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Introduction

The E.Z.N.A.® Blood DNA Midi Kit is designed for isolation of genomic DNA from up to 10 ml of fresh, whole blood treated with any common anticoagulant such as heparin, EDTA, or acid-citrate-dextrose. 10ml of blood typically yields 200-250 µg of genomic DNA. The procedure completely removes contaminants and enzyme inhibitors making total DNA isolation fast, convenient, and reliable. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation, and precipitation with isopropanol or LiCl, are eliminated. DNA purified using the E.Z.N.A.® Blood DNA Midi method is ready for applications such as PCR*, Restriction digestion, Southern blot and so on.

The E.Z.N.A.® Blood DNA Midi Kit uses the reversible binding properties of HiBind® matrix, a new silica-based material. This is combined with the speed of midi-column spin technology. Red blood cells are selectively lysed and white cells collected by centrifugation. After lysis of white blood cells under denaturing conditions that inactivate DNases, genomic DNA is purified on the HiBind® DNA Midi column. A specifically formulated high salt buffer system allows DNA molecules greater than 200 bases to bind to the matrix. Cellular debris and other contaminants (such as hemoglobin) are effectively washed away and high quality DNA is finally eluted in Elution Buffer.

Storage and Stability

All components of the E.Z.N.A.® Blood DNA Midi Kit, except the OB Protease, RNase A should be stored at 22ºC-25ºC. Once reconstituted in water, OB Protease must be stored at -20ºC. Store RNase A at -20ºC. 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 37ºC to dissolve. Store Buffer BL at room temperature.

Expiration Date: All E.Z.N.A.® Blood DNA Midi Kit components are guaranteed for at least 24 months from the date of purchase when stored as above.

Binding Capacity

Each HiBind™ DNA Midi column can bind approximately 0.5 mg Genomic DNA. Using greater than 10 ml whole blood 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 Number	D3494-00	D3494-01	D3494-03	D3494-04
Purification times	2 Preps	10 Preps	50 Preps	100 Preps
HiBind™ DNA Midi Columns	2	10	50	100
15 ml Collection Tubes	2	10	50	100
10 x Buffer ERL	15 ml	70 ml	2 x 200 ml	3x 250 ml
Buffer TL	8 ml	30 ml	120 ml	230 ml
Buffer BL	8 ml	30 ml	120 ml	230 ml
Buffer HB	10 ml	35 ml	170 ml	2 x 160ml
DNA Wash buffer	10 ml	20 ml	2 x 40 ml	3 x 50 ml
OB Protease	6 mg	30 mg	150 mg	300 mg
Protease Storage Buffer	1 ml	1.8 ml	8 ml	16 ml
RNase A	50 µl	220 µl	1050 µl	2x1050 µl
Elution Buffer	10 ml	20 ml	100 ml	200 ml
Instruction Manual	1	1	1	1

Materials to Be Provided by User

Please take a few minutes to read this booklet thoroughly and become familiar with the protocol. Prepare all materials required before starting to reach optimize performance.

- All the subsequent steps must be performed using a centrifuge capable of at least 4,000-8,000 x g. Ensure that an appropriate rotor adaptor is in place to prevent damage to the collecting tube.
- **Water bath -** set to 70ºC.
- **Absolute ethanol -** approximately 3 ml per sample
- Have a shaking water bath set to 55ºC.
- 50 ml centrifuge tubes and clean 15-20 ml centrifuge tubes.
- Laboratory centrifuge equipped with **swinging-bucket** rotor.

Before Starting

IMPORTANT

1 **Buffer ERL** is supplied as a 10 X concentrate and must be diluted with sterile deionized water as follows.

D3494-00	Empty contents of the bottle supplied into an appropriately sized vessel and add 135ml deionized water.
D3494-01	Empty contents of the bottle supplied into an appropriately sized vessel and add 630ml deionized water.
D3494-03	Empty contents of the bottle supplied into an appropriately sized vessel and add 1800 ml deionized water per bottle of Buffer ERL.
D3494-04	Empty contents of the bottle sipplied into an appropriately sized vessel and add 2250 ml deionized water per bottle of Buffer ERL.

2 **Reconstitute OB Protease** in 0.3ml (2 preps), 1.5ml (10 preps), 7.5 ml (50 Prep) and 15 ml for (100 preps) Prosease Storage Buffer. Vortex vial briefly prior to use. We recommend that you aliquot and store vials of reconstituted protease at -20ºC.

3 **DNA Wash buffer concentrate** must be diluted with absolute ethanol before use.

D3494-00	Add 40 ml 96-100% ethanol
D3494-01	Add 80 ml 96-100% ethanol
D3494-03	Add 160 ml 96-100% ethanol to each bottle
D3494-04	Add 200 ml 96-100% ethanol to each bottle

All centrifugation steps must be carried out at room temperature.

Harvesting and Storage of Blood

E.Z.N.A.® Blood DNA Midi Kit is designed for purification of genomic DNA from up to 10 ml whole blood. The system is not limited by DNA binding capacity of HiBind® Midi columns (which can bind up to 500 ug of DNA), but by lysis volume. Due to the abundance of erythrocytes and proteins, greater than 10 ml whole blood will significantly lower DNA quality. Storage of blood samples without previous treatment leads to reduced yields of genomic DNA. For best result, blood samples should be proceeded as following.

1. For short-term storage (up to a week), collect blood in tubes containing EDTA as anticoagulant, and store at 4°C.

2. For long-term storage, collect blood in tubes containg an anticoagulant and store at -70°C. Thawed frozen blood sample at 37°C with gently agtitaion before used.

Standard Protocol (for 2 ml of whole blood sample)

This protocol allows rapid isolation of genomic DNA (include viral DNA presented in blood samples) from up to 2 ml blood sample. Yield vary depend on source. Add up to 2 ml whole blood to a 15 ml centrifuge tube. If the sample less than 2 ml, bring the volume up to 2 ml with Elution Buffer provided.

1. **For 2 ml whole blood sample, add 150 µl OB Protease, 2.1 ml Buffer BL and 20 µl RNase A.** Vortex at maximum speed for 1 minutes to mix thoroughly.

2. **Incubate sample at 65°C for 15-20 min.** Briefly vortex the tube serval times during incubation.

3. **Add 2.2 ml of absolute ethanol** to lysate and mix thoroughly by vortexing at maximum speed for 30s.

4. Insert a HiBind® DNA Midi column in a 15 ml collection tube (provided). **Transfer 3.5ml of the lysate from Step 3 into the column and centrifuge at 4,000 x g for 5 min to bind DNA**. Discard flow-through liquid.

5. **Insert the column back in the same collection tube, transfer the remaining lysate from step 3 into column and centrifuge as above.** Discard flow through liquid and re-use the collection tube in next step.

6. **Place the column into the collection tube and wash by pipetting 3 ml of HB Buffer.** Centrifuge as above. Discard the liquid and re-use the collection tube.

7. Place the column into the collection tube and wash by pipetting 3 ml of DNA Wash Buffer diluted with ethanol. Centrifuge as above. Discard the flow-through liquid and re-use the collection tube.

   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 DNA Wash Buffer must be brought to room temperature before use.

8. Using the **same centrifuge tube**, wash the column with a second 3 ml of DNA Wash Buffer and centrifuge as above. Discard flow-through.

9. Using the same 15 ml collection tube, centrifuge at 4,000 x g for 10-15 min to dry the column. ***This step is critical for removal of trace ethanol that might otherwise interfere with downstream applications***.

10. Place the column into a nuclease-free 15 ml centrifuge tube (not provided) and add 500 µl **of preheated (70°C) Elution Buffer**. Allow tubes to sit for 5 min at room temperature.

11. **To elute DNA from the column, centrifuge at 4,000 x g for 5 min.** Retain flow-through containing the DNA. Place column into a second 15 ml tube and repeat elution step with another 500 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 give >80%. However, increasing elution volume reduces the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using same 0.5 ml Elution to perform second elution.

Maxi-Yield Protocol (for up to 10 ml whole blood)

1. **To 1 volume of whole blood (maximum of 10 ml) add 5 volumes of 1 x Buffer ERL.** For example add 5 ml Buffer ERL to 1 ml blood. Mix by vortexing.

   Note: Buffer ERL is supplied as a 10 x concentrate and must be diluted with sterile deionized water before use. Refer to page 4 or label on bottle for directions.

2. **Incubate on ice for 15 min, mixing by brief vortexing twice.** Lysis of red blood cells is indicated when the solution becomes translucent. Blood samples from individuals with an elevated hematocrit or ECR, extend incubation time to 20 min.

3. **Pellet leukocytes by centrifuging at 450 x g for 10 min at 4°C.** Discard the supernatant containing lysed red blood cells.

4. Wash the white blood cell pellet with 2 volumes of Buffer ERL per volume of whole blood used in step 1. Thoroughly vortex to resuspend cells.

   Tip: If you used 10 ml of whole blood, wash with 20 ml of Buffer ERL.

5. **Centrifuge at 450 x g for 10 min at 4°C.** Discard the supernatant without disturbing the visible white pellet. Leave about 100 µl of residue liquid in the tube.

6. Vortex the tube vigorously until the white blood cells are completely re-suspended.

7. **Add 2 ml TL buffer to the re-suspended white blood cells** and vortex at maxi speed for 20s.

8. **Add 150 ul of OB protease and vortex to mix well. Incubate at 55°C in a shaking water bath to effect complete lysis**. If no shaking water bath is available, vortex every 20-30 minutes. Lysis time depend on amount and type of tissue, but usually under 2 hours. -

9. **Add 2.1 ml Buffer BL, 20 ul RNase A and mix by vortexing at maxi speed for 20s. Incubated at 65°C for 10 minutes.**

10. **Add 2.2 ml absolute ethanol and mix thoroughly by vortexing at maxi speed for 30s.** If precipitation can be seen at this point, break the precipitation by passing through a needle using a syringe.

    IMPORTANT: THIS AND ALL SUBSEQUENT STEP MUST BE PERFORMED USING A CENTRIFUGE EQUIPPED WHICH IS CAPABLE OF 4,000-6,000 X g.

11. Assemble an HiBind® DNA Midi column in a 15 ml collection tube (provided). Transfer the 3.5 ml sample from step 10 into the column including any precipitate that may have formed. Centrifuge at 4,000-6,000 x g for 5 min to bind DNA. Discard both flow-through liquid and reuse the collection tube.

    Note: Since the HiBind® DNA Midi column can only contains around 4 ml sample volume, it is necessary to load the column twice.

12. **Place the column back into the 15 ml collection tube and load the rest of the sample from step10 into the column.** Centrifuge at 4,000-6,000 x g for 5 minutes. Discard the flow-through and collection tube.

13. **Proceed wash and elution procedure as step 6-11 of standard protocol on page 5-6.**

Troubleshooting Guide

Problem	Possible Cause	Suggestions
Clogged Column	Incomplete lysis	Extend incubation time of lysis with Buffer TL and protease. 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	using more than 20 ml is not recommend for this kit
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	DNWash Buffer must be diluted with absolute (100%) ethanol as specified on page 5 before use.
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.
	Incomplete cell lysis or protein degradation due to insufficient incubation.	Increase incubation time with Buffer Tland protease. Ensure that no visible pieces of tissue remain.
	Samples are rich in protein.	After applying to column, wash with 1ml of a 1:1 mixture of Buffer BL and ethanol and then with DNA Wash Buffer.
No DNA eluted	Poor cell lysis due to improper mixing with Buffer BL.	Mix thoroughly with Buffer BL prior to loading HiBind® column.
	Poor protein lysis in Buffer TL.	Increase incubation time at 55°C with Buffer TL to ensure that tissue is completely lysed.
	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 DNA Wash Buffer	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.
Washing leaves colored residue in column	Incomplete lysis due to improper mixing.	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.