omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Blood DNA Maxi Kit

D2492-00	2 preps
D2492-03	50 preps

Manual Date: July 2019
Revision Number: v8.0

For Research Use Only

Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

omega-bio-tek
omegabiotek
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Introduction

E.Z.N.A.® Blood DNA Maxi Kits are designed for isolation of total DNA (include genomic, mitochondrial, and viral DNA) from 2-10 mL (with the standard protocol) and up to 20 mL (with the 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 PCR.

Overview

The E.Z.N.A.® Blood DNA Maxi Kits combine the reversible binding properties of HiBind® matrix, a new silica-based material, with the speed of spin column technology to provide fast and high-quality DNA. The sample is first mixed with BL Buffer which lyses the cells and releases DNA under denaturing conditions which inactivate DNases. The cell lysate is then loaded onto the HiBind® DNA Maxi Column. DNA binds to HiBind® matrix while impurities are effectively removed after few quick wash steps. Genomic DNA is purified on the HiBind® DNA Maxi Column. The ready-to-use high-quality DNA can be eluted in Elution Buffer or water.

New in this Edition:

July 2019:
- DNA Wash Buffer, HBC Buffer, and Elution Buffer have been increased as necessary to meet standardized packaging sizes.

June 2018:
- The amount of DNA Wash Buffer supplied with D2492-03 has been increased.

September 2017:
- The HiBind® DNA Maxi Columns have been updated to improve functionality.
- Additional Elution Buffer has been provided to account for the increase in elution volume.

April 2014:
- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- Equilibration Buffer (used in the Troubleshooting section) is no longer included with this kit.
- Equilibration Buffer can be replaced with 3M NaOH provided by the user.

Kit Contents

Product	D2492-00	D2492-03
Preparations	2	50
HiBind® DNA Maxi Columns	2	50
50 mL Collection Tubes	2	50
TL Buffer	15 mL	375 mL
BL Buffer	25 mL	600 mL
HBC Buffer	10 mL	200 mL
DNA Wash Buffer	25 mL	2 x 200 mL
RNase A	50 µL	1.1 mL
Proteinase K Solution	550 µL	13 mL
Elution Buffer	30 mL	500 mL
User Manual	✓	✓

Storage and Stability

E.Z.N.A.® Blood DNA Maxi Kits should be stored at room temperature. Proteinase K Solution can be stored at room temperature for 12 months. For long-term storage (>12 months), store Proteinase K Solution at 2-8°C. Under cool ambient conditions, a precipitate may form in the BL Buffer. If a precipitate is present, heat the bottle at 37°C to dissolve. All the kit components are guaranteed for at least 12 months from date of purchase when properly stored.

Preparing Reagents

- Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D2492-00	100 mL
D2492-03	800 mL per bottle

- Dilute HBC Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
D2492-00	4 mL
D2492-03	78 mL

E.Z.N.A.® Blood DNA Maxi Kit Protocol - up to 10 mL Whole Blood

Materials and Equipment to be Supplied by User:

- Water bath or incubator
- Shaking water bath
- Nuclease-free 50 mL centrifuge tubes
- Centrifuge equipped with swing bucket rotor capable of at least 4,000 x *g* (do not use a fixed-angle rotor)
- 100% ethanol
- 100% isopropanol
- Optional: 10 mM Tris-HCl or PBS

Before Starting:

- Prepare DNA Wash Buffer according to the "Preparing Reagents" section on Page 4
- Set water bath or incubator to 70°C
- Heat Elution Buffer to 70°C

1. Transfer up to 10 mL whole blood to a 50 mL centrifuge tube (not provided).

   Note: If the sample is less than 10 mL, bring the volume to 10 mL with 10 mM Tris-HCl, PBS, or Elution Buffer (provided).

2. Add 250 µL Proteinase K Solution. Vortex to mix thoroughly.

3. Add 10.2 mL BL Buffer. Vortex for 5 minutes to mix thoroughly.

4. Add 20 µL RNase A.

5. Incubate sample at 70°C for 10 minutes. Vortex once during incubation.

6. Add 10.3 mL 100% ethanol or 100% isopropanol. Vortex to mix thoroughly.

   Note: For buffy coat, isolated leukocytes, and cultured cells, yields will improve if 100% ethanol is used in place of isopropanol.

7. Insert a HiBind® DNA Maxi Column in a 50 mL Collection Tube (provided).

8. Transfer the sample from Step 6 to the column.

9. Centrifuge at 4,000 x *g* for 5 minutes.

10. Discard the filtrate and reuse the Collection Tube.

11. Repeat Steps 8-10 until all the sample has been transferred to the column.

12. Transfer the HiBind® DNA Maxi Column into a new 50 mL Collection Tube.

13. Add 5 mL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol. Please see the instructions on Page 4.

14. Centrifuge at 3,000 - 5,000 x *g* for 3 minutes.

15. Discard the filtrate and reuse the Collection Tube.

16. Add 15 mL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol. Please see the instructions on Page 4.

17. Centrifuge at 3,000 - 5,000 x *g* for 5 minutes.

18. Discard the filtrate and reuse the Collection Tube.

19. Repeat Steps 16-18 for a second DNA Wash Buffer wash step.

20. Centrifuge at ≥4,000 x *g* for 10 minutes to dry the membrane.

    Note: It is critical to remove any trace of ethanol that may otherwise interfere with downstream applications.

Optional: If the centrifugal force is less than 4,000 x *g*, it may be necessary to dry the membrane further in a 70°C oven for 10 minutes.

21. Transfer the HiBind® DNA Maxi Column into a nuclease-free 50 mL centrifuge tube (not provided).

22. Add 2 mL Elution Buffer heated to 70°C.

23. Let sit for 3 to 5 minutes at room temperature.

24. Centrifuge at 4,000 x *g* for 5 minutes.

25. Repeat Steps 22-24 for a second elution step.

    Note: Each elution typically yields 60%-70% of the DNA bound to the column. Thus two elutions generally give >80%. However, elution volumes less than 2 mL reduce yields. To obtain DNA at higher concentrations, elution can be carried out using 500 µL Elution Buffer. Volumes lower than 500 µL greatly reduce yields. Alternatively, use the first eluate to perform the second elution.

26. Store DNA at -20°C.

E.Z.N.A.® Blood DNA Maxi Kit Protocol - 10 to 20 mL Whole Blood

Materials and Equipment to be Supplied by User:

- Water bath or incubator
- Shaking water bath
- Nuclease-free 50 mL centrifuge tubes capable of 4,000 x *g*
- Refrigerated centrifuge equipped with swing bucket rotor capable of at least 4,000 x *g* (do not use a fixed-angle rotor)
- 100% ethanol
- 100% isopropanol
- PBS
- Ammonium chloride (NH_4Cl)
- Potassium bicarbonate ($KHCO_3$)
- Disodium EDTA (Na_2EDTA)

Before Starting:

- Prepare DNA Wash Buffer according to the "Preparing Reagents" section on Page 4
- Set water bath or incubator to 70°C
- Set shaking water bath to 55°C
- Heat Elution Buffer to 70°C

1. Prepare Red Blood Cell Lysis Buffer according to the table below.

Red Blood Cell Lysis Buffer	
NH_4Cl	155 mM
$KHCO_3$	10 mM
Na_2EDTA	0.1 mM
Adjust to pH 7.4	

2. Divide blood sample evenly into two 50 mL centrifuge tubes (not provided).

3. Add 4 volumes Red Blood Cell Lysis Buffer.

4. Incubate for 15 minutes on ice. Vortex twice during incubation.

   Note: Lysis of red blood cells is indicated when the solution becomes translucent. For blood samples from individuals with an elevated hematocrit or ESR, extend the incubation time to 20 minutes.

5. Centrifuge at 450 x *g* for 10 minutes at 4°C.

6. Aspirate and discard the supernatant.

7. Add 2 volumes Red Blood Lysis Buffer per 1 volume whole blood used in Step 2. Vortex to resuspend cells.

8. Centrifuge at 450 x *g* for 10 minutes at 4°C.

9. Aspirate and discard the supernatant.

10. Add 0.5 mL PBS. Vortex vigorously to completely resuspend the cell pellet.

11. Add 3 mL TL Buffer. Vortex to mix thoroughly.

12. Combine the sample into one 50 mL centrifuge tube (not provided).

13. Add 250 µL Proteinase K Solution. Vortex to mix thoroughly.

14. Incubate at 55°C in a shaking water bath.

    Note: If a shaking water bath is not available, vortex every 20-30 minutes. Lysis time depends on the amount and source of blood, but is usually less than 2 hours.

15. Add 20 µL RNase A.

16. Incubate at 70°C for 10 minutes.

17. Add 7 mL BL Buffer. Vortex to mix thoroughly.

    Note: A wispy precipitate may form upon addition of BL Buffer; it will not interfere with DNA recovery.

18. Add 7 mL 100% ethanol. Vortex to mix thoroughly.

    Note: If any precipitation can be seen at this point, pass the precipitant through a needle.

19. Insert a HiBind® DNA Maxi Column in a 50 mL collection tube (provided).

20. Transfer the entire sample from Step 18 to the HiBind® DNA Maxi Column, including any precipitate that may have formed.

21. Centrifuge at 3,000 - 5,000 x *g* for 5 minutes.

22. Discard the filtrate and reuse the Collection Tube.

23. Add 5 mL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol. Please see the instructions on Page 4.

24. Centrifuge at 3,000 - 5,000 x *g* for 3 minutes.

25. Discard the filtrate and the Collection Tube.

26. Insert the HiBind® DNA Maxi Column into a new 50 mL Collection Tube.

27. Add 15 mL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol. Please see the instructions on Page 4.

28. Centrifuge at 3,000 - 5,000 x *g* for 5 minutes.

29. Discard the filtrate and reuse the Collection Tube.

30. Repeat Steps 27-29 for a second DNA Wash Buffer wash step.

31. Centrifuge at ≥4,000 x *g* for 10 minutes to dry the membrane.

    Note: It is critical to remove any trace of ethanol that may otherwise interfere with downstream applications.

Optional: If the centrifugal force is less than 4,000 x *g*, it may be necessary to dry the membrane further in a 70°C oven for 10 minutes.

32. Transfer the HiBind® DNA Maxi Column into a nuclease-free 50 mL centrifuge tube.

33. Add 2 mL Elution Buffer heated to 70°C.

34. Let sit for 3 to 5 minutes at room temperature.

35. Centrifuge at 4,000 x *g* for 10 minutes.

36. Repeat Steps 33-35 for a second elution step.

    Note: Each elution typically yields 60%-70% of the DNA bound to the column. Thus two elutions generally give >80%. However, elution volumes less than 2 mL reduce yields. To obtain DNA at higher concentrations, elution can be carried out using 500 µL Elution Buffer. Volumes lower than 500 µL greatly reduce yields. Alternatively, use the first eluate to perform the second elution.

37. Store DNA at -20°C.

E.Z.N.A.® Blood DNA Maxi Kit Protocol - Vacuum Protocol

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

Materials and Equipment to be Supplied by User:

- Vacuum Manifold (Cat# VAC-08)
- Water bath or incubator
- Shaking water bath
- Nuclease-free 50 mL centrifuge tubes capable of 4,000 x *g*
- Centrifuge equipped with swing bucket rotor capable of at least 5,000 x *g* (do not use a fixed-angle rotor)
- 100% ethanol
- 100% isopropanol
- Optional: 10 mM Tris-HCl or PBS

Before Starting:

- Prepare DNA Wash Buffer according to the "Preparing Reagents" section on Page 4
- Set water bath or incubator to 70°C
- Set shaking water bath to 55°C
- Heat Elution Buffer to 70°C

1. Transfer up to 10 mL whole blood to a 50 mL centrifuge tube (not provided).

   Note: If the sample is less than 10 mL, bring the volume to 10 mL with 10 mM Tris-HCl, PBS, or Elution Buffer (provided).

2. Add 250 µL Proteinase K Solution. Vortex to mix thoroughly.

3. Add 10.2 mL BL Buffer. Vortex for 5 minutes to mix thoroughly.

4. Add 20 µL RNase A.

5. Incubate sample at 70°C for 10 minutes. Vortex once during incubation.

6. Add 10.3 mL 100% ethanol or 100% isopropanol. Vortex to mix thoroughly.

   Note: For buffy coat, isolated leukocytes, and cultured cells, yields will improve if 100% ethanol is used in place of isopropanol.

7. Insert the HiBind® DNA Maxi Column on a outlet of the vacuum manifold.

8. Transfer one-half volume of sample to the HiBind® DNA Maxi Column.

9. Apply the vacuum until all the sample has passed through the membrane.

10. Turn off the vacuum source.

11. Transfer the remainder of the sample to the HiBind® DNA Maxi Column.

12. Apply the vacuum until all the sample has passed through the membrane.

13. Turn off the vacuum source.

14. Add 5 mL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol. Please see the instructions on Page 4.

15. Apply the vacuum until all the buffer has passed through the membrane.

16. Turn off the vacuum source.

17. Add 15 mL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol. Please see the instructions on Page 4.

18. Apply the vacuum until all the buffer has passed through the membrane.

19. Repeat Steps 16-18 for a second DNA Wash Buffer wash step.

20. Continue to apply maximum vacuum for an additional 50 minutes to dry the membrane.

    Note: It is critical to dry the membrane. Residual ethanol may interfere with downstream applications.

21. Turn off the vacuum source.

Optional: It may be necessary to dry the column further in a 70°C oven for 10 minutes.

22. Transfer the HiBind® DNA Maxi Column into a nuclease-free 50 mL centrifuge tube (not provided).

23. Add 2 mL Elution Buffer heated to 70°C.

24. Let sit for 3 to 5 minutes at room temperature.

25. Centrifuge at 4,000 x *g* for 5 minutes.

26. Repeat Steps 23-25 for a second elution step.

    Note: Each elution typically yields 60%-70% of the DNA bound to the column. Thus two elutions generally give >80%. However, elution volumes less than 2 mL reduce yields. To obtain DNA at higher concentrations, elution can be carried out using 500 µL Elution Buffer. Volumes lower than 500 µL greatly reduce yields. Alternatively, use the first eluate to perform the second elution.

27. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Clogged Column	Incomplete lysis	Extend incubation time of lysis with BL Buffer and proteinase. Incubate for specified time at 70°C. It may be necessary to extend incubation time beyond 10 minutes.
	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 or PBS proportionately.
Problem	**Cause**	**Solution**
Low DNA Yield	Poor elution	Repeat elution or increase elution volume. Incubate column at 70°C for 5 minutes with Elution Buffer.
	Improper washing	DNA Wash Buffer must be diluted with 100% ethanol as instructed on Page 4 before use.
		HBC Buffer must be diluted with 100% isopropanol as instructed on Page 4 before use.
	Poor cell lysis due to improper mixing with BL Buffer	Repeat the procedure, make sure to vortex the sample with BL Buffer immediately and thoroughly.
	Incomplete cell lysis or protein degradation due to insufficient incubation	Increase incubation time with BL Buffer and proteinase.
	Samples are rich in protein	After applying to column, wash with 3 mL of a 1:1 mixture of BL Buffer and ethanol and then proceed with HBC Buffer and DNA Wash Buffer.
	Columns need to be activated	Add 3 mL 3M NaOH to the column prior to loading the sample. Centrifuge at 3,000-5,000 x *g* for 3 minutes. Discard the filtrate.

Troubleshooting Guide

Problem	Cause	Solution
No DNA Eluted	Poor cell lysis due to improper mixing with BL Buffer	Mix thoroughly with BL Buffer prior to loading HiBind® column.
	Ethanol was not added to DNA Wash Buffer	DNA Wash Buffer must be diluted with 100% ethanol as instructed on Page 4 before use.
Problem	**Cause**	**Solution**
Washing Leaves Colored Residue in Column	Poor cell lysis due to improper mixing with BL Buffer	BL Buffer is viscous and the sample must be vortexed thoroughly.
	Ethanol was not added to DNA Wash Buffer	DNA Wash Buffer must be diluted with 100% ethanol as instructed on Page 4 before use.

For more purification solutions, visit **www.omegabiotek.com**

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