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Revised November 2007

Introduction

The E-Z 96® Blood DNA Kit allows rapid and reliable isolation of high-quality genomic DNA or viral DNA from a wide variety of sample sources including fresh, frozen, or anticoagulated whole blood, serum, plasma, bone marrow, body fluids; lymphocytes and cultured cells. This kit incorporates the reversible nucleic acid-binding properties of HiBind® matrix in a high-throughput 96-well format to eliminate proteins, nucleases and other enzyme inhibitors or contaminators from blood or body fluids. Up to 96 samples can be simultaneously processed in a single E-Z 96® DNA plate. The newly designed E-Z 96® DNA plate has a binding capacity of 50 µg DNA per well. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions, thus reducing plastic waste and hands-on time to allow up to 96 samples to be processed at one time.

Overview

If using the E-Z 96® Blood DNA Kit for the first time, please read this manual in its entirety to become familiar with the procedures. Blood or other body fluid samples are added to a specially formulated buffer containing detergent and mixed with proteinase. Binding conditions are then adjusted and the sample is applied to the E-Z 96® DNA plate. Two rapid wash steps remove trace contaminants and enzyme inhibitors and pure DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the E-Z 96® Blood DNA Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer BL. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffers at room temperature and the OB protease at -20°C.

Kit Contents

Product Number	D1192-00	D1192-01	D1192-02
E-Z 96® DNA Plate	1	4	20
96-Well Collection Plate (2 mL)	1*	2*	4*
Round Well Plate (1.2mL)	1	4	20
Caps for Round-well Plate	24 x 8	96 x 8	480 x 8
Racked Microtubes	1	4	20
Caps for Racked Microtubes	12 x 8	48 x 8	240 x 8
Buffer BL	30 mL	100 mL	500 mL
OB Protease	50 mg	200 mg	1.0 g
Buffer HB	45 mL	170 mL	820 mL
DNA Wash Buffer	80 mL	300 mL	3 x 400mL
Elution Buffer	40 mL	160 mL	2 x 250 mL
AeraSeal Film	5	20	100
Instruction Booklet	1	1	1

Note: 96-Well Collection Plates (2mL) can be used to collect flow-through from the E-Z 96® DNA plate. They are designed for repeated use. Wash the plates thoroughly in tap water after each use. Incubate 5 minutes at room temperature in 0.5M HCl. Rinse with distilled water. Used plates can also be autoclaved after washing.

Materials to be provided by user

- Laboratory centrifuge capable of at least 5,000 x g equipped with swinging-bucket rotor.
- Rotor adapter for deep well microplates
- Waterbath equilibrated to 65°C
- Absolute (96%-100%) ethanol
- Multichannel pipet with tips
- **Optional:** RNase A stock solution (20 mg/mL)
- Incubator or vacuum oven preset at 65°C

Before Starting

Please read the entire manual to become familiar with the E-Z 96® Blood DNA Kit procedures.

Important

Prepare an **OB Protease** stock solution with **Elution Buffer** and aliquot into adequate portions. Store each aliquot at -20°C and thaw before use. Each sample will require 25 µL of this solution.

D1192-00 Dissolve with 2.5 mL Elution Buffer
D1192-01 Dissolve with 10.0 mL Elution Buffer
D1192-02 Dissolve with 50.0 mL Elution Buffer

Dilute **DNA Wash Buffer** Concentrate with **absolute ethanol** as follows and store at room temperature.

D1192-00 Add 120 mL (96%-100%) ethanol
D1192-01 Add 450 mL (96%-100%) ethanol
D1192-02 Add 600 mL (96%-100%) ethanol per bottle

Preheat Elution Buffer at 65°C

Adjust the volume of samples to 250 µL. For samples smaller than 250 µL, add appropriate volume of PBS to bring them to 250 µL. For samples larger than 250 µL, split each sample into two 250 µL aliquots and use two wells of the 1.2 mL round well plate for lysis. Load the combined lysates into each well of the E-Z 96® DNA Plate.

Storage of Blood Samples

Storage of blood samples without previous treatment leads to reduced yields of genomic DNA. For the 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.

Protocol 1: E-Z 96® Blood DNA Protocol

1. Pipet 25 µL OB Protease (or Proteinease K stock, 20mg/mL) into the bottom of each well of the 1.2 mL round well plate. Make a chart to record the position of each sample. Use multichannel pipet to dispense the protease solution deep inside of each well to avoid potential contamination.

2. Add samples to each well of the 1.2 mL round-well plate by touching the inside of the well without touching the rims with tip ends. Use 250 µL whole blood, serum or body fluids for each well of the round-well deep well plate. (Up to 6 x 10^6 lymphocytes or cultured cells in PBS can be used in each well.)

   Note: For sample volumes smaller or larger than 250 µl, adjust the sample volume to 250 µL. (See the Before Starting section on Page 3 for details.)

3. Add 250 µL Buffer BL to each sample. Take care not to touch the rims of the wells with tip ends, which might lead to cross-contamination.

4. **Optional:** Add 5 µL RNase A solution (20mg/mL) to each sample to remove RNA. Optionally, a 5 µL RNase A solution per 250 µL Buffer BL mixture (20µL RNase A/1 mL Buffer BL) can be prepared in advance of Step 2 above to simplify delivery; in which case 255 Buffer BL/RNase A solution mixture should be added to each sample in Step 2.

5. Seal the round-well plate with 8-strip caps (supplied) and mix the samples thoroughly by vortexing or vigorously shaking the plate (side to side) for 30 seconds.

   Note: Shake the rack side to side, not up and down, to prevent possible leakage around microtube caps.

6. Centrifuge briefly at 2000 x g to collect any solution from caps.

7. Incubate at 65°C for 10 minutes in an incubator or oven. Mix occasionally during incubation by rotating the plate gently.

   Note: Incubation for more than 30 min at 65° C can cause DNA degradation.

8. Centrifuge briefly at 2000 x g to collect any solution fro caps. Remove the microtube caps and add 250 µl of absolute ethanol (96-100%) to each well.

9. Seal the round-well plate using **new caps (supplied)**.

10. Mix the samples by vortexing or vigorously shaking the plate (side to side) for 1 minute. Centrifuge briefly at 2000 x g to collect any liquid from the caps.

11. Place the E-Z 96® DNA plate on top of a 2 mL 96-well Collection Plate (supplied). Mark the E-Z 96® DNA plate for later identification.

12. Transfer all the samples from Step 10 to each well of the E-Z 96® DNA plate.

13. Seal the E-Z 96® DNA plate with AeraSeal film cover. Centrifuge at 5,000 x g for 5-10 minutes. Make sure all the samples have passed through the membrane in each well of the E-Z 96® DNA plate.

14. Discard the flow-through in the 96-well Collection Plate before the following wash step.

15. Remove the adhesive film cover, then add 400 µL HB Buffer to each well.

16. Seal the plate with new AeraSeal film cover; then centrifuge the plate at 5000 x g for 5 minutes. Discard the flow-through in the 96-well Collection Plate.

17. Remove the adhesive film cover, then add 700 µL DNA wash buffer diluted with absolute ethanol to each well.

    Note that DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle or page 4. If refrigerated, the diluted wash buffer must be brought to room temperature before use.

18. Seal the plate with new AeraSeal film cover; then centrifuge the plate at 3000-5000 x g for 5 minutes. Discard the flow-through in the 96-well collection plate.

19. Remove the adhesive film cover and again add 700 µL DNA wash buffer diluted with absolute ethanol to each well. Place the E-Z 96® DNA plate on top of the 2 mL Collection plate, seal the E-Z 96® DNA plate with adhesive film cover and centrifuge at maxi speed (≥5,000 x g) for 10 minutes.

20. Remove the adhesive film cover and incubate the E-Z 96® DNA plate in a vacuum oven or incubator preset at 70°C for 7 minutes to dry the membrane.

    Note: These drying steps are *critical* for removal of trace amounts of ethanol that might otherwise interfere with downstream applications.

21. Place the E-Z 96® DNA plate on top of a new racked microtunes (supplied). Add 200 µL Elution Buffer preheated at 65°C to each well of the E-Z 96® DNA plate. Incubate at room temperature for two to four minutes or in incubator set at 65°C for one to two minutes.

22. Seal the E-Z 96® DNA plate with new adhesive film cover and centrifuge the plate at 5,000 x g for 5 minutes to elute DNA.

Note: First elution typically yields 60%-70% of the DNA bound to the column. Second elution with 200 µL Elution Buffer can increase the yield by 20%. However, increasing elution volume reduces the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 100 µL Elution Buffer. Volumes lower than 50 µL greatly reduce yields.

Protocol 2: E-Z 96® DNA Protocol for Viral DNA

1. Integrated viral DNA or proviral DNA can be isolated by using the same standard protocol as for genomic DNA

2. Viral DNA or RNA from extracellular viruses can be isolated with the E-Z 96® Viral RNA kit. To avoid genomic DNA contamination, cell free samples are recommended. Use 10-12 µg of carrier DNA (such as poly dA or Poly dT) for each 250 µL sample. Adjust binding condition by add 280 µL of ethanol instead of 250 at Step 8 of the standard protocol.

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl buffer, or Elution Buffer as blank. DNA concentration is calculated as:

[DNA] = (Absorbance$_{260}$) x (0.05 µg/µL) x (Dilution factor)

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio of (A_{260}/A_{280}) of 1.7-1.9 corresponds to 85%-95% purity

Toubleshooting Guide

Problem	Possible Cause	Suggestions
Clogged well	Incomplete lysis	Add the correct volume of Buffer BL and incubate for specified time at 65°C. It may be necessary to extend incubation time by 10 min.
	Sample too large	If using more than 250 µL of blood, increase volumes of Protease, Buffer BL, and isopropanol. Pass lysate through one well successively.
	Sample too viscous	Divide sample into multiple wells, adjust volume to 250 µL with PBS.
Low DNA yield	Poor elution	Repeat elution or increase elution volume. Incubation of plate at 70oC for 5 min with Elution Buffer may increase yields. Make sure the pH of the water is more than 7.5
	Improper washing	DNA Wash Buffer Concentrate must be diluted with absolute (100%) ethanol as specified on Page 3 .
Low A_{260}/A_{280} ratio	Extended centrifugation during elution step.	Resin from the plate 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 lyse for improper mixing with BL	making sure to vortex the sample with Buffer BL immediately and completely.
	Incomplete cell lysis n due to insufficient incubation.	Increase incubation time with Buffer BL and protease.
	Samples are rich in protein.	After applying to wells, wash with 300 µL of a 1:1 mixture of Buffer BL and ethanol and then with DNA Wash Buffer.
	Silica fine interference	Remove the silica fines by centrifugation and check the OD again
Smeared DNA from gel	Endonuclease Contamination	Ensure to wash the plate with HB Buffer
	Silica fines interference	Remove the silica fines by centrifugation and run the gel again
No DNA eluted	Poor lysis for improper mixing with Buffer BL.	Mix thoroughly with Buffer BL prior to loading to the DNA plate.
	Absolute ethanol not added to sample.	Before applying sample to column, ethanol must be added as prescribed in protocol
	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 with Buffer BL.	Buffer BL is viscous and the sample must be vortexed thoroughly.
	No ethanol added to DNA Wash Buffer.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.