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Introduction

E.Z.N.A.™ Blood DNA Midiprep Kits are designed for isolation of total DNA (include genomic, mitochondrial and viral DNA) from 0.2-2 mL (with standard protocol) and up to 10 mL (with maximum yield protocol) of fresh, whole blood treated with any common anticoagulant such as heparin, EDTA, or acid-citrate-dextrose. This kit can also be used to purify DNA from buffy coat, lymphocytes, serum, plasma and bone marrow. 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 method is ready for applications such as RT-PCR*.

Principle

The E.Z.N.A.™ Blood DNA Kits combine the reversible binding properties of HiBind® matrix, a new silica-based material, with the speed of midi-column spin technology to provide fast and high quality DNA. The standard protocol Sample is first mixed with BL buffer which lyses the cell and releases DNA under denaturing conditions that inactivate DNases. The cell lysate is then loaded into the HiBind® Midi-spin. DNA binds to HiBind® matrix while impurities are effectively removed after a few quick wash steps. genomic DNA is purified on the HiBind® Midi spin column.

Storage

E.Z.N.A.™ Blood DNA Midiprep Kits should be stored at room temperature. During shipment crystals may form in the BL Lysis Buffer. Warm to 37°C to dissolve. All the components are guaranteed for at least 24 months from date of purchase.

*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

E.Z.N.A.™ Blood DNA Kits	D3494-00	D3494-01	D3494-03	D3494-04
Purification	2	10	50	100
HiBind™ DNA Midi Columns	2	10	50	100
15 mL Collection Tubes	2	10	50	100
Buffer TL	5 mL	25 mL	120 mL	240 mL
Buffer BL	5 mL	25 mL	120 mL	240 mL
Buffer HB	8 mL	40 mL	160 mL	320 mL
Equilibration Buffer	3 mL	12 mL	60 mL	125 mL
DNA Wash buffer	5 mL	15 mL	80 mL	200 mL
OB Protease	6 mg	30 mg	150 mg	300 mg
Proteinase Storage Buffer	270 µl	3 mL	10 mL	20 mL
RNase A	25 µL	110 µL	550 µL	1.2 mL
DNA Elution Buffer	5 mL	20 mL	100 mL	200 mL
Instruction Manual	1	1	1	1

Before Starting

Important	Reconstitute OB Protease with elution buffer before use
	<p>D3494-00 Reconstitute OB Protease with 250 µL Proteinase Storage Buffer</p> <p>D3494-01 Reconstitute OB Protease with 1.5 mL Proteinase Storage Buffer</p> <p>D3494-02 Reconstitute OB Protease with 7.5 mL Proteinase Storage Buffer</p> <p>D3494-03 Reconstitute OB Protease with 15 mL Proteinase Storage Buffer</p> <p>*We recommend that you aliquot and store vials of reconstituted protease at -20°C. Vortex briefly before use</p>
	<p>DNA Wash buffer must be diluted with absolute ethanol before use</p> <p>D3494-00 Add 20 ml of 100% ethanol to each bottle</p> <p>D3494-01 Add 60 mL of 100% ethanol to each bottle</p> <p>D3494-03 Add 320 mL of 100% ethanol to each bottle</p> <p>D3494-04 Add 800 mL of 100% ethanol to each bottle</p>

Harvesting and Storage of Blood

E.Z.N.A.™ DNA Midiprep Kits are 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 µg of DNA), but by lysis volume. Due to the abundance of erythrocytes and proteins, greater than 20 mL whole blood will significantly lower DNA quality. The relatively low DNA content of leukocytes means that the maximum binding capacity of HiBind® DNA columns can not be reached.

Samples should be collected in the presence of an anticoagulant (preferably acid-citrate-dextrose) and processed within a few hours. Minimize storage prior DNA isolation as leukocyte transcripts generally have variable stabilities.

Standard Protocol (for up to 2 mL of whole blood sample)

Equipments to be supplied by user

- Swing bucket centrifuge capable of 3,000-5,000 x g.
- Water bath or heating block - preset to 70°C.
- Have a shaking water bath set to 55°C.
- Absolute ethanol (96-100%)
- Isopropanol
- Sterile 50 mL centrifuge tubes and 15 mL centrifuge tubes.
- Vacuum manifold (for Vacuum protocol)
- Vacuum source (-200 to 600 mBa) (for vacuum protocol)

1. **Add up to 2 mL sample** into a 15 mL centrifuge tube (not provided). If the sample less than 2 mL, bring the volume up to 2 mL with 10 mM Tris-HCl, PBS, or Elution Buffer provided.
2. **Add 140 µL OB Protease** and mix the sample thoroughly by vortexing.
3. **Add 2.1 mL of Buffer BL.** Vortex 5 minutes to mix thoroughly.
4. **Add 10 µL RNase A** solution to each sample to remove RNA.
5. Incubate sample at 70°C for 10 min.
6. Briefly vortex the tube once during incubation.
7. **Add 2.2 mL of isopropanol** to lysate and mix.

Note: We recommend that you aliquot and store vials of reconstituted protease at -20°C. Vortex briefly before use
8. Take a HiBind® DNA Midi Column pre-inserted in a 15 ml collection tube (supplied). **Add 1 ml Equilibration Buffer into the column. Let the column sit for 4 minutes at room temperature to equilibrate the membrane.** Centrifuge at 3,000-5,000 x g for 3 minutes
9. **Transfer the half volume of sample** into the column and centrifuge at 3,000-5,000 x g for 3 min to bind DNA. Discard the flow-through and re-use the collection tube.
10. Add remainder of lysate to column. Centrifuge at 3,000-5,000 x g for 3 minutes. Discard the flow-through and re-use the collection tube.
11. **Add 3 mL of HB Buffer.** Centrifuge at 3,000-5,000 x g for 5 minutes. Discard the flow-through liquid and re-use the collection tube.

12. **Add 3.5 mL of DNA Wash Buffer.** Centrifuge at 3,000-5,000 x g for 3 minutes. Discard the flow-through liquid and re-use the collection tube.
13. **Add 3.5 mL of DNA Wash Buffer.** Centrifuge at 3,000-5,000 x g for 3 minutes. Discard the flow-through liquid and re-use the collection tube.
14. **With the collection tube empty, spin the column at 3,000-5,000 x g for 15 minutes to completely dry the HiBind® matrix.**

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

15. Place the column into a nuclease-free 15 mL centrifuge tube (not provided) and **add 200-500 µL of preheated (70°C) Elution Buffer** (10mM Tris-HCl, pH 8.5) or water. Allow tubes to sit for 2 min at room temperature.
16. **To elute DNA from the column,** centrifuge at 4,000 x g for 5 min. Retain flow-through containing the DNA. For maximum yield, place column into a second 1.5 mL tube and repeat elution step with another 200-500 µL of preheated Elution Buffer or water. Discard column.

Note: First elution typically yields 60%-70% of the DNA bound to the column. Second elution can increase the yield by 20%. However, increasing elution volume reduces the concentration of the final product. Volumes lower than 200 µL greatly reduce yields. reduces the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out by load the eluted DNA back into the column and perform the second elution.

Maximum Yield Protocol (for up to 10 mL whole blood)

Prepare the Red Blood Lysis Buffer (RBL) as following	
NH ₄ Cl	155mM
KHCO ₃	10mM
Na ₂ EDTA	0.1mM
Adjust to pH 7.4 with 1M Hcl or NaOH	

1. **To 1 volume of whole fresh blood (maximum of 10 mL) add 5 volumes of RBL Buffer.** For example add 5 mL Buffer RBL to 1 mL blood. Mix by vortexing.

2. **Incubate for 15 min on ice,** 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. Completely remove and discard the supernatant containing lysed red blood cells.
4. Wash the white blood cell pellet with 2 volumes of Buffer RBL 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 RBL.
5. Centrifuge at 450 x g for 10 min at 4°C. Again, completely remove and discard the supernatant.
6. **Add 2 mL TL buffer** to the pelleted white blood cells and vortex thoroughly to mix.
7. **Add 140ul of OB protease** solution, vortex to mix well, and 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.
8. **Add 10 uL RNase A** and mix by briefly vortexing.
9. **Add 2.1 mL Buffer BL** and vortex to mix. Incubate at 70°C for 10 minutes. A wispy precipitate may form on addition of Buffer BL, but does not interfere with DNA recovery.
10. **Add 2.2 mL absolute ethanol** and mix thoroughly by vortexing. If precipitation can be seen at this point, break the precipitation by passing through a needle using a syringe.

11. Take a HiBind® DNA Midi Column pre-inserted in a 15 ml collection tube (supplied). **Add 1 ml Equilibration Buffer into the column. Let the column sit for 4 minutes at room temperature to equilibrate the membrane.** Centrifuge at 3,000-5,000 x g for 3 minutes
12. Transfer 3mL of the sample to the HiBind® column including any precipitate that may have formed. Centrifuge at 3,000-5,00x g for 5 min to bind DNA. Discard flow-through liquid and re-use the collection tube.

Note: Since the column can only contains around 4 mL sample volume, it is necessary to load the column twice.

13. Add reminder of lysate to column. Centrifuge at 3,000-5,000 x g for 5 minutes. Discard the flow-through and re-use the collection tube.
14. **Add 3 mL of Buffer HB.** Centrifuge at 3,000-5,000 x g for 3 mins. Discard

the flow-through liquid and re-use the collection tube

15. **Add 3.5 mL of DNA Wash Buffer.** Centrifuge at 3,000-5,000 x g for 3 min. Discard the flow-through liquid and re-use the collection tube.
16. **Add 3.5 mL of DNA Wash Buffer.** Centrifuge at 3,000-5,000 x g for 3 min. Discard flow-through liquid and collection tube.
17. **With the collection tube empty, spin the column at 3,000-5,000 x g for 15 minutes to completely dry the HiBind® matrix.**

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

18. **Place column into a clean 15 mL centrifuge tube. Add 200-500 µL Elution Buffer** (10 mM Tris-HCl, pH 8.5) or sterile deionized water onto the center of column matrix and centrifuge 5 min at 4500 x g to elute DNA. This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration. Preheating the water to 70°C and allowing the column to soak 2 min at room temperature before elution may significantly increase yields.

Vacuum Protocol (for up to 2 mL of whole blood sample)

1. **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 10 mM Tris-HCl, PBS, or Elution Buffer provided.
2. **Add 140 µL OB Protease** and mix the sample thoroughly by vortexing.
3. **Add 2.1 mL of Buffer BL.** Vortex 5 minutes to mix thoroughly.
4. **Add 10 µL RNase A** solution to each sample to remove RNA.
5. Incubate sample at 70°C for 10 min.
6. Briefly vortex the tube once during incubation.
7. **Add 2.2 mL of isopropanol** to lysate and mix. For buffy coat, isolated leukocytes, and cultured cells, yields will improve if 260 µL absolute ethanol is used in place of isopropanol.
8. Insert the HiBind® DNA Midi-spin column on a outlet of vacuum manifold. **Add 1 ml Equilibration Buffer into the column. Let the column sit for 4 minutes at room temperature to equilibrate the membrane.** Apply the vacuum until all the sample passes through the membrane. Turn off the

vacuum

9. Transfer half volume of sample into the column. Apply the vacuum until all the sample passes through the membrane. Turn off the vacuum.
10. Load the remainder of the sample into the column. Apply the vacuum until all the sample passes through the membrane. Turn off the vacuum.
11. **Add 3 mL HB Buffer.** Apply the vacuum until all the liquid passes through the column. Turn off the vacuum.
12. **Add 3.5 mL DNA wash Buffer.** Apply the vacuum until all the liquid passes through the column. Turn off the vacuum.
13. **Add another 3.5 mL DNA wash Buffer.** Apply the vacuum until all the liquid passes through the column.
14. After all the liquid pass through the membrane, apply maximum vacuum for additional 15 minutes to dry the column.

Note: It is critical for removing traces of ethanol that may otherwise interfere with downstream applications.
15. Turn off the vacuum and remove the column from manifold. Insert a HiBind® DNA Midi-spin column in a 15 mL collection tube (provided).
16. **Add 200-500 µL Elution Buffer** (10 mM Tris-HCl, pH 8.5) or sterile deionized water onto the center of column matrix.
17. To elute DNA from the column, centrifuge at 3,000-5,000 x g for 5 min. Retain flow-through containing the DNA. For maximum yield, place column into a second 15 mL tube and repeat elution step with another 200-500 µL of preheated Elution Buffer or water. 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 by load the eluted DNA back into the column and perform the second.

Yield and quality of DNA: determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

$$\text{DNA concentration} = \text{Absorbance}_{260} \times 50 \times (\text{Dilution Factor}) \mu\text{g/mL}$$

High copy number plasmids generally yield up to 1 mg of DNA from 500 mL culture. The ratio of $(\text{Abs}_{260})/(\text{Abs}_{280})$ gives an indication of nucleic acid purity. A value greater than 1.8 indicates greater than 90% nucleic acid.

Alternatively, quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations. Typically, the majority of the DNA eluted is in monomeric supercoil form, though concatamers may also be present.

Troubleshooting Guide

Problem	Possible Cause	Suggestions
Clogged Column	Incomplete lysis	Extend incubation time of lysis with Buffer BL and protease. Incubate for specified time at 70°C. It may be necessary to extend incubation time beyond 10 min.
	Sample too large	Do not use more than maximum starting sample volume specified in the protocol.
	Sample too viscous	Divide sample into multiple tubes, adjust volume with 10 mM Tris-HCl proportionately. Replace ethanol instead of isopropanol in step 7.
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 ethanol (96%-100%) as specified on page 3 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 sure 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 BL and protease.
	Samples are rich in protein.	After applying to column, wash with 3 mL 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.
	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.