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Introduction

The E.Z.N.A.® family of products is an innovative system that radically simplifies the extraction and purification of nucleic acids from a variety of sources. The key to this system is the new HiBind® matrix that specifically, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or a low salt buffer.

The E.Z.N.A.® Blood DNA Kit provides an easy and rapid method for the isolation of genomic DNA for consistent PCR and Southern analysis. Up to 1 µl - 250 µl of fresh, frozen or anticoagulated whole blood can be readily processed at one time. Omega Bio-Tek Inc.'s E.Z.N.A.® Blood DNA Kit can also be used for the preparation of genomic DNA from buffy coat, serum, plasma, saliva, buccal swab and other body fluids. The E.Z.N.A.® Blood DNA Kit allows for the single or multiple simultaneous processing of samples. There is no need for phenol/chloroform extractions, and time-consuming steps are eliminated (e.g. precipitation using isopropanol or ethanol). Purified DNA obtained with the E.Z.N.A.® Blood DNA Kit will be ready for applications such as PCR, Southern Blotting, and Restriction Digestion.

New in This Edition

A new column Equilibration Buffer is introduced in this kit to improve the DNA yield and kit performance.

Storage and Stability

All E.Z.N.A.® Blood DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: Reconstituted OB Protease -20 °C, and all other materials at RT (22-25 °C). 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 approximately 100µg DNA. Using greater than 250 µl of whole Blood or of buffy coat is not recommended.

Kit Contents

Product Number	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	120 ml
Equilibration Buffer	1.5 ml	7 ml	25 ml
DNA Wash Buffer Concentrate	12 ml	40 ml	3 x 40 ml
Elution Buffer*	5 ml	40 ml	160 ml
OB Protease	3 mg	30 mg	120 mg
Instruction Booklet	1	1	1

* Elution Buffer = 10mM Tris-HCl, pH 8.5

*Buffer BL contains Chaotropic salts. Equilibration Buffer contains Sodium Hydroxide. Use gloves and protective eyeware when handling this solution.

Before Starting

It is strongly advised that you familiarize yourself with the entire booklet before starting. E.Z.N.A.® Kits are designed to be simple, fast, and reliable provided that all steps are followed diligently.

Important	
	Reconstitute each vial of **OB Protease** in 150µl (5 preps), 1.5 ml (50 preps), or 6.0 ml (200preps) of **Elution Buffer**. Vortex vial briefly prior to use. We recommend that you aliquot, and store vials of reconstituted OB Protease at -20 °C.
	Dilute **DNA Wash Buffer** with **absolute ethanol** as follows **D3392-00** Add 18ml of absolute ethanol to each bottle **D3392-01** Add 60ml of absolute ethanol to each bottle **D3392-02** Add 60ml of absolute ethanol to each bottle

Blood & Body Fluid DNA Protocol

Materials provided by user

- Tabletop microcentrifuge
- Sterile 1.5ml centrifuge tubes
- Waterbath
- RNase stock solution
- Absolute ethanol
- OB Protease stock solution
- PBS Buffer

CARRY OUT ALL CENTRIFUGATION STEPS AT ROOM TEMPERATURE

NOTE: The procedure below has been optimized for the use with **FRESH** or **FROZEN** blood samples of 1 - 250µl in volume. Anticoagulated Blood, Saliva, Serum, Buffy Coat or other Body Fluids can also be used. In addition, ≤ 10^7 of leukocytes or cultured cells may be used with this procedure. For DNA extraction from tissue and mouse tail we suggest that you use the *E.Z.N.A.® Tissue DNA Kit* (Product No. D3396). To isolate Viral RNA from serum or other non-cellular body fluids we recommend using *the E.Z.N.A.® Viral RNA Kit* (Product No. R6874).

1. **Transfer the sample into a sterile microcentrifuge tube and bring the volume up to 250 µl with 10mM Tris-HCl, PBS, or Elution Buffer** (provided).

2. **Add 25µl of Reconstituted OB Protease and 250 µl of Buffer BL. Vortex at maximum speed for 15 seconds to mix thoroughly.** If RNA-Free Genomic DNA is required, add 5µl of RNase A (50mg/ml) to each sample.

3. **Incubate the sample at 65°C for 10 minutes. BRIEFLY vortex the tube once during incubation.**
   Note: During incubation step 5 can be processed.

4. **Add 260 µl of absolute ethanol** (RT, 96-100%) **to lysate. Vortex at maximum speed for 20 seconds to mix thoroughly. Briefly centrifuge the tube to collect any drops from the inside of the lid.**

5. **Insert a HiBind® DNA Mini Column into a 2 ml collection tube (**provided). **Add 100µl Equilibration Buffer into the column. Let the column sit for 4 minutes at room temperature. Spin at maximum speed for 20 seconds.**

6. **Transfer the lysate from step 4 into the column, and centrifuge at ≥10,000 x g for 1 min to bind DNA. Discard the collection tube and flow-through liquid.**

7. **Place the HiBind® DNA Mini Column into a NEW provided 2 ml collection tube. Add 500µl of Buffer HB, and centrifuge as above. Discard collection tube, and flow-through liquid.**

8. **Place the HiBind® DNA Mini Column into the SAME 2 ml collection tube from step 7, and wash by pipetting in 700 µl of DNA Wash Buffer diluted with ethanol. Centrifuge at ≥10,000 x g for 1 min. Discard collection tube, and flow-through liquid.**

   NOTE: DNA Wash Buffer is provided as a concentrate and must be diluted with absolute

ethanol as indicated on the bottle, and on page 4. If refrigerated, the diluted DNA Wash Buffer must be brought to room temperature before use.

9. **Using a NEW 2 ml collection tube, REPEAT step 8 with an ADDITIONAL 700µl of DNA Wash Buffer diluted with absolute ethanol, and centrifuge as above. Discard flow through.** (Collection tube will be re-used in the following step)

10 **Place the empty column into the SAME 2ml collection tube from step 9, centrifuge at a maximum speed of (≥13,000 x g) for 2 min to dry the column.**
This step is crucial for ensuring optimal elution in the following step.

11. **Place the HiBind® DNA Mini Column into a sterile 1.5 ml microfuge tube, and add 100-200µl of preheated (65°C) Elution Buffer (10mM Tris-HCl, pH 8.5, 65 °C). Allow to sit at room temperature for 5 minutes.**

12. **To elute DNA from the HiBind® DNA Mini Column, centrifuge at (≥13,000 x g) for 1 min. Retain flow-through containing the DNA. Place the column into a new 1.5ml tube. Elute DNA again as indicated in previous step. Discard column, and store the Eluted DNA at -20 °C.**

IB: Vacuum/Spin Protocol

1. **Prepare the lysate and column by following steps 1-4 of the Spin Protocol on page 5.**

2. **Insert a HiBind® DNA Mini Column into the vacuum manifold. CAREFULLY apply the lysate to the column. Turn on the vacuum source to draw all of the liquid through the column. Turn off the vacuum.**

   NOTE: If at this stage the lysate has difficulty passing through the column, place the column into a collection tube (supplied). Close the lid and centrifuge at ≥13,000 x g for 2 minutes or until all of the liquid has passed through. Place the column into another collection tube (supplied), and proceed to step 6 of the spin protocol on page 5.

3. **Add 500 µl of Buffer HB into the column. Turn on the vacuum source to draw all of the liquid through the column. Turn off the vacuum.**

4. **Wash the column by pipetting 700µl of DNA Wash Buffer diluted with ethanol into the column. Turn on the vacuum source to draw all of the liquid through the column. Turn off the vacuum.**

5. **Repeat Step 4 with another 700µl of DNA Wash Buffer.**

6. **Close the lid of the HiBind® DNA Mini Column, remove it from the vacuum manifold. Insert the column into a collection tube** (supplied)**, and centrifuge at maximum speed (≥13,000 x g) for 2 minutes to completely dry the column.**

7. **Place the HiBind® DNA Mini Column into a sterile 1.5 ml microfuge tube and add 100-200 µl of preheated (65°C) Elution Buffer (10mM Tris-HCl, pH 8.5). Allow tubes to sit for 5 min at room temperature.**

8. **To elute DNA from the column, centrifuge at ≥13,000 x g for 1 min. Retain flow through containing the DNA. Place the column into a second 1.5 ml tube. Elute DNA again as indicated in the previous step. Discard column, and store the Eluted DNA at -20 °C.**

NOTES ON ELUTION
The first elution will typically produce yields of 60-70% of the DNA bound to the column. Thus two elutions will generally give > 90% yields. However, increasing the elution volume will also reduce the concentration of the final product. To obtain DNA at higher concentrations, the elution can be carried out using 50 µl - 100 µl of Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µl greatly reduce yields. In some instances yields may be increased by incubating the column at 70°C (rather than at room temperature) upon the addition of Elution Buffer.

If necessary the DNA can be concentrated. Add sodium chloride to reach 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 minutes. Centrifuge at 10,000 x g for 15 min and discard supernatant. Add 700µl of 70% ethanol and centrifuge at 10,000 x g for 2 min. Discard supernatant, air dry the pellet for 2 min, and resuspend the DNA in 20 µl of sterile deionized water or 10 mM Tris-HCl, pH 8.5. The expected yield from 250 µl of blood is approximately 4-12µg of DNA.

E.Z.N.A.® Buccal Swab DNA Protocol

IMPORTANT NOTE: This protocol requires an increased volume of Buffer BL; fewer preparations can be performed. Additional Buffer BL can be purchased separately (see page 4).
ALL CENTRIFUGATION STEPS MUST BE CARRIED OUT AT ROOM TEMPERATURE

1. **Place the Buccal Swab in a 2 ml centrifuge tube and add 500µl of PBS Buffer to the tube.** If RNA-free DNA is required for downstream applications, add 4µl of RNase A Stock Solution (50mg/ml) into the sample.

2. **Add 25µl of Reconstituted OB Protease and 500 µl of Buffer BL. Vortex at maximum speed for 30 seconds to mix thoroughly. Incubate the sample at 65°C for 10 minutes. Note: step 5 can be processed while incubating sample.**

3. **Remove the Buccal Swab.**

4. **Add 500 µl of absolute ethanol** (room temperature, 96-100%) **to the sample, and mix by vortexing. Collect any liquid drops remaining on the lid by briefly centrifugating.**

5. **HiBind® DNA Mini Column into a 2 ml collection tube (**provided). **Add 100µl Equilibration Buffer into the column. Let the column sit for 4 minutes at room temperature. Spin at maximum speed for 20 seconds.**

6. **CAREFULLY transfer 750µl of the sample from step 4 into the column, and centrifuge at 10,000 x g for 1 minute. Discard the collection tube and flow-through liquid.**

7. **Place the HiBind® DNA Mini Column into a NEW provided 2 ml collection tube. CAREFULLY apply the remainder of the sample from step 4 to the column. Centrifuge as above. Discard collection tube, and flow-through liquid.**

8. **Place the HiBind® DNA Mini Column into a NEW 2 ml collection tube. Add 500µl of HB Buffer to the column. Centrifuge as above. Discard the flow-through and reuse the collection tube in the following step.**

9. **Place the HiBind® DNA Mini Column into the SAME 2 ml collection tube from step 8, and add 700 µl of DNA Wash Buffer diluted with ethanol. Centrifuge as above. Discard flow-through liquid, and reuse the collection tube in the following step.**

NOTE: DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle, and on page 4. If refrigerated, the diluted DNA Wash Buffer must be brought to room temperature before use.

10 **REPEAT wash step 8 with another 700 µl of DNA Wash Buffer diluted with ethanol.**

11. **Place the column into the same 2 ml collection tube from step 9. Centrifuge at ≥13,000 x g for 2 minutes to completely dry the HiBind® DNA column. Discard the flow-through and the collection tube.**

12 **Place the column into a sterile 1.5 ml microcentrifuge tube, and add 100-200 µl of preheated Elution Buffer** (10mM Tris-HCl, pH 8.5, 65 °C). **Allow tubes to sit for 5 min at room temperature.**

13. **To elute DNA from the column, centrifuge at maximum speed for 1 min. Retain flow through containing the DNA. Place the column into a second 1.5 ml tube. Elute DNA again as described above. Discard column, and store the Eluted DNA at -20 °C.**

Vacuum/Spin Protocol

1. Prepare Buccal Swab Lysate by following steps 1-4 of the Spin Protocol on page 7.

2. **Insert the HiBind® DNA Mini Column into the vacuum manifold. CAREFULLY, apply the lysate to the column. Turn on the vacuum source to draw the lysate from step 4 of the Spin Protocol on page 7 through the column. Turn off the vacuum. REPEAT this step until all of the lysate has passed through the column.**

3. **Pipet 500 µl of Buffer HB into the column. Turn on the vacuum source to draw all of the liquid through the column. Turn off the vacuum.**

4. **Wash the column by pipetting 700µl of DNA Wash Buffer diluted with ethanol into the column. Turn on the vacuum source to draw all of the liquid through the column. Turn off the vacuum.**

5. **REPEAT wash step 4 with another 700µl of DNA Wash Buffer diluted with ethanol .**

6. **Close the lid of the HiBind® DNA Mini Column, remove it from the vacuum manifold. Insert the column into a collection tube** (supplied)**, and centrifuge at ≥13,000 x g for 2 minutes to completely dry the column.**

7. Proceed with steps 10 and 11 of the Spin Protocol on page 7.

E.Z.N.A.® Dried Blood DNA Spin Protocol

1. **Cut or punch out the blood spot from the filter paper (up to 200µl of blood can be used per spot). Tear or cut the filter paper into small pieces and place them into a microfuge tube.**

2. **Add 250 µl of PBS Buffer, and incubate at 65°C for 1 hour, while vortexing every 20 min for proper mixing.**

3. **Add 25µl of OB Protease stock solution and mix well. Incubate for 30 min at 65°C with occasional mixing.**

Note: Step 6 can be performed during incubation time.

4. **Centrifuge at ≥13,000 x g for 5 min at room temperature.**

5. **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. Collect any drops remaining on the lid by briefly centrifuging.**

6. **Insert a HiBind® DNA Mini Column into a 2 ml collection tube (**provided). **Add 100µl Equilibration Buffer into the column. Let the column sit for 4 minutes at room temperature. Spin at maximum speed for 20 seconds.**

7. **Transfer the lysate from step 5 into the column, and centrifuge at 10,000 x g for 1 min to bind the DNA. Discard the flow-through liquid and the collection tube.**

8. **Place the HiBind® DNA Mini Column into a NEW 2 ml collection tube. Add 500µl of Buffer HB to the column, and centrifuge as above. Discard the flow-through and reuse the collection tube in the following step.**

9. **Place the HiBind® DNA Mini Column into the SAME 2 ml collection tube from step 7, and wash by pipetting in 700 µl of DNA Wash Buffer diluted with ethanol. Centrifuge as above. Dispose of the collection tube and flow-through liquid.**

NOTE: DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle, and on page 4. If refrigerated, the diluted DNA Wash Buffer must be brought to room temperature before use.

10 **Using a NEW collection tube** (provided), **wash the column with a second 700 µl of DNA Wash Buffer diluted with ethanol. Centrifuge as above. Discard flow-through liquid, and reuse the collection tube in the following step.**

11. **Place the empty column into the same 2 ml collection tube from step 10. Centrifuge at maximum speed (≥13,000 x g) for 2 minutes to completely dry the column. Discard the flow-through and the collection tube.**

NOTE: This step is crucial for ensuring optimal elution in the following steps.

12 **Place the column into a sterile 1.5 ml microcentrifuge tube, and add 100-200 µl of preheated Elution Buffer** (10mM Tris-HCl, pH 8.5, 65 °C)**. Allow tubes to sit for 5 min at room temperature.**

13. **To elute DNA from the column, centrifuge at maximum speed (≥15,000 x g) for 1 min. Retain flow through containing the DNA. Discard column, and store the Eluted DNA at -20 °C.**

NOTE : Blood Spots from finger pricks usually contain no more than 50 µl of blood, and yield approximately 500 ng to 1 µg of DNA. This is usually sufficient for PCR analysis. To obtain higher DNA concentrations, elute with 50 µl of preheated elution buffer (volumes lower than 50 µl greatly reduce yields). Alternatively, the first eluate can be used to perform a second elution.

E.Z.N.A.® Buffy Coat DNA Spin Protocol

The buffy coat fraction of whole blood is enriched with WBC's, and usually gives at least 5-folds more of 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** while making sure not to disturb the layer of concentrated leukocytes. The buffy coat can be drawn off with a pipette, and be 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 a blank. Dilute the DNA in TE buffer and calculate the concentration using the following equation:

[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 (A_{260}/A_{280}) ratio of 1.7-1.9 corresponds to 85%-95% purity.

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

Troubleshooting Guide

Problem	Cause	Possible Solution
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 is too Large	If using more than 250 µl of Blood, increase volumes of OB Protease, Buffer BL, and Isopropanol. Pass aliquots of lysate through one column successively.
	Sample is too viscous	Divide sample into multiple tubes, and adjust the volume to 250µl with 10 mM Tris-HCL.
Low DNA Yield	Clogged Column	See above
	Poor elution	Repeat elution or increase elution volume (see notes on elution on page 6) 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 4 before use.
	Buffy Coat Used	With Buffy Coat samples, use absolute ethanol, rather than isopropanol .
Low A_{260}/A_{280} Ratio	Extended centrifugation during elution.	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 the column, wash once with 300µl of Buffer BL.
No DNA Eluted	Poor Cell Lysis due to improper mixing with Buffer BL	Mix thoroughly with Buffer BL prior to loading the column.
	Absolute Ethanol not added to Buffer BL.	Before applying the sample to the column, and aliquot of Buffer BL/ethanol solution must be added.
	No Ethanol added to Wash Buffer Concentrate	Dilute Wash Buffer with the indicated volume of absolute ethanol before use (page 4).
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 is too Large	Reduce sample volume, and proceed with protocol.
	Hemoglobin remains in column	After applying sample, wash column once with 300ul of Buffer BL.

Ordering Information

Cat. No.	Product Name and Preps	Sample Size
D3471-00	SE Blood DNA Kit (5)	100µl - 1 ml
D3471-01	SE Blood DNA Kit (50)	100µl - 1 ml
D3471-02	SE Blood DNA Kit (200)	100µl - 1 ml
D3392-00	Blood DNA Kit (5)	100µl - 1 ml
D3392-01	Blood DNA Kit (50)	100µl - 1 ml
D3392-02	Blood DNA Kit (200)	100µl - 1 ml
D3494-01	Blood DNA Midi Kit (10)	up to 10 ml
D3494-03	Blood DNA Midi Kit (50)	up to 10 ml
D3494-04	Blood DNA Midi Kit (100)	up to 10 ml
D2492-01	Blood DNA Maxi Kit (10)	up to 30 ml
D2492-02	Blood DNA Maxi Kit (20)	up to 30 ml
D1192-01	E-Z 96® Blood DNA Isolation 4 x 96	up to 200µl
D1192-02	E-Z 96® Blood DNA Isolation 20 x 96	up to 200µl
M6221-01	Mag-Bind® Blood DNA Kit (50)	up to 200µl
M6221-02	Mag-Bind® Blood DNA Kit (200)	up to 200µl
M6220-01	Mag-Bind® Blood DNA Midi Kit (10)	up to 2 ml
M6220-02	Mag-Bind® Blood DNA Midi Kit (50)	up to 2 ml
M6222-01	Mag-Bind® Blood DNA Maxi Kit (10)	up to 10 ml
M6222-02	Mag-Bind® Blood DNA Maxi Kit (50)	up to 10 ml

Product	Size	Product No.
Buffer BL	**100 ml**	**PD062**
Buffer HB	**250 ml**	**PS009**
DNA Wash Buffer	**(40 ml; add 60ml ETOH)**	**PDR044**
DNA Wash Buffer	**(100 ml; add 150ml ETOH)**	**PS010**
DNA Wash Buffer	**(500 ml; add 750ml ETOH)**	**PS011**
Elution Buffer	**100 ml**	**PDR048**
RNase A	**400µl / 5ml**	**AC117/AC118**
OBProtease	**30mg/100mg**	**AC113/AC114**
Proteinase K	**30mg/100mg/1g**	**AC110/AC111/AC112**
2ml capless collection tubes	**500/BAG**	**SS1-1370-00**
1.5ml DNase/RNase Free Centrifuge Tubes	**500/BAG**	**SS1-1210-00**

For Technical Questions, Pricing and Availability, or any other concerns please contact us.
Tel: 770-931-8400 (US)
Fax: 770-931-0230 (US)
Tel: 1-800-832-8896 (Toll free)
Fax: 1-888-624-1688 (Toll free)
e-mail: info@omegabiotek.com
www.omegabiotek.com