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Introduction

E-Z 96® Tissue DNA Kits allow rapid and reliable isolation of high-quality total cellular DNA from a wide variety of animal tissues or cell cultures in a 96 well plate format. Up to 30 mg tissue or two 0.6 cm mouse tail pieces can be processed in each well. The system combines the reversible nucleic acid-binding properties of the HiBind™ matrix with the speed and versatility of the E-Z 96® DNA plate to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from tissue lysates. The newly designed E-Z 96® DNA Binding Plate has a capacity of 100 µg. 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® Tissue DNA Kit for the first time, please read this booklet to become familiar with the procedures and their various modifications. Tissue or tail samples are cut into small pieces, and then lysed in a specially formulated buffer containing proprietary detergent solution with proteinase. Binding conditions are then adjusted and the sample is applied to a E-Z 96® DNA plate. Two rapid wash steps remove trace contaminants such as residual polysaccharides, 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® Tissue 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 precipitates. Store Buffers at room temperature and the proteinase at -20°C..

Kit Contents

Product Number	D1196-01	D1196-02
E-Z 96™ DNA Plate	4	20
96 well Collection Plate (2 ml)	2*	10*
Microcollection tube (1.2ml)	8	40
Microcollection tube caps	150x 8	730 x 8
Buffer TL	100mL	500ml
Buffer BL	100 ml	500 ml
Buffer HB	200 ml	2 x 500 ml
OB protease	240 mg	1.2 g
DNA Wash Buffer Concentrate	300ml	3 x 500ml
Instruction Booklet	1	1

* 2ml 96 well plate is reusable, see page 4 for cleaning instructions.

Before Starting

- Please read the entire booklet to become familiar with the E-Z 96® Tissue DNA Kit procedure.
- Prepare an OB protease stock solution 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**.

D1196-01, 4 x 96 preps Dilute with 450 ml absolute (96%-100%) ethanol to each bottle (1.5:1).

D1196-02, 20 x 96 preps Dilute with 750 ml absolute (96%-100%) ethanol to each bottle (1.5:1).

- Choose the most appropriate protocol to follow. Procedures are described for each of dried and fresh (or frozen) specimens. In addition, a short protocol is given for isolation of DNA for PCR reactions.

A. Tissue sample For processing ≤ 20 mg ground tissue in each well. Yield can be up to 70 µg depending on the tissue type.

B. Mouse tail For processing two 0.6 cm mouse tails pieces, yield is similar to A.

- 2ml deep well plates can be used to collect flow-through from the E-Z 96® DNA binding plate; they are designed for repeated use. Wash the plate thoroughly in tap water after each use. Incubate 5 minutes at room temperature in 0.5M HCl. Rinse with distilled water. Used plate can also be autoclaved after washing.

Materials to be provided by user:

- Laboratory centrifuge capable of 6000 rpm equipped with swinging-bucket rotor.
- Adapter for deep well microplate
- Waterbath equilibrated to 55°C
- Equilibrate sterile dH₂O water or 10 mM Tris pH 9.0 at 65°C.
- Isopropyl alcohol (isopropanol)
- Absolute (96%-100%) ethanol
- RNase A stock solution at 20 mg/ml
- Liquid nitrogen for freezing/disrupting samples (for tissue samples)
- Multichannel pipet with tips
- Adhesive film/tape for microplate
- Incubator or vacuum oven preset at 70°C

Protocol for Tissue and Mouse Tail

OPTIONAL: Although no mechanical homogenization of tissue is necessary, pulverizing the samples in liquid nitrogen will improve lysis and reduce incubation time. Once the liquid nitrogen has evaporated, transfer the powdered tissue to a clean 1.5 ml tube. Add 200 µl Buffer TL and proceed to step 2 below.

Note: do not use too much starting material; otherwise the lysates will be too viscous and may clog the DNA columns.

1. **Mince 20 mg tissue and place into each 1.2ml microtube.** Make a chart to record the position of each sample. For mouse tail, cut the tail to 0.6 cm pieces and place two pieces into each microtube.
2. **Prepare a OB Protease/TL buffer working solution by adding 25 µl protease to each 200ul TL Buffer for each sample.** Seal the microtube properly using the micro tube caps (supplied).
3. **Mix the samples by inverting the micro tube rack.** Briefly spin the collection micro tube rack at 2500 -3000rpm to collect any residue solution from the caps. It is very important that samples are completely submerged in the solution.
4. **Incubate at 65°C overnight or until the samples are completely lysed.** The lysate should be clear and viscous after complete digestion. Mix

occasionally during the incubation by rotating the plate gently. Make sure that the samples are completely lysed; **otherwise** they will clog the DNA binding plate in step 12.

5. **Shake the micro tubes vigorously.** Hold the microtube caps to ensure the micro tubes are sealed properly. Ensure the lysate is completely homogenous after shaking. If a gelatinous mass is visible, a further digestion is required.
6. **Optional: Add 20 μ l RNase A solution (20mg/ml) to each sample and incubate at room temp 5 minutes to remove any RNA.**
7. **Remove the micro tube caps and add equal volume of Buffer BL to each sample.**
8. **Add 210 μ l ETOH to each sample.** A white precipitate may form at this step; it will not interfere with the DNA isolation. Seal the micro tubes with new caps (supplied).
9. **Mix the sample by shaking the micro tube rack vigorously for 1 minute.** Centrifuge the micro tube at 3000 rpm for 1 minute to collect any liquid from the caps.
10. **Place the E-Z 96[®] DNA Binding Plate on top of a 2.0 ml deep-well plate. Mark the E-Z 96[®] DNA Binding Plate for later identification.**
11. **Transfer all the supernatant in STEP 9 to each well of the E-Z 96[®] DNA Binding Plate.**
12. **Seal the E-Z 96[®] DNA Binding Plate with adhesive film.** Centrifuge at 5000-6000 rpm for 15 minutes. Ensure all the sample has passed through the membrane in each well of the DNA plate. Longer centrifugation may be required if any lysate remains in any of the wells.
13. **Removal the adhesive film, and add 300 μ l HB buffer to each well.**
14. **Seal the plate with new adhesive film and centrifuge the plate at 5000 rpm for 5 minutes.**
15. **Removal the adhesive film, and add 600 μ l DNA wash buffer to each well.**
16. **Seal the plate with new adhesive film and centrifuge the plate at 5000 rpm for 5 minutes.**
17. **Remove the adhesive film. Add 600 μ l DNA wash buffer to each well. Discard the flow-through in the deep-well collection plate. Place the E-Z 96[®] DNA Binding Plate on top of the deep well plate.**

18. **Seal the plate with new adhesive film and centrifuge the plate at 5000 rpm for 10 minutes.**
19. **Remove the adhesive film, place the E-Z 96[®] DNA Binding Plate on top of a new rack of collection micro tubes.** Incubate the plates in a incubator or vacuum oven preset at 70°C to dry the membrane.
Note: *This step is critical for removing traces of ethanol that may otherwise interfere with subsequent downstream applications.*
20. **Add 200 μ l water or 10mM Tris-HCl (pH 9.0) preheated at 65°C to each well of the DNA Binding plate.**
21. **Seal the plate with new adhesive film and centrifuge the plate at 5500-6000 rpm for 5 minutes to elute DNA.**

Protocol for Cultured Cells:

1. For cells grown in suspension, harvest the cells by centrifugation at 300 x g for 5 minutes. Resuspend cells with 200 ul of TL Buffer followed by addition of 25ul proteinase solution. Incubate 2-4 hours at 65°C. Continue the protocol with step 5 on page 4.
2. For cells grown monolayer, release the cells with trypsin. Collect the cells by centrifugation at 300 x g. Resuspend cells with 200 ul of TL Buffer followed by addition of 25ul proteinase solution. Incubate 2-4 hours at 65°C. Continue the protocol with step 5 on Page 5.

Modified Vacuum Manifold Protocol for Tissue DNA Isolation:

The following protocol has been tested for only cultured cells and limited types of animal tissues. It may not be suitable for some types of animal tissue samples rich in polysaccharides.

1. Prepare the cell lysates by following the centrifugation protocol (Steps 1-9). Use CBL Buffer instead of using BL Buffer. (CBL is not supplied with this kit; it can ordered separately.)
2. Assemble the plate on the vacuum manifold according the manifold's manufacturer's instructions.
3. Apply the vacuum to draw the lysate through the E-Z 96[®] DNA Plate.
4. Wash the plate twice with 700 ul DNA Buffer.
5. Wash the plate with 700 ul absolute ethanol once.
6. Dry the plate by applying vacuum for an additional 10 minutes.
7. Place the plate in a vacuum oven preset to 70°C to further dry the plate. It is very crucial to *completely* dry the plate before elution (important).
8. Add 100ul DNA Elution Buffer or water to each well. Apply the vacuum to elute DNA into collection plate.

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:

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

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 to 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	Extend incubation time of lysis with Buffer TL and protease. 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 30 mg tissue, increase volumes of OB Protease or Proteinase K, Buffer TL, Buffer BL, and ethanol. Pass aliquots of lysate through one column successively.
	Sample too viscous	Divide sample into multiple tubes, adjust volume to 250 μl with 10 mM Tris-HCl.
Low DNA yield	Clogged well	See above
	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 (100%) ethanol as specified on page 5 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 TL and protease. Ensure that no visible pieces of tissue remain.

Problem	Possible Cause	Suggestions
	Samples are rich in protein.	After applying to column, 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.
	Poor cell and/or protein lysis in Buffer TL.	Tissue sample must be cut or minced into small pieces. Increase incubation time at 65°C 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.

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