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E-Z 96® Tissue DNA Kit

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Introduction

E-Z 96® Tissue DNA Kits allows 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 20 mg tissue or two 0.6 cm mouse tail segments can be processed in each well. The system combines the reversible nucleic acid-binding properties of Omega Bio-Tek's HiBind® matrix with the speed and versatility of 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 binding 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. Tissue or tail samples are cut into smaller pieces, and then lysed in a specially formulated buffer and 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 such as residual polysaccharides, and pure DNA is eluted with the Elution Buffer provided. 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. Warm at 37°C and gently shake the containers to dissolve. Store buffers at room temperature and the proteinase at -20°C.

Kit Contents

Product Number	D1196-00	D1196-01	D1196-02
E-Z 96® DNA Plate	1	4	20
96-well Round Well Plate (1.2 mL)	1	4	20
Caps for Round well Plate	24 x 8	100 x 8	500 x 8
96-Well Collection Plate (2 mL)	1*	4*	4*
Racked Microtubes (1.2 mL)	1	4	20
8-Strip Microtube Caps	12 x 8	50 x 8	250 x 8
Buffer TL	30 mL	100 mL	500 mL
Buffer BL	30 mL	100mL	500 mL
HB Buffer	70 mL	220 mL	1050 mL
OB Protease	60 mg	240 mg	1.2 g
Elution Buffer	50 mL	200 mL	1000 mL
DNA Wash Buffer	40 mL	100 mL	3 x 200 mL
AeraSeal Sealing film	4	16	80
Instruction Booklet	1	1	1

*** 2 mL 96-well Collection Plates are reusable; see below for cleaning instructions.**

Before Starting

- Please read the entire booklet to become familiar with the E-Z 96® Tissue DNA Kit procedures.
- Prepare an OB protease stock solution at 20 mg/mL with DNA 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.
- Dilute DNA Wash Buffer with ethanol **(96%-100%)** as follows and **store at room temperature**:

  D1196-00 Add 160 mL absolute ethanol to each bottle

  D1196-01 Add 400 mL absolute ethanol to each bottle

  D1196-02 Add 800 mL absolute ethanol to each bottle

- Prepare **Buffer BL/ethanol mixture** as follows and **store at room temperature**:

  D1196-00 Add 30 mL absolute (96%-100%) ethanol

  D1196-01 Add 100 mL absolute (96%-100%) ethanol

  D1196-02 Add 500 mL absolute (96%-100%) ethanol

- The 2 mL 96-well Plate provided should be used to collect flow-through from the E-Z 96® DNA binding plate. It is designed for repeated use. **Washing Instructions:** Wash the plate thoroughly with tap water after each use. Incubate 5 minutes at room temperature in 0.5M HCl. Rinse with distilled water. Used plate can also beautoclaved after washing.

Materials to be provided by user:

- Laboratory centrifuge capable of 3,000-5,000 x g equipped with swinging-bucket rotor.
- Adapter for deep-well microplate
- Waterbath equilibrated to 56°C
- Equilibrated sterile dH_2O water or Elution Buffer at 70°C.
- 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

E-Z 96® Tissue DNA Protocol for Tissues 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 column.

1. Mince 10-20 mg tissue and place into each 1.2 mL round well plate. Make a chart to record the position of each sample. For mouse tails, cut the samples to 0.6cm pieces; for rat tails, cut the samples to 0.3 cm pieces. Place two pieces into each well.

2. Prepare an OB Protease/Buffer TL working solution by mixing 25 µl protease with 200ul Buffer TL for each sample. For each 96 sets of sample, prepare the Protease/TL stock working solution by mix 2.5 mL protease with 20 mL Buffer TL. Pipet 225 µl protease/TL working solution into each well. Seal the plate properly using the caps supplied.

3. Mix the samples by inverting the plate. Briefly spin the plate at 2,500 -3,000 x g to collect any residue solution from the caps. It is very important that samples are completely submerged in the solution. If the protease/TL solution does not completely cover the sample, increase the sample volume to 300 µl. (Additional reagent can be purchased separately).

4. Incubate at 56°C over night or until the samples are completely lysed. The lysate should be clear and viscous after digestion is complete. Mix occasionally during the incubation by rotating the plate gently. Make sure the samples are completely lysed; otherwise they will clog the DNA binding plate in step 11.

5. Shake or vortex the plate vigorously from side to side. (Do not shake up and down to avoid leaking around the caps.) Hold the caps to ensure the plate is sealed properly.

Ensure the lysate is completely homogenous after shaking. If a gelatinous mass is visible, further digestion is required.

6. **Optional:** Certain tissues such as liver have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. For isolation of RNA-Free genomic DNA, spin briefly to collect any drops and add 20µl RNase A solution (20mg/mL) to each sample and incubate 10-20 minutes to remove the RNA.

7. **Optional:** Spin at 3,000-5,000 x g for 10 minutes at room temperature to remove undigested particles. Transfer the supernatant into a new 1.2 ml plate (no provided). This step should be done when DNA Binding plate is clogged in step 11.

8. Remove the caps and add two volumes of Buffer BL/ethanol mixture (about 450ul) to each sample. (See the preparation instructions on page 3). A white precipitate may form at this step; it will not interfere with DNA isolation. Seal the plate with new caps (supplied).

9. Mix the sample by shaking or vortex the plate vigorously (side to side) for 1 minute. Spin briefly to collect any liquid from the caps (the centrifugation speed should be less than 300 x g).

10. Place the E-Z 96® DNA Binding Plate on top of a 2.0 mL collection plate (supplied). Mark the E-Z 96® DNA Binding Plate for later identification.

11. Remove the caps and transfer all liquid include any pellet in STEP 9 to each well of the E-Z 96® DNA Plate.

12. Seal the E-Z 96® DNA Plate with sealing film. Centrifuge at 3,000-5,000 x g for 10 minutes. Ensure that each sample has passed through the membrane in each well of the E-Z 96® DNA Plate. Longer centrifugation may be required if any lysate remains in any of the wells. If some lysates are still left in the wells even with increased centrifugation time, proceed to next step.

13. Remove the sealing film and add 500 µl HB Buffer to each well. Centrifuge at 3,000-5,000 x g for 5 minutes. Discard the flow-through and re-use the collection plate in the next step.

14. Add 600µl DNA Wash Buffer to each well, seal the plate with new sealing film and centrifuge at 3,000-5,000 x g for 5 minutes. Discard flow-through and re-use the collection plate in the next step.
15. Repeat step 14, but centrifuge at 3,000-5,000 x g for 15 minutes.
    Note: This step is critical for removing trace residual ethanol that might otherwise

interfere with downstream applications. The plate can be further dried by placing the plates in an incubator or vacuum oven preset at 70ºC to dry the membrane.

16. Remove the sealing film, place the E-Z 96® DNA Plate on top of a new rack of collection microtubes (supplied).

17. Add 150 µl water or Elution Buffer (10mM Tris-HCl, pH 9.0) preheated at 70ºC to each well of the E-Z 96® DNA Plate. Seal the DNA plate with new sealing film and incubate at room temperature for 5 minutes. Centrifuge at 3,000-5,000 x g for 5 minutes to elute DNA.

18. Elute DNA with another 150 µl water or Elution Buffer (10mM Tris-HCl, pH 9.0) preheated at 70ºC by repeating step 17.

19. Cap the collection tube and store the eluted sample at -20°C.
    Note: A second elution will increase total DNA yield; however, due to increased elution volume, the DNA concentration will be reduced. If higher DNA concentration is desired, the second elution can be performed with the 150 µl eluate from first elution, reheated to 70°C.

E-Z 96® DNA Protocol for Cultured cells:

1. For cells grown in suspension, harvest cells with centrifugation at 300 x g for 5 minutes. Resuspend cells with 200 ul Buffer TL followed by 25 µl proteinase solution. Incubate 2-4 hours at 60° C. Continue the protocol with step 5 on age 4.

2. For cells grown in a mono-layer, release the cells with trypsin. Collect the cells with centrifugation at 300 x g for 5 minutes. Resuspend cells with 200 µl Buffer TL followed by 25 µl proteinase solution. Incubate 2-4 hours at 60° C. Continue the protocol with step 5 on page 4.

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 work for some types of animal tissue samples rich in polysaccharides.

1. Prepare the cell lysates by following the centrifugation protocol (steps 1 - 8).
2. Assemble the plate on the vacuum manifold according the manufacturer's instructions.
3. Apply the vacuum to draw the lysate through the E-Z 96® DNA Plate.
4. Wash the plate once with 500 µl HB Buffer.
5. Wash the plate twice with 600 µl DNA Wash Buffer.
6. Wash the plate once with 700 µl absolute ethanol (96%-100%).
7. Dry the plate by applying vacuum for an additional 10 minutes.
8. Centrifuge at 5000 x g for 10minutes to completely dry the membrane.

9. Place the plate in a vacuum oven preset on 70°C to further dry the plate. It is very critical to completely dry the plate before elution.
10. Assemble the manifold by placing the racked microtubes inside the based of manifold. Place the E-Z 96® DNA Plate on top part of the manifold.
11. Add 200µl DNA Elution Buffer or water to each well. Apply the vacuum to elute DNA into the collection plate.

Troubleshooting Guide

Problem	Possible Cause	Suggestions
Clogged well	Incomplete lysis	Extend lysis incubation time with Buffer TL and protease. 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 30 mg tissue, increase proportionately volumes of OB Protease or Proteinase K, Buffer TL, Buffer BL, and ethanol. Pass aliquots of lysate through one column successively.
	Incomplete lysis from sample preparation	Increase the centrifugation time by another 10 minutes. Or add HB Buffer and proceed with next step.
	Sample too viscous	Following lysis, divide sample into multiple tubes, adjust volume to 250 µl with 10 mM Tris-HCl. Proceed at step 7.
Low DNA yield	Clogged well	See above
	Poor elution	Repeat elution or increase elution volume (see note on page 6). 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 (96%-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 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 TL and protease. Ensure that no visible pieces of tissue remain.
	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.