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Updated May 2004

Introduction

E-Z 96® Blood DNA Kits allow rapid and reliable isolation of high-quality genomic DNA from a wide variety of sample sources including fresh, frozen, or anticoagulated whole blood; serum; plasma; bone marrow; other 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 contaminants 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 100 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 TL or BL. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffers at room temperature and the proteinase at -20°C..

Kit Contents

Product Number	D1192-00 (Trial Sample)	D1192-01 (4 x 96)	D1192-02 (20 x 96)
E-Z 96 [®] DNA Plate	1	4	20
96-Well Collection Plate (2 ml)	1*	2*	10*
96-Well Racked Microtubes (1.2ml)	2	8	40
Microtube Caps	36 x 8	144 x 8	720 x 8
Buffer BL	30 ml	4 x 30 ml	5 x 110 ml
OB Protease	50 mg	200 mg	1.0 g
HB	50 ml	200 ml	2 x 1000
DNA Wash Buffer Concentrate	60 ml	4 x 60 ml	5 x 200ml
DNA Elution Buffer	25 ml	2 x 45 ml	4 x 110 ml
Microplate Adhesive Film Covers	3	12	60
Instruction Booklet	1	1	1

Note: 2 ml plates are reusable. See below for cleaning instructions.

Before Starting

- Please read the entire manual to become familiar with the E-Z 96[®] Blood DNA Kit procedures.
- Prepare an OB Protease stock solution or Proteinase K at 20 mg/ml and aliquot into adequate portions. Store each aliquot at -20°C and thaw before use. Each sample will require 25 µl of this solution.
- Dilute Wash Buffer Concentrate with ethanol as follows and **store at room temperature:**

D1192-00 , 1 x 96 Sample	Dilute bottle with 90 ml absolute (96%-100%) ethanol.
D1192-01 , 4 x 96 preps	Dilute each bottle with 90 ml absolute (96%-100%) ethanol.
D1192-02 , 20 x 96 preps	Dilute each bottle with 300 ml absolute (96%-100%) ethanol.
- Equilibrate DNA Elution Buffer at 65°C
- **Adjust the volume of samples to 250µl. For samples smaller than 250µl, add appropriate volume of PBS to bring them to 250µl. For samples larger than 250µl, split each sample into two 250µl aliquots and use two of the 1.2 ml microtubes for lysis. Load the combined**

lysates into one well of the E-Z 96[®] DNA binding plate.

- 2ml deep well plates 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 5,000 x g equipped with swinging-bucket rotor.
- Rotor adapter for deep well microplates
- Waterbath equilibrated to 65°C
- Isopropyl alcohol (isopropanol)
- Absolute (96%-100%) ethanol
- Multichannel pipet with tips
- *Optional:* RNase A stock solution (20 mg/ml) [See Step 4 below]
- Microplate adhesive film cover
- Incubator or vacuum oven preset at 65°C

E-Z 96[®] Blood DNA Protocol

1. Pipet 25µl OB Protease or Proteinase K stock into the bottom of each tubule of the 96-well 1.2 ml microtube rack. Make a chart to record the position of each sample. Use multichannel pipet to dispense the protease solution deep inside the microtube to avoid potential contamination.
2. Add samples to each tubule of the microtube rack by touching the inside of the tubes without touching the rims with tip ends. Use 250 µl whole blood, serum or body fluids for each tube. (Up to 4 x 10⁵ lymphocytes or cultured cells in PBS can be used each tube.)

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

3. Add 250µl Buffer BL to each sample. Take care not to touch the rims of the tubes 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 (20 µl 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 microtubes with microtube caps and mix the samples thoroughly

by vortexing or vigorously shaking the microtube rack (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 1500 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. Remove the microtube caps and add 250µl of absolute ethanol (96-100%) to each tube.
9. Seal the microtube rack using **new caps**.
10. Mix the samples by vortexing or vigorously shaking the microtube rack (side to side) for 1 minute. Centrifuge the microtube rack at 1500 x g for 1 minute to collect any liquid from the caps.
11. Place the E-Z 96® DNA plate on top of a 2.0 ml deep-well plate. 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 microplate adhesive film cover. Centrifuge at 3,000-5,000 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 2ml collection plate before the following wash step.
15. Remove the adhesive film cover, then add 300 µl HB Buffer to each well.
16. Seal the plate with new microplate adhesive film cover; then centrifuge the plate at 3000-5000 x g for 5 minutes. Discard the flow-through in the deep-well collection plate.
17. Remove the adhesive film cover, then add 600 µl DNA wash buffer to each well.
18. Seal the plate with new microplate adhesive film cover; then centrifuge the plate at 3000-5000 x g for 5 minutes. Discard the flow-through in the

deep-well collection plate.

19. Remove the adhesive film cover and again add 600 µl DNA wash buffer to each well. Place the E-Z 96® DNA plate on top of the deep well plate, seal the E-Z 96® DNA plate with adhesive film cover and centrifuge at 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. Add 50-200 µl DNA Elution Buffer preheated at 65°C to each well of the DNA Binding 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 3,000-5,000 x g for 5 minutes to elute DNA.

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:

$$[DNA] = (Absorbance_{260}) \times (0.05 \mu g/\mu l) \times (Dilution \ factor)$$

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.

Expected yields vary with both amount, and type of tissue used. 30 mg of fresh tissue will yield 10-40 µg DNA with two elutions (each 200 µl).

Troubleshooting Guide

Problem	Possible Cause	Suggestions
Clogged well	Incomplete lysis	Add the correct volume of Buffer BL and incubate for specified time at 70°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 OB Protease/Proteinase K, Buffer BL, and isopropanol. Pass aliquots of lysate through one well successively.
	Sample too viscous	Divide sample into multiple tubes, adjust volume to 250 µl with PBS.
Low DNA yield	Clogged well	See above
	Poor elution	Repeat elution or increase elution volume. Incubation of plate at 70°C for 5 min with Elution Buffer may increase yields. Make sure the pH of the water is more than 7.5
	Improper washing	Wash Buffer Concentrate must be diluted with absolute (100%) ethanol as specified on Page 3 before use.
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 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 wells, wash with 300 µl 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 to the DNA plate.

Problem	Possible Cause	Suggestions
	Absolute ethanol not added to sample.	Before applying sample to column, ethanol must be added as prescribed in protocol
	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.

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