, wash the column with a second 700 μ l of DNA Wash Buffer and centrifuge as above. Discard flow-through and re-use the collection tube for next step.
- Place the empty column into the same 2 ml collection tube from step 9, centrifuge at maximum speed (15,000 x g) for 2 min to dry the column. **This step is crucial for ensuring optimal elution in the following step.**
- Place the column into a sterile 1.5 ml microfuge tube and add 100-200 μ l of preheated (65°C) Elution Buffer. Allow tubes to sit for 5 min at room temperature.
- 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. Elute DNA again as step 11-12. Discard column and store the eluted DNA at -20°C.

Note: Each elution typically yields 60%-70% of the DNA bound to the column. Thus two elution generally give >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 μ l to 100 μ l Elution Buffer. Volumes lower than 50 μ l 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 2 x volume of absolute (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 μ l 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 μ l sterile deionized water or 10 mM Tris-HCl, pH 8. The expected yield from 250 μ l blood is approximately 4-12 μ g DNA.

B. Blood & Body Fluid DNA Vacuum/Spin Protocol

Material and equipments supplied by user

- **Tabletop microcentrifuge and sterile 1.5 ml tubes**
- **Vacuum Manifold**
- **Water bath** - set to 65°C.
- **Ethanol** -approximately 0.3 ml per sample.
- **RNase A** - Prepare a stock solution of RNase A at 50mg/ml.

- Prepare the lysate by following step 1-5 of Protocol A, Spin protocol on page 4.
- Insert the HiBind® DNA Mini column into the vacuum manifold. Carefully apply the lysate to an HiBind® DNA column. Turn on the vacuum source to draw all liquid through the column. Turn off the vacuum.
Note: If the lysate has difficulty to pass through the column at this stage. Place the column into a collection tube (supplied). Close the lid and centrifuge at 8000 x g for 5 minutes or until all liquid pass through the column. Place the column into another collection tube (supplied) and continue step 7 of the spin protocol.
- Pipet 500 μ l of Buffer HB into the column. Turn on the vacuum source to draw all liquid through the column. Turn off the vacuum.
- Wash the column by pipetting 750 μ l of DNA Wash Buffer diluted with ethanol into the column. Turn on the vacuum source to draw all liquid through the column. Turn off the vacuum.
- Close the lid of HiBind® DNA column, remove it from the vacuum manifold. Insert the column into a collection tube (supplied) and centrifuge at 15,000 x g for 2 minute to completely dry the column.
- Elute DNA as Step 11-12 on page 5.

C. Buccal Swab DNA Spin Protocol

Important Note:

- Due to the increased volume of Buffer BL that required for buccal swab protocol, fewer preparation can be performed. Additional BL Buffer can be purchased separately. (Product # BL-100).
- All centrifugation steps are carried out at room temperature.

Material and Equipments required

- **Tabletop microcentrifuge and sterile 1.5 ml tubes**
- **Water bath** - set to 65°C
- **PBS Buffer**
- **Absolute Ethanol** - approximately 0.3 ml per sample
- **RNase A(Optional)** - Prepare a stock of RNase A at 50mg/ml

1. Place the buccal swab in a 2 ml centrifuge tube. Add 500 µl PBS in the tube. If RNA-free DNA is required for downstream application, add 4 µl RNase A (50mg/ml) into the sample.
2. Add 25µl OB Protease and 500µl Buffer BL into sample. Mix by vortexing at maxi speed for 30s.
3. Incubate at 65°C for 10 minutes. Collect any liquid drop from lid by brief centrifugation.
4. Add 500µl absolute ethanol (room temperature, 96-100%) to the sample and mix by vortexing at maxi speed for 15 seconds. Collect any liquid drop from lid by brief centrifugation.
5. Carefully apply 750µl sample from step 4 into HiBind® DNA Mini column pre-inserted in a 2 ml collection tube (provided). Centrifuge at 8000 x g for 1 minute. Discard the flow-through and collection tube.
6. Place the column into a new 2 ml collection tube (provided). Carefully apply remaining sample from step 4 into HiBind® DNA column. Centrifuge at 8000 x g for 1 minute. Discard the flow-through and collection tube.
7. Place the column into a new 2 ml collection tube (provided). Add 500µl of HB Buffer into the column. Centrifuge at 8000 x g for 1 minute. Discard the flow-through and reuse the collection tube in next step.
8. Place the column into a same 2 ml collection tube from step 7. Add 700µl of DNA Wash Buffer into the column. Centrifuge at 8000 x g for 1 minute. Discard

the flow-through and reuse the collection tube in next step.

Note that DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle and page 3. If refrigerated, the diluted wash buffer must be brought to room temperature before use.

9. Place the column into a same 2 ml collection tube from step 8. Centrifuge at 15,000 x g for 2 minutes to completely dry the HiBind® DNA Mini column. Discard the flow-through and the collection tube.
10. Place the column into a sterile 1.5 ml microfuge tube and add 50-100 µl of preheated (65°C) Elution Buffer. Allow tubes to sit for 5 min at room temperature.
11. 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. Elute DNA again as step 10-11. Discard column and store the eluted DNA at -20°C.

D. Buccal Swab DNA Vacuum/Spin Protocol

Material and Equipments required

- **Tabletop microcentrifuge and sterile 1.5 ml tubes**
- **Vacuum Manifold**
- **Water bath** - set to 65°C
- **PBS Buffer**
- **Absolute Ethanol** - approximately 0.3 ml per sample.
- **RNase A (Optional)**- Prepare a stock of RNase A at 50 mg/ml.

1. Prepare Buccal Swab lysate by following step 1-4 of Spin Protocol on page 7.
2. Insert the HiBind® DNA column into the vacuum manifold. Carefully apply the lysate to an HiBind® DNA column. Turn on the vacuum source to draw all liquid through the column. Turn off the vacuum.
Note: If the lysate has difficulty to pass through the column at this stage. Place the column into a collection tube (supplied). Close the lid and centrifuge at 8000 x g for 5 minutes or until all liquid pass through the column. Place the column into another collection tube (supplied) and continue step 7 of the spin protocol.
3. Apply remaining lysate into the column. Turn on the vacuum source to draw all liquid through the column. Turn off the vacuum.
4. Pipet 500 µl of Buffer HB into the column. Turn on the vacuum source to draw

all liquid through the column. Turn off the vacuum.

5. Wash the column by pipetting 750 μ l of DNA Wash Buffer diluted with ethanol into the column. Turn on the vacuum source to draw all liquid through the column. Turn off the vacuum.
6. Close the lid of HiBind[®] DNA column, remove it from the vacuum manifold. Insert the column into a collection tube (supplied) and centrifuge at 15,000 x g for 2 minute to completely dry the column.
7. Proceed Step 10-11 of Baccul Swab DNA Spin Protocol on page 7-8.

E. Dried Blood Samples DNA Spin Protocol

The following protocol is designed for isolating genomic DNA from dry blood sample collected with filter paper or commercial blood collecting card.

Material and equipments supplies by user

- **Tabletop microcentrifuge and sterile 1.5 ml tubes**
- **Water bath** - set to 65°C
- **PBS Buffer**
- **Absolute Ethanol** - approximately 0.3 ml per sample.
- **RNase A (Optional)** - Prepare a stock of RNase A at 50 mg/ml.

1. Cut or punch out the blood spot from the filter paper. (Up to 200 μ l blood can be used for each spot.) Tear or cut filter into small pieces and place into a microfuge tube.
2. Add 250 μ l PBS and incubate at 65°C for 1 hour. Vortex to mix every 20 min.
3. Add 25 μ l OB protease and mix well. Incubate 30 min at 60°C with occasional mixing.
4. Centrifuge at 10,000 x g for 5 min at room temperature. Transfer the supernatant to a clean microfuge tube and add **ONE** volume of Buffer BL followed by **ONE** volume of absolute ethanol. Vortex thoroughly to mix at maxi speed for 30s. Collect any drop from the lid by briefly centrifugation.
5. Assemble an HiBind[®] DNA column in a 2 ml collection tube (provided). Transfer the lysate from step 4 into the column and centrifuge at 8,000 x g for 1 min to bind DNA. Discard the collection tube and flow-through liquid.
6. Place the column into a **second 2 ml tube** and wash by pipetting 500 μ l of Buffer

HB. Centrifuge at 8,000 x g for 1 min. Again, Discard flow-through liquid and reuse the collection tube for next step.

7. Place the column into a **same 2 ml tube** from step 7 and wash by pipetting 700 μ l of DNA Wash Buffer diluted with ethanol. Centrifuge at 8,000 x g for 1 min. Again, dispose of collection tube and flow-through liquid.
8. Using a **new collection tube (provided)**, wash the column with a second 700 μ l of DNA Wash Buffer diluted with ethanol and centrifuge as above. Discard flow-through and re-use the collection tube for next step.
9. Place the empty column into the same 2ml collection tube from step 8, centrifuge at maximum speed (15,000 x g) for 2 min to dry the column. **This step is crucial for ensuring optimal elution in the following step.**
10. Place the column into a sterile 1.5 ml microfuge tube and add 50-100 μ l of preheated (65°C) Elution Buffer. Allow tubes to sit for 5 min at room temperature.
11. To elute DNA from the column, centrifuge at 8,000 x g for 1 min. Retain flow-through containing the DNA. Discard column and store the eluted DNA at -20°C.

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

F. Buffy Coat DNA Spin Protocol

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, 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.[®] Blood DNA Protocol, or frozen at -70°C for storage.

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
	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 5, page 6.
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 sure 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 AL.

Problem	Possible Cause	Suggestions
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 mixed 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.