Contents

Introduction. 2

Principle. 2

Storage. 2

Kit Contents. 2

Important Notes. 3

Before Starting. 3

Standard Protocol (for up to 10 mL blood). 4

Maximum yield protocol (for 10- 20 mL blood). 5

DNA Quality. 6

Troubleshooting Tips. 7

Introduction

E.Z.N.A.™ Blood DNA Maxiprep Kits are designed for isolation of total DNA (include genomic, mitochondrial and viral DNA) from 2-10 mL (with standard protocol) and up to20 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 an dbone 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 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 Maxi-column spin technology to provide fast and high quality DNA . The standard protocol Sample is first mixed with BL buffer which lyse the cell and release DNA under denaturing conditions that inactivate DNases. The cell lysate is then loaded into the HiBind® Maxi- spin. DNA binds to HiBind® matrix while impurities are effectively removed after few quick wash steps. genomic DNA is purified on the HiBind® Maxi spin column. The ready-to-use high quality DNA can be eluted in DNA Elution Buffer or water.

Storage

E.Z.N.A.® Blood DNA Maxiprep Kits should be stored at room temperature. During shipment, crystals may form in the Buffer BL. Warm to 37°C and gently shake container to dissolve. All the kit components are guaranteed for at least 24 months from date of purchase when properly stored.

Kit Components

Blood DNA Maxi Kit	D 2492-00	D 2492-01	D 2492-02	D 2492-03
Purification	2	10	20	50
HiBind® DNA Maxi Columns	2	10	20	50
50 mL Collection Tubes	2	10	20	50
Buffer TL	10mL	35mL	70mL	160mL
Buffer BL	25mL	120mL	240mL	600mL
Buffer HB	15mL	60mL	120mL	300mL
Equilibration Buffer	10mL	35mL	75mL	2 x 85mL
DNA Wash Buffer	40mL	100mL	200mL	3 x 200mL
RNase A	50mL	250µL	500µL	2.2mL
OB Protease	20mg	60mg	120mg	300mg
DNA Elution Buffer	10mL	30mL	60mL	120mL
Instruction Manual	1	1	1	1

Harvesting and Storage of Blood

E.Z.N.A.™ DNA Maxiprep Kits are designed for purification of genomic DNA from 3-10 mL (with standard protocol) and up to20 mL (with maximum yield protocol) whole blood. The system is not limited by the DNA binding capacity of HiBind® Maxi columns (which can bind up to 1.5 mg of DNA), but by lysis volume. Due to the abundance of erythrocytes and proteins, greater than 20 mL whole blood can significantly lower DNA quality. The relatively low DNA content of leukocytes means that the maximum binding capacity of HiBind® DNA Maxi 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 time prior DNA isolation, as leukocyte transcripts generally have variable stabilities.

Before Starting

IMPORTANT

Reconstitute **OB Protease** in **Elution Buffer** or **Tris-Buffer** (10mM, pH 8). Vortex vial briefly prior to use. Store vials of reconstituted OB Protease at -20°C.

D2492-00 Add 1mL Elution Buffer
D2492-01 Add 3mL Elution Buffer
D2492-02 Add 6mL Elution Buffer
D2492-03 Add 15mL Elution Buffer

Dilute **DNA Wash buffer** with **absolute ethanol** as follows

D2492-00 Add 60mL ethanol
D2492-01 Add 150mL ethanol
D2492-02 Add 300mL ethanol
D2492-03 Add 300mL ethanol

Materials and Equipments to be provided by user

- Water bath or incubator set to 70°C
- Absolute ethanol (96%-100%)
- Isopropanol (absolute)
- Shaking water bath set to 55°C
- 50 mL centrifuge tubes capable of 4,000 x g
- Laboratory centrifuge equipped with **swinging-bucket** rotor
- All subsequent steps must be performed using a centrifuge capable of at least 5,000 x g. Ensure that an appropriate rotor adaptor is in place to prevent damage to the collection tube. **DO NOT USE A FIXED-ANGLE ROTOR.**

A. Standard Protocol (for up to 10 mL whole blood)

All centrifugation steps must be carried out at room temperature for standard protocol.

1. Add up to 10 mL whole blood to a 50 mL centrifuge tube. If the sample is less than10 mL, bring the volume up to 10 mL with 10 mM Tris-HCl, PBS, or Elution Buffer provided.

2. Add 250 µL OB Protease and vortex. Add 10.2 mL Buffer BL. Vortex

5 min to mix thoroughly.

3. Add 20 µL RNase A solution to each sample to remove RNA.

4. Incubate sample at 70°C for 10 min.

5. Briefly vortex the tube once during incubation.

6. Add 10.3 mL of ethanol or isopropanol to lysate and mixthroughly by vortexing. ***For buffy coat, isolated leukocytes and cultured cells, yields will improve if 260 µL absolute ethanol is used in place of isopropanol.***

7. Insert a HiBind® DNA Maxi-spin column in a 50 mL collection tube (provided). Transfer the solution from Step 6 into the column and centrifuge at 4,000 x g for 5 min to bind DNA. **Discard the collection tube and flow-through liquid**.

8. Place the column into a **second 50 mL tube** and wash by pipetting 5 mL of HB Buffer. Centrifuge at 3,000 x g for 5 minutes Discard the flow-through liquid and re-use the collection tube in the next step.

9. Place the column into the **same 50 mL tube** from step 8 and wash by pipetting 10 mL of DNA Wash Buffer diluted with ethanol. Centrifuge at 3000 x g for 5 minutes. Discard the collection tube and reuse the collection tube.

10. Using a **same centrifuge tube from step 9**, wash the column with a second 10 mL of DNA Wash Buffer and centrifuge as above. Discard flow-through and reuse the collection tube.

11. Using the same 50 mL collection tube, centrifuge at 4500 x g for 10 min to dry the column.
NOTE: It is critical for removing traces of ethanol that may otherwise interfere with downstream applications. Remove any traces of ethanol from the column using a pipette.

Optional: If the centrifugal force is less than 4000 x g It might be necessary to dry the column further by placing the column in a vacuum oven at 70°C for 10 minutes.

12. Place the column into a nuclease-free 50 mL centrifuge tube and add 1 mL of preheated (70°C) Elution Buffer (1mM Tris-HCl, pH 8.5). Allow tubes to incubate for 3 to 5 min at room temperature.

13. To elute DNA from the column, centrifuge at 4,500 x g for 5 min. Retain flow-through containing the DNA. Place column into a second 50 mL tube and repeat elution step 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***.

B. Maximum Yield Protocol (for 10- 20 mL whole blood)

Prepare the Red Blood Lysis Buffer as following	
NH_4Cl	155mM
$KHCO_3$	10mM
Na_2EDTA	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 1x Buffer 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 briefly vortexing twice. Lysis of red blood cells is indicated when the solution becomes translucent. For blood samples from individuals with an elevated hematocrit or ECR, extend the 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 Red Blood Lysis Buffer per volume of whole blood used in step 1. Thoroughly vortex to resuspend cells.

Example: If you used 10 mL of whole blood, wash with 20 mL of Red Blood Lysis Buffer .

5. Centrifuge at 450 x g for 10 min at 4°C. Again, completely remove and discard the supernatant.

6. Resuspend the cells by adding 0.5 mL PBS Buffer and 3 mL TL Buffer to the pelleted white blood cells in each tube. 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 (20mg/mL 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 depends on the amount and source of blood, but is usually less than 2 hours.

8. Add 20 µL RNase A solution at this point if high levels of RNA can be seen from gel analysis. Incubate the mixture at **70°C** for 10 minutes.

9. Add 7 mL Buffer BL and vortex to mix. A wispy precipitate may form upon addition of Buffer BL; it will not interfere with DNA recovery.

10. Add 7 mL absolute ethanol (96%-100%) and mix thoroughly by vortexing. 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 CAPABLE OF 5,000 X g.

11. Assemble a HiBind® DNA Maxi spin column in a 50 mL collection tube (provided). Apply the entire sample from step 11 into the column, including any precipitate that may have formed. Centrifuge at 3,000 x g for 5 min to bind DNA. Discard both flow-through liquid and reuse the collection tube.

12. Wash the column by pipetting 5 mL of HB Buffer. Centrifuge at 4,000 x g for 3 min. **Discard the flow-through liquid and collection tube.** **Note:** Remove any liquid from the thread of the 50 mL tube before reinserting the column.

13. Place the column into a new 50 mL collection tube. Add 10 mL DNA Wash Buffer to the column. Centrifuge at 4,000 x g for 5 min. **Discard the flow-through liquid and re-use the collection tube.** **NOTE:** Remove any liquid from the threads of the 50 mL tube before reinserting the column.

14. Place the column back into the same 50 mL tube and wash by pipetting 10 mL DNA Wash Buffer into the column. Centrifuge at 4,000 x g for 5 min. Re-use the collection tube and discard flow-through liquid. **Note:** Remove any liquid from the thread of the 50 mL tube before re-inserting the column.

15. Centrifuge the empty capped column for 15-20 min at 4000 x g to dry the column matrix. **Do not skip this step - it is critical for removing traces of ethanol that may otherwise interfere with downstream applications. Remove any traces of ethanol that remain from the column using a pipette.**

    Optional: If the centrifuge force is below 4000 x g, it might be necessary to dry the column further by placing the column in a vacuum oven at 65° C for 10 minutes.

16. Place column into a clean 50 mL centrifuge tube. Add 1-2 mL (depending on desired final concentration) Elution Buffer, pre-heated to 70° C, onto the column matrix. Incubate at room temperature for 3 to 5 min, then centrifuge 10 min at 4,000 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.

C. Vacuum Protocol (for up to 10 mL of whole blood sample)

Note: This protocol required more HB and DNA Wash Buffer, additional HB and DNA wash Buffer can be purchased separately from Omega Bio-tek and its distributors.

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 and mix the sample throughly by vortexing.

3. Add 12 mL of Buffer BL. Vortex 5 minutes to mix thoroughly.

4. Add 20 µL RNase A solution to each sample to remove RNA.

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

6. Add 10 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.

7. Insert the HiBind® DNA maxi-spin column on a outlet of vacuum manifold. Transfer the half volume of sample into the column. Apply the vacuum until all the sample pass through the membrane.

8. Load the remainder of the sample into the column. Apply the vacuum until all the sample pass through the membrane. Turn off the vacuum.

9. Add 10 mL HB Buffer into the column. Apply the vacuum until all the liquid pass through the column. Turn off the vacuum.

10. Add 15 mL DNA wash Buffer into the column. Apply the vacuum until all the liquid pass through the column. Turn off the vacuum.

11. Add another 15 mL DNA wash Buffer into the column. Apply the vacuum until all the liquid pass through the column. After all the liquid pass through the membrane, apply maximum vacuum for additional 50 minutes to dry the column.
NOTE: It is critical for removing traces of ethanol that may otherwise interfere with downstream applications. Remove any traces of ethanol from the column using a pipettor. It might be necessary to dry the column further by placing the column in a vacuum oven at 70°C for 10 minutes.

12. Turn off the vacuum and remove the column from manifold. Insert a HiBind® DNA Maxii-spin column in a 50 mL collection tube (provided).

13. Apply 1 mL DNA Elution Buffer into the center of the membrane. Place the column into a nuclease-free 50 mL centrifuge tube (not provided) and apply 1 mL of preheated (70°C) Elution Bufferor water onto the center of the membrane. Allow tubes to sit for 2 min at room temperature.

14. 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 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.

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.
Low DNA Yield	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 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 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.
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.