Contents

Introduction

The E.Z.N.A.® Blood DNA Maxi Kit is designed for isolation of genomic DNA from up to 25 ml of fresh, whole blood treated with any common anticoagulant such as heparin, EDTA, or acid-citrate-dextrose. 20ml of blood typically yields 700–1000 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 method is ready for applications such as PCR*, Restriction digestion, Southern blot and so on.

The E.Z.N.A.® Blood DNA Maxi Kit uses the reversible binding properties of HiBind® matrix, a new silica-based material. This is combined with the speed of maxi-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® Maxi spin 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 Maxi Kit, except the OB Protease, RNase A should be stored at 22°C-25°C. Once reconstituted in water, OB Protease must be stored at -20°C. Store RNase A 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 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 Maxi Kit components are guaranteed for at least 24 months from the date of purchase when stored as above.

Binding Capacity

Each HiBind™ DNA Maxi column can bind approximately 2 mg Genomic DNA. Using greater than 25 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	D2492-01	D2492-02	D2492-03
Purification times	5 Preps	20 Preps	50 Preps
HiBind™ DNA Maxi Columns	5	20	50
50 ml Collection Tubes	5	20	50
Buffer TL	20 ml	70 ml	180 ml
Buffer BL	60 ml	220 ml	530 ml
Buffer HB	30 ml	120 ml	300 ml
DNA Wash buffer Concentrate	25 ml	2 x 50 ml	5 x 50 ml
RNase A	110 µl	440 µl	1.1ml
OB Protease	25 mg	100 mg	260 mg
Elution Buffer	20 ml	80 ml	200 ml
Instruction Manual	1	1	1

Before Starting

Take a few minutes to read this booklet thoroughly and become familiar with the protocol.

IMPORTANT

1 Reconstitute OB Protease in 1.4 ml (5 preps), 5 ml (20 Prep) and 13ml (50 preps) 10 mM Tris-HCl, pH 8.0. Vortex vial briefly prior to use. We recommend that you aliquot and store vials of reconstituted protease at -20°C.

2 DNA Wash buffer Concentrate must be diluted with absolute ethanol before use.

D2492-01 Add 100 ml 96-100% ethanol

D2492-02 Add 200 ml 96-100% ethanol to each bottle

D2492-03 Add 200 ml 96-100% ethanol to each bottle

All centrifugation steps must be carried out at room temperature.

Materials to be provided by User

Prepare all materials required before starting to reach optimize performance.

- All the subsequent steps must be performed using a centrifuge capable of at least 4000 x g. Ensure that an appropriate rotor adaptor is in place to prevent damage to the collecting tube. DO NOT USE A FIXED-ANGLE ROTOR.
- Water bath - set to 70°C.
- Absolute ethanol - approximately 3 ml per sample
- Have a shaking water bath set to 55°C.
- 50 ml centrifuge tubes capable of 4000 x g
- Laboratory centrifuge equipped with swinging-bucket rotor.

Harvesting and Storage of Blood

The E.Z.N.A.® DNA Maxi Kit is designed for purification of genomic DNA from up to 20 ml whole blood. The system is not limited by DNA binding capacity of HiBind® DNA Maxi columns (which can bind up to 2000 ug of DNA), but by lysis volume. Due to the abundance of erythrocytes and proteins, greater than 30 ml whole blood will significantly lower DNA quality. The relatively low DNA content of leukocytes means that the maximum binding capacity of HiBind® Maxi DNA columns can not be reached.

Storage of blood samples without previous treatment leads to reduced yields of genomic DNA. For best result, blood samples should be proceeded as following.

- For short-term storage (up to a week), collect blood in tubes containing EDTA as anticoagulant, and store at 4°C.
- 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 Up to 10 ml Whole Blood

This protocol allows rapid isolation of genomic DNA from up to 10 ml blood sample. Yield vary depend on source. Do not use more than 2 x 10^8 cells

1. Add up to 10 ml whole blood to a 50 ml centrifuge tube. If the sample less than 10 ml, bring the volume up to 10 ml with 10 mM Tris-HCl, PBS, or Elution Buffer provided.

2. Add 250 µl OB Protease, 10.2 ml Buffer BL and 20 µl RNase A. Vortex at max speed for 5 minutes to mix thoroughly.

3. Incubate sample at 70°C for 10 min. Briefly vortex the tube once during incubation.

4. Add 10.3 ml absolute ethanol (room temperature, 96-100%) to lysate and mix throughly by vortexing at max speed for 30s.

5. Insert a HiBind® DNA Maxi column in a 50 ml collection tube (provided). Transfer 20 ml of the lysate from Step 4 into the column and centrifuge at 4,000 x g for 5 min to bind DNA. Discard the flow-through liquid and re-use the collection tube.

   NOTE: Since the HiBind® DNA Maxi column can only contains around 20 ml sample volume, it is necessary to load the column twice.

6. Place the column back into the 50 ml collection tube and load the remaining of the lysate for step 4 into the column. Centrifuge as above. Discard the flow-through and re-use the collection tube.

7. Place the column into the same 50 ml tube and wash by pipetting 5 ml of HB Buffer. Centrifuge at 4,000 x g for 5 minutes Discard the flow-through liquid and re-use the collection tube.

8. Place the column into the same 50 ml tube from step 7 and wash by pipetting 10 ml of DNA Wash Buffer **diluted with ethanol**. Centrifuge at 4,000 x g for 5 minutes. Discard the collection tube and flow-through liquid.

9. Using a new 50 ml centrifuge tube, wash the column with a second 10 ml of Wash Buffer **diluted with ethanol** and centrifuge as above. Discard flow-through.

10. Using the same 50 ml collection tube, centrifuge at 4000 x g for 10 min to dry the column. *This step is critical for removal of trace ethanol that might otherwise interfere with downstream applications*.

11. Place the column into a nuclease-free 50 ml centrifuge tube and add 1 ml of preheated (70°C) Elution Buffer. Allow tubes to sit for 5 min at room temperature.

12. 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 50 ml tube and repeat elution step 11-12 with another 1 ml 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 0.5 ml Elution Buffer. Volumes lower than 500 µl greatly reduce yields. *Alternatively, use the first eluate to perform the second elution*

Maxi-Yield protocol for 10- 20 ml Whole Blood

This Modified protocol allows isolation of genomic DNA from up to 20 ml blood sample. Yield vary depend on source. Using more than 25 ml blood is not recommend. Before starting, prepare the Red Blood Lysis Buffer (ERL Buffer) as following:

Red Blood Lysis Buffer (ERL Buffer)

NH4Cl	155mM
KHCO3	10mM
Na2EDTA	0.1mM

Adjust to pH 7.4 with 1M Hcl or NaOH

1. Divide blood sample into two 50 ml tube with equal volume. To 1 volume of whole fresh blood add 5 volumes of 1 x Red Blood Lysis Buffer (ERL Buffer. For example, add 50 ml Buffer ERL to 10 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 ERL Buffer 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. Again, completely remove and discard the supernatant.

6. Resuspend the cell pellet with 0.5 ml PBS Buffer and 3ml TL buffer to the pelleted white blood cells in each tube and vortex thoroughly to mix. Combine the sample into one 50 ml tube, there should be 7ml total sample after the combination.

7. Add 250ul of OB protease and vertex 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.

8. Add 20 ul RNase A, 7 ml Buffer BL and mix thoroughly by vortexing at max speed for 30s. Incubate the mixture at 70°C for 10 minutes. A wispy precipitate may form on addition of Buffer BL, but does not interfere with DNA recovery.

9. Add 7 ml absolute ethanol and mix thoroughly by vortexing at max 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 4000 X g.

10. Proceed step 5-12 of standard protocol on page 5.

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:

DNA concentration = Absorbance$_{260}$ × 50 × (Dilution Factor) µg/ml

The ratio of (Abs_{260})/(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.

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 70oC. It may be necessary to extend incubation time by 10 min.
	Sample too large	Do not use more than 25 ml of whole blood
Low DNA yield	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume (see note on page 6). Incubation of column at 70oC for 5 min with Elution Buffer may increase yields.
	Improper washing	DNA Wash Buffer Concentrate must be diluted with absolute (100%) ethanol.
Low A260/A280 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 lysis for incompletely mixing with Buffer BL	making sure to vortex 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 5ml 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 cell and/or protein lysis in Buffer TL.	Increase incubation time at 55oC 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 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.