

## **E-Z 96 Tissue DNA Kit**

D1196-00	1 x 96 preps
D1196-01	4 x 96 preps
D1196-02	20 x 96 preps

**May 2013**



# E-Z 96 Tissue DNA Kit

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Manual Revision: May 2013



# Introduction and Principle

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## Introduction

The E-Z 96 Tissue DNA Kit 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 30 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. Purified DNA is suitable for PCR, restriction digestion, and hybridization applications. 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.

## Principle

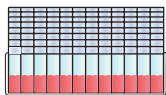
If using the E-Z 96 Tissue DNA Kit for the first time, please read this booklet to become familiar with the procedures. Tissue or tail samples are cut into smaller pieces, and then lysed in specially formulated buffer and protease. Binding conditions are then adjusted and the sample is applied to the E-Z 96 DNA Plate. Three 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.

### New In this Edition

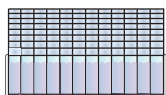
- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- Equilibration Buffer (used in an Optional Column Equilibration protocol) is no longer included with this kit.
- Equilibration Buffer can be replaced with 3M NaOH provided by the user.
- OB Protease is now supplied in a liquid form eliminating the step to resuspend prior to use. OB Protease Solution can also be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

# Centrifugation Protocol

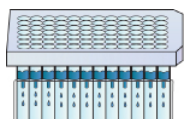
# Vacuum Protocol



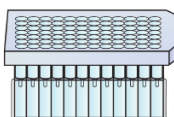
Lyse the Samples



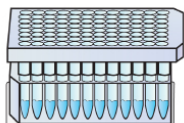
Adjust the Binding Conditions



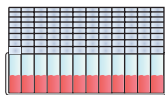
Bind and Wash 3x



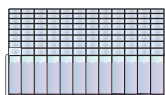
Dry Membrane



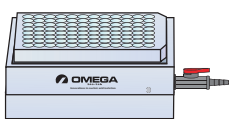
Elute



Lyse the Samples

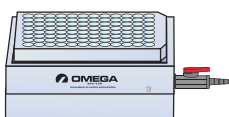


Adjust the Binding Conditions

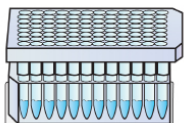


Bind and Wash 3x

Vacuum



Dry Membrane



Elute

## 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 Square-well Plate (2.2 mL)	1	4	20
96-well Racked Microtubes (1.2 mL)	1	4	20
Caps for Racked Microtubes	12 x 8	50 x 8	250 x 8
TL Buffer	25 mL	100 mL	500 mL
BL Buffer	25 mL	100 mL	500 mL
HBC Buffer	40 mL	200 mL	3 x 270 mL
OB Protease Solution	3 mL	12 mL	60 mL
Elution Buffer	50 mL	200 mL	1000 mL
DNA Wash Buffer	25 mL	100 mL	3 x 200 mL
AeraSeal™ film	5	20	100
User Manual	✓	✓	✓

## Storage and Stability

All of the E-Z 96 Tissue DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. OB Protease Solution can be stored at room temperature for up to 12 months. For long-term storage, store OB Protease Solution at 2-8°C. During shipment or storage in cool ambient conditions, precipitates may form in the buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

## Preparing Reagents

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

Kit	100% Ethanol to be Added
D1196-00	100 mL
D1196-01	400 mL
D1196-02	800 mL per bottle

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

Kit	Isopropanol to be Added
D1196-00	16 mL
D1196-01	78 mL
D1196-02	109 mL per bottle

- For Protocols other than Blood, dilute BL Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D1196-00	25 mL
D1196-01	100 mL
D1196-02	500 mL

**Important: DO NOT dilute BL Buffer with ethanol if the Blood Protocol is to be used.**

# Guideline for Vacuum Manifold

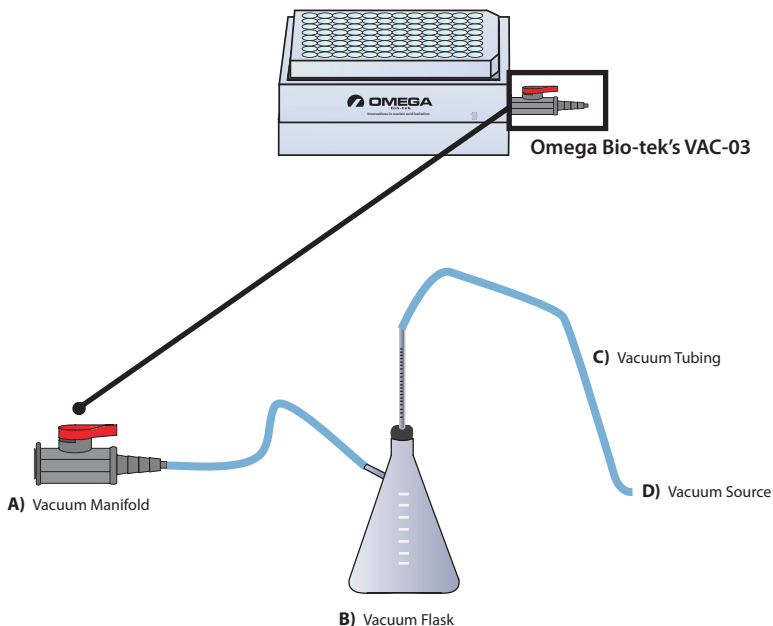
The following is required for use with the Vacuum Protocol:

- A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-03)  
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, Promega Vacman®, or manifold with standard Luer connector
- B) Vacuum Flask
- C) Vacuum Tubing
- D) Vacuum Source (review tables below for pressure settings)

Manifold	Recommended Pressure (mbar)
VAC-03	-200 to -400

Conversion from millibars:	Multiply by:
Millimeters of Mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of Mercury (inchHg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

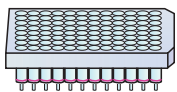
## Illustrated Vacuum Setup





# Guideline for Vacuum Manifold

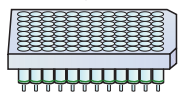
## Lysate Clearance Setup



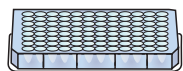
Lysate Clearance Plate



Vacuum Manifold Top



E-Z 96 DNA Plate

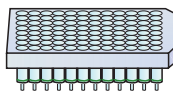


300 µL Microplate



Vacuum Manifold Bottom

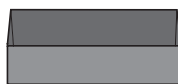
## DNA Bind Setup



E-Z 96 DNA Plate



Vacuum Manifold Top

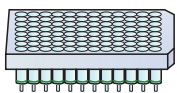


Waste Collection

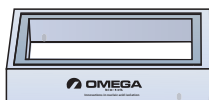


Vacuum Manifold Bottom

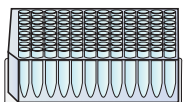
## Standard Elution Setup



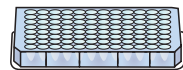
E-Z 96 DNA Plate



Vacuum Manifold Top



96-well Racked Microtubes

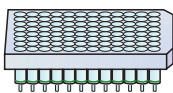


300 µL Microplate

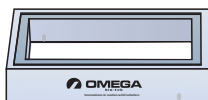


Vacuum Manifold Bottom

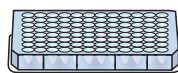
## Optional Elution Setup



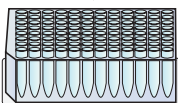
E-Z 96 DNA Plate



Vacuum Manifold Top



300 µL Microplate



96-well Racked Microtubes



Vacuum Manifold Bottom

# E-Z 96 Tissue DNA Kit Protocols

## E-Z 96 Tissue DNA Kit Protocol - Tissues and Mouse Tail

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 each well of the 96-well Round-well Plate (supplied) and proceed to Step 2 below.

**Note:** Do not use too much starting material; the lysate will be too viscous and may clog the E-Z 96 DNA Plate.

### Materials and Equipment to be Supplied by User:

- Centrifuge capable of 4,000 x *g* with swing bucket rotor
- Centrifuge adapter for deep-well microplates
- Water baths, incubators, or heat blocks capable of 70°C
- 100% ethanol
- Isopropanol
- Multichannel pipet with tips
- Vortexer
- Optional: RNase A (20 mg/mL)
- Optional: Liquid nitrogen for freezing/disrupting samples
- Optional: 3M NaOH

### Before Starting:

- Prepare reagents according to “Preparing Reagents” section on Page 5
  - Set a water bath, incubator, or heat block to 60°C
  - Heat Elution Buffer to 70°C
1. Mince 20 mg tissue and place into the 96-well Round-well Plate (provided). For mouse tails, cut the samples to 0.6 cm pieces; for rat tails, cut the samples to 0.3 cm pieces. Place two pieces into each well.
  2. Add 200  $\mu$ L TL Buffer and 25  $\mu$ L OB Protease Solution. Seal with Caps for Round-well Plates. Vortex to mix thoroughly.

**Note:** It is very important that samples are completely submerged in the solution. If the TL Buffer does not completely cover the sample, increase the sample volume to 300  $\mu$ L. (Additional reagent can be purchased separately). TL Buffer and OB Protease can be made as a mastermix before adding.

## E-Z 96 Tissue DNA Kit Protocols

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3. Briefly spin the plate at 2,500 -3,000 x *g* to collect any residue solution from the caps.
4. Incubate at 60°C overnight or until the samples are completely lysed. Mix occasionally during incubation by rotating the plate gently.

**Note:** The lysate should be clear and viscous after digestion is complete. Make sure the samples are completely lysed. Undigested material may clog the E-Z 96 DNA 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 homogeneous after shaking. If a gelatinous mass is visible, further digestion is required.

**Optional:** Add 5 µL RNase A solution (20 mg/mL, not provided) to each sample and let sit at room temperature for 5 minutes.

6. Add two volumes BL Buffer (about 450 µL) to each sample. A white precipitate may form at this step; it will not interfere with DNA isolation. Seal the plate with new Caps for Round-well Plates. Mix the sample by shaking or vortex the plate vigorously (side to side) for 1 minute.

**Note:** BL Buffer must be diluted with 100% ethanol before use. Please follow the instructions on Page 5.

7. Briefly spin the plate at 2,500 -3,000 x *g* to collect any residue solution from the caps.

**Note:** Do not centrifuge for a prolonged time. Once the speed reaches 2,500 -3,000 x *g* stop the centrifuge.

**Optional Column Equilibration Protocol:** Place the E-Z 96 DNA Plate on top of a 96-well Square-well Plate (provided). Add 100 µL 3M NaOH into each well of the plate and let the plate sit for 4 minutes at room temperature. Centrifuge at 4,000 x *g* for 3 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

8. Place the E-Z 96 DNA Plate on top of a 96-well Square-well Plate (provided).
9. Transfer all the lysate from Step 7 to each well of the E-Z 96 DNA Plate.

## E-Z 96 Tissue DNA Kit Protocols

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10. Seal the E-Z 96 DNA Plate with AeraSeal film.

11. Centrifuge at  $\geq 4,000 \times g$  for 10 minutes.

**Note:** 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 lysate is still left in the wells even with increased centrifugation time, proceed to next step.

12. Remove and discard the AeraSeal film.

13. Add 500  $\mu\text{L}$  HBC Buffer to each well. Seal the plate with new AeraSeal film.

**Note:** HBC Buffer must be diluted with isopropanol before use. Please follow the instructions on Page 5.

14. Centrifuge at  $\geq 4,000 \times g$  for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate.

15. Remove and discard the AeraSeal film.

16. Add 600  $\mu\text{L}$  DNA Wash Buffer to each well. Seal the plate with new AeraSeal film.

**Note:** DNA Wash Buffer must be diluted with 100% ethanol before use. Please follow the instructions on Page 5.

17. Centrifuge at  $\geq 4,000 \times g$  for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate.

18. Repeat Steps 15-17 for a second DNA Wash step.

19. Centrifuge at  $\geq 4,000 \times g$  for 15 minutes. Discard the filtrate and the 96-well Square-well Plate.

**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 70°C to dry the membrane.

## E-Z 96 Tissue DNA Kit Protocols

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20. Transfer the E-Z 96 DNA Plate to a set of 96-well Racked Microtubes (provided).
21. Remove and discard the AeraSeal film.
22. Add 200  $\mu\text{L}$  Elution Buffer heated at 70°C to each well of the E-Z 96 DNA Plate. Seal the E-Z 96 DNA Plate with new AeraSeal film.
23. Let sit at room temperature for 2-5 minutes.
24. Centrifuge at  $\geq 4,000 \times g$  for 5 minutes.

**Optional:** Repeat Steps 21-24 for a second elution step.

**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 200  $\mu\text{L}$  eluate from first elution, reheated to 70°C.

25. Seal the 96-well Racked Microtubes with the Caps for Racked Microtubes and store the eluted DNA at -20°C.

# E-Z 96 Tissue DNA Kit Protocols

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## E-Z 96 DNA Kit Protocol - Cultured cells

### Materials and Equipment to be Supplied by User:

- Centrifuge capable of 4,000 x *g* with swing bucket rotor
- Adapter for deep-well microplates
- Water baths, incubators, or heat blocks capable of 70°C
- 100% ethanol
- Isopropanol
- Multichannel pipet with tips
- Ice bucket
- Vortexer
- Trypsin
- PBS
- Optional: RNase A (20 mg/ mL)
- Optional: 3M NaOH

### Before Starting:

- Prepare reagents according to “Preparing Reagents” section on Page 5
- Set a water baths, incubators, or heat blocks to 60°C
- Heat Elution Buffer to 70°C
- Chill PBS on ice

1. Harvest and resuspend the cells using one of the following protocols.
  - A. For cells grown in suspension, do not use more than  $5 \times 10^6$  cells.
    1. Centrifuge cells at 300 x *g* for 5 minutes.
    2. Resuspend cells with 200  $\mu$ L cold (4°C) PBS and transfer to a 96-well Round-well Plate (provided).
    3. Add 25  $\mu$ L OB Protease Solution and seal the plate with Caps for Round-well Plate.
    4. Incubate at 60°C for 10 minutes.
    5. Continue the protocol with Step 2.

## E-Z 96 Tissue DNA Kit Protocols

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- B. For cells grown in a monolayer, do not use more than  $5 \times 10^6$  cells.
  1. Release the cells with trypsin.
  2. Centrifuge at  $300 \times g$  for 5 minutes.
  3. Resuspend cells with 200  $\mu\text{L}$  cold ( $4^\circ\text{C}$ ) PBS and transfer to a 96-well Round-well Plate (provided).
  4. Add 25  $\mu\text{L}$  OB Protease Solution and seal the plate with Caps for Round-well Plate.
  5. Incubate at  $60^\circ\text{C}$  for 10 minutes.
  6. Continue the protocol with Step 2.
2. 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 homogeneous after shaking. If a gelatinous mass is visible, further digestion is required.

**Optional:** Add 5  $\mu\text{L}$  RNase A solution (20 mg/mL, not provided) to each sample and let sit at room temperature for 5 minutes.

3. Add two volumes BL Buffer (about 450  $\mu\text{L}$ ) to each sample. A white precipitate may form at this step; it will not interfere with DNA isolation. Seal the plate with new Caps for Round-well Plates. Mix the sample by shaking or vortex the plate vigorously (side to side) for 1 minute.

**Note:** BL Buffer must be diluted with 100% ethanol before use. Please follow the instructions on Page 5.

4. Briefly spin the plate at  $2,500 - 3,000 \times g$  to collect any residue solution from the caps.

**Note:** Do not centrifuge for a prolonged time. Once the speed reaches  $2,500 - 3,000 \times g$  stop the centrifuge.

**Optional Column Equilibration Protocol:** Place the E-Z 96 DNA Plate on top of a 96-well Square-well Plate (provided). Add 100  $\mu\text{L}$  3M NaOH into each well of the plate and let the plate sit for 4 minutes at room temperature. Centrifuge at  $4,000 \times g$  for 3 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

5. Place the E-Z 96 DNA Plate on top of a 96-well Square-well Plate (provided).
6. Transfer all the lysate from Step 4 to each well of the E-Z 96 DNA Plate.

## E-Z 96 Tissue DNA Kit Protocols

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7. Seal the E-Z 96 DNA Plate with AeraSeal film.

8. Centrifuge at  $\geq 4,000 \times g$  for 10 minutes.

**Note:** 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 lysate is still left in the wells even with increased centrifugation time, proceed to next step.

9. Remove and discard the AeraSeal film.

10. Add 500  $\mu\text{L}$  HBC Buffer to each well. Seal the plate with new AeraSeal film.

**Note:** HBC Buffer must be diluted with isopropanol before use. Please follow the instructions on Page 5.

11. Centrifuge at  $\geq 4,000 \times g$  for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

12. Remove and discard the AeraSeal film.

13. Add 600  $\mu\text{L}$  DNA Wash Buffer to each well. Seal the plate with new AeraSeal film.

**Note:** DNA Wash Buffer must be diluted with 100% ethanol before use. Please follow the instructions on Page 5.

14. Centrifuge at  $\geq 4,000 \times g$  for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

15. Repeat Steps 12-14 for a second DNA Wash step.

16. Centrifuge at  $\geq 4,000 \times g$  for 15 minutes. Discard the filtrate and the 96-well Square-well Plate.

**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 70°C to dry the membrane.



## E-Z 96 Tissue DNA Kit Protocols

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17. Transfer the E-Z 96 DNA Plate to the 96-well Racked Microtubes (provided).
18. Remove and discard the AeraSeal film.
19. Add 200  $\mu$ L Elution Buffer heated at 70°C to each well of the E-Z 96 DNA Plate. Seal the E-Z 96 DNA Plate with new AeraSeal film.
20. Let sit at room temperature for 2-5 minutes.
21. Centrifuge at  $\geq 4,000 \times g$  for 5 minutes.

**Optional:** Repeat Steps 18-21 for a second elution step.

**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 200  $\mu$ L eluate from first elution, reheated to 70°C.

22. Seal the 96-well Racked Microtubes with the Caps for Racked Microtubes and store the eluted DNA at -20°C.

# E-Z 96 Tissue DNA Kit Protocols

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## E-Z 96 DNA Kit Protocol - Bacteria

### Materials and Equipment to be Supplied by User:

- Centrifuge capable of 4,000 x *g* equipped with swinging-bucket rotor
- Adapter for deep-well microplates
- 100% ethanol
- Isopropanol
- Water baths, incubators, or heat blocks capable of 70°C
- Shaking water bath capable of 56°C
- TE Buffer
- Lysozyme (50 mg/mL)
- RNase A (20 mg/mL)
- Vortexer
- Deep-well culture plate with mat or caps
- Optional: 3M NaOH

### Before Starting:

- Prepare reagents according to "Preparing Reagent" section
- Set a shaking water bath to 56°C
- Set a water baths, incubators, or heat blocks to 30°C
- Heat Elution Buffer to 70°C

1. Collect and resuspend the bacteria using one of the following protocols.

A. For 2 mL gram-positive bacteria culture:

1. Centrifuge bacteria at 4,000 x *g* for 10 minutes.
2. Remove and discard the supernatant.
3. Add 180  $\mu$ L TE Buffer and resuspend the pellet.
4. Add 18  $\mu$ L lysozyme (50 mg/mL) and vortex to mix.
5. Incubate at 30°C for 10 minutes.
6. Centrifuge digested cell at 4,000 x *g* for 5 minutes.
7. Aspirate supernatant leaving ~20  $\mu$ L residual liquid.
8. Vortex to resuspend pellet.
9. Continue the protocol with Step 2.

B. For 2 mL gram-negative bacteria culture:

1. Centrifuge bacteria at 4,000 x *g* for 10 minutes.
2. Remove and discard the supernatant.
3. Add 20  $\mu$ L TE Buffer and resuspend the pellet.
4. Continue the protocol with Step 2.

## E-Z 96 Tissue DNA Kit Protocols

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2. Add 200  $\mu$ L TL Buffer and 25  $\mu$ L OB Protease Solution. Seal with mat or caps. Vortex to mix thoroughly.

**Note:** TL Buffer and OB Protease Solution can be made as a mastermix before adding.

3. Incubate at 56°C in a shaking water bath to complete lysis.

**Note:** Usually no more than 1 hour is required for bacterial lysis. If no shaking water bath is available, incubate and shake or briefly vortex the samples every 20-30 minutes.

4. Add 5  $\mu$ L RNase A (20 mg/mL, not provided). Vortex to mix.

5. Let sit at room temperature for 5 minutes.

6. Add 450  $\mu$ L BL Buffer. A precipitate may form at this point but does not interfere with DNA isolation. Vortex for 30 seconds to mix thoroughly.

**Note:** BL Buffer must be diluted with 100% ethanol before use. Please follow the instructions on Page 5.

**Optional Column Equilibration Protocol:** Place the E-Z 96 DNA Plate on top of a 96-well Square-well Plate (provided). Add 100  $\mu$ L 3M NaOH into each well of the plate and let the plate sit for 4 minutes at room temperature. Centrifuge at 4,000  $\times g$  for 3 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

7. Place the E-Z 96 DNA Plate on top of a 96-well Square-well Plate (provided).

8. Transfer all the lysate from Step 6 to each well of the E-Z 96 DNA Plate.

9. Seal the E-Z 96 DNA Plate with AeraSeal film.

## E-Z 96 Tissue DNA Kit Protocols

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10. Centrifuge at  $\geq 4,000 \times g$  for 10 minutes.

**Note:** 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 lysate is still left in the wells even with increased centrifugation time, proceed to next step.

11. Remove and discard the AeraSeal film.

12. Add 500  $\mu\text{L}$  HBC Buffer to each well. Seal the plate with new AeraSeal film.

**Note:** HBC Buffer must be diluted with isopropanol before use. Please follow the instructions on Page 5.

13. Centrifuge at  $\geq 4,000 \times g$  for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

14. Remove and discard the AeraSeal film.

15. Add 600  $\mu\text{L}$  DNA Wash Buffer to each well. Seal the plate with new AeraSeal film.

**Note:** DNA Wash Buffer must be diluted with 100% ethanol before use. Please follow the instructions on Page 5.

16. Centrifuge at  $\geq 4,000 \times g$  for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

17. Repeat Steps 14-16 for a second DNA Wash step.

18. Centrifuge at  $\geq 4,000 \times g$  for 15 minutes. Discard the filtrate and the 96-well Square-well Plate.

**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 70°C to dry the membrane.

## E-Z 96 Tissue DNA Kit Protocols

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19. Transfer the E-Z 96 DNA Plate to the 96-well Racked Microtubes (provided).
  
20. Remove and discard the AeraSeal film.
  
21. Add 200  $\mu$ L Elution Buffer heated at 70°C to each well of the E-Z 96 DNA Plate. Seal the E-Z 96 DNA Plate with new AeraSeal film.
  
22. Let sit at room temperature for 2-5 minutes.
  
23. Centrifuge at  $\geq 4,000 \times g$  for 5 minutes.

**Optional:** Repeat Steps 20-23 for a second elution step.

**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 200  $\mu$ L eluate from first elution, reheated to 70°C.

24. Seal the 96-well Racked Microtubes with the Caps for Racked Microtubes and store the eluted DNA at -20°C.

# E-Z 96 Tissue DNA Kit Protocols

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## E-Z 96 DNA Kit Protocol - Blood

**Important: DO NOT dilute BL Buffer with ethanol prior to performing this protocol.**

### Materials and Equipment to be Supplied by User:

- Centrifuge capable of 4,000 x *g* with a swing bucket rotor
- Adapter for deep-well microplates
- Water bath, incubator, or heat block capable of 60° C
- 100% ethanol
- Isopropanol
- Multichannel pipet with tips
- Incubator or vacuum oven preset at 70°C
- Vortexer
- Optional: RNase A (20 mg/ mL)
- Optional: 3M NaOH

### Before Starting:

- Prepare reagents according to “Preparing Reagents” section on Page 5
- Set a water bath, incubator, or heat block to 60°C
- Heat Elution Buffer to 70°C

1. Pipet 25  $\mu$ L OB Protease Solution into the bottom of each well of a 96-well Round-well Plate (provided).
2. Add 250  $\mu$ L whole blood, serum, or body fluid to each well of the 96-well Round-well Plate. (Up to  $6 \times 10^6$  lymphocytes can be used in each well.)

**Note:** For sample volumes smaller or larger than 250  $\mu$ L, adjust the sample volume to 250  $\mu$ L.

3. Add 250  $\mu$ L BL Buffer. A white precipitate may form at this step; it will not interfere with DNA isolation. Seal the plate with Caps for Round-well Plates. Mix the samples by shaking or vortex the plate vigorously (side to side) for 1 minute.

**Note: DO NOT dilute BL Buffer with ethanol prior to performing this protocol.** Shake the plate side to side, not up and down, to prevent possible leakage around caps.

## E-Z 96 Tissue DNA Kit Protocols

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**Optional:** Add 20  $\mu\text{L}$  RNase A solution (20 mg/mL, not provided) to each sample and let sit at room temperature for 5 minutes.

4. Centrifuge briefly at 2,500-3,000  $\times g$  to collect any solution from the caps.
5. Incubate at 60°C for 10 minutes in an incubator or oven. Mix occasionally during incubation by rotating the plate gently.

**Note:** Incubation for more than 30 minutes at 60°C can cause DNA degradation.

6. Centrifuge briefly at 2,500-3,000  $\times g$  to collect any solution from the caps. Remove the caps and add 250  $\mu\text{L}$  100% ethanol to each well.
7. Seal the 96-well Round-well Plate (provided) using new Caps for Round-well Plates.
8. Mix the samples by vortexing or vigorously shaking the plate (side to side) for 1 minute. Centrifuge briefly at 3,000 rpm to collect any liquid from the caps.

**Optional Column Equilibration Protocol:** Place the E-Z 96 DNA Plate on top of a 96-well Square-well Plate (provided). Add 100  $\mu\text{L}$  3M NaOH into each well of the plate and let the plate sit for 4 minutes at room temperature. Centrifuge at 4,000  $\times g$  for 3 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

9. Place the E-Z 96 DNA Plate on top of a 96-well Square-well Plate (provided).
10. Transfer all the lysate from Step 8 to each well of the E-Z 96 DNA Plate.
11. Seal the E-Z 96 DNA Plate with AeraSeal film.
12. Centrifuge at  $\geq 4,000 \times g$  for 10 minutes.

**Note:** 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 lysate is still left in the wells even with increased centrifugation time, proceed to next step.

## E-Z 96 Tissue DNA Kit Protocols

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13. Remove and discard the AeraSeal film.

14. Add 500  $\mu$ L HBC Buffer to each well. Seal the plate with new AeraSeal film.

**Note:** HBC Buffer must be diluted with isopropanol before use. Please follow the instructions on Page 5.

15. Centrifuge at  $\geq 4,000 \times g$  for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

16. Remove and discard the AeraSeal film.

17. Add 600  $\mu$ L DNA Wash Buffer to each well. Seal the plate with new AeraSeal film.

**Note:** DNA Wash Buffer must be diluted with 100% ethanol before use. Please follow the instructions on Page 5.

18. Centrifuge at  $\geq 4,000 \times g$  for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

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

20. Centrifuge at  $\geq 4,000 \times g$  for 15 minutes. Discard the filtrate and the 96-well Square-well Plate.

**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 70°C to dry the membrane.

21. Transfer the E-Z 96 DNA Plate to the 96-well Racked Microtubes (provided).

22. Remove and discard the AeraSeal film.



## E-Z 96 Tissue DNA Kit Protocols

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23. Add 200  $\mu$ L Elution Buffer heated at 70°C to each well of the E-Z 96 DNA Plate. Seal the E-Z 96 DNA Plate with new AeraSeal film.
24. Let sit at room temperature for 2-5 minutes.
25. Centrifuge at  $\geq 4,000 \times g$  for 5 minutes.

**Optional:** Repeat Steps 22-25 for a second elution step.

**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 200  $\mu$ L eluate from first elution, reheated to 70°C.

26. Seal the 96-well Racked Microtubes with the Caps for Racked Microtubes and store the eluted DNA at -20°C.

# E-Z 96 Tissue DNA Kit Protocols

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## E-Z 96 DNA Kit Protocol - Vacuum Protocol

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

### Materials and Equipment to be Supplied by User:

- Vacuum Manifold

1. Prepare the lysate using one of the previous protocols, E-Z 96 Tissue Protocol Steps 1-7, E-Z 96 Cultured Cells Protocol Steps 1-4, Bacterial DNA Protocol Steps 1-6, or Blood DNA Protocol Steps 1-8.
2. Assemble the plate on the vacuum manifold according the manufacturer's instructions.

**Optional Column Equilibration Protocol:** Place the E-Z 96 DNA Plate on top of a 96-well Square-well Plate (provided). Add 100  $\mu$ L 3M NaOH into each well of the plate and let the plate sit for 4 minutes at room temperature. Turn on the vacuum source until all the liquid passes through the E-Z 96 DNA Plate.

3. Transfer the lysate to the E-Z 96 DNA Plate.
4. Turn on the vacuum source to draw the lysate completely through the E-Z 96 DNA Plate.
5. Turn off the vacuum source.
6. Add 500  $\mu$ L HBC Buffer to each well.

**Note:** HBC Buffer must be diluted with isopropanol before use. Please follow the instructions on Page 5.

7. Turn on the vacuum source to draw the liquid completely through the E-Z 96 DNA Plate.
8. Turn off the vacuum source.

## E-Z 96 Tissue DNA Kit Protocols

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9. Add 600  $\mu$ L DNA Wash Buffer to each well.

**Note:** DNA Wash Buffer must be diluted with 100% ethanol before use. Please follow the instructions on Page 5.

10. Turn on the vacuum source to draw the liquid completely through the E-Z 96 DNA Plate.
11. Turn off the vacuum source.
12. Repeat Steps 9-11 for a second DNA Wash step.
13. Add 600  $\mu$ L ethanol to each well.
14. Turn on the vacuum source to draw the liquid completely through the E-Z 96 DNA Plate.
15. Continue to apply the vacuum for 10 minutes after all liquid has passed through the E-Z 96 DNA Plate.
16. 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.
17. Place the 96-well Racked Microtubes inside the base of the manifold.
18. Place the E-Z 96 DNA Plate on top of the manifold.
19. Add 200  $\mu$ L Elution Buffer heated to 70°C to each well.
20. Turn on the vacuum source to draw the liquid completely through the E-Z 96 DNA Plate.
21. Seal the 96-well Racked Microtubes with the Caps for Racked Microtubes and store the eluted DNA at -20°C.

# Troubleshooting Guide

Please use this guide to solve any problems that may arise. We hope that it will aid in clearing up any questions for you. If for any reason you need further assistance, please contact our technical support staff at our **Toll Free Number 1-800-832-8896**.

## Possible Problems and Suggestions

Problem	Cause	Solution
96-well plate is clogged	Incomplete lysis	Extend lysis incubation time with TL Buffer and OB protease Solution. Add the correct volume of BL Buffer and incubate for specified time at 60°C.
	Sample amount too large	If using more than 30 mg tissue, increase proportionately volumes of OB Protease Solution, TL Buffer, BL Buffer, and ethanol. Pass aliquots of lysate through successively.
	Incomplete lysis from sample preparation	Increase the centrifugation time by an additional 10 minutes. Or add HBC Buffer and proceed with next step.
	Sample too viscous	Following lysis, divide sample into multiple tubes, adjust volume to 250 $\mu$ L with 10 mM Tris-HCl.
	Improper washing	DNA Wash Buffer must be diluted with 100% ethanol as instructed on Page 5 before use.
Problem	Cause	Solution
Washing leaves colored residue in column	Incomplete lysis due to improper mixing with BL Buffer	BL Buffer is viscous and the sample must be vortexed thoroughly.
	Ethanol was not added to the DNA Wash Buffer	Dilute DNA Wash Buffer with the indicated volume of ethanol before use.

## Troubleshooting Guide

Problem		Solution
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 digestion.
	Poor cell lysis due to incomplete mixing with BL Buffer	Repeat the procedure and make sure to vortex the sample with BL Buffer immediately and completely.
	Incomplete cell lysis or protein degradation due to insufficient incubation.	Increase incubation time with TL Buffer and OB Protease Solution. Ensure that no visible pieces of tissue remain.
	Samples are rich in protein.	After applying to the plate, wash with 300 $\mu$ L of a 1:1 mixture of BL Buffer and ethanol and then with HBC Buffer and DNA Wash Buffer.
Problem		
No DNA eluted	Poor cell lysis due to improper mixing with BL Buffer	Mix thoroughly with BL Buffer 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 60°C with TL Buffer to ensure that tissue is completely lysed.
	Ethanol was not added to BL Buffer	Before applying sample to column, an aliquot of BL Buffer/ethanol must be added. See protocol above.
	Ethanol was not added to the DNA Wash Buffer	Dilute DNA Wash Buffer with the indicated volume of ethanol before use.
	Isopropanol was not added to the HBC Buffer	Dilute HBC Buffer with the indicated volume of isopropanol before use.
	Poor elution	Repeat elution or increase elution volume (see note on Page 11). Incubation at 70°C for 5 minutes with Elution Buffer may increase yields.

## Ordering Information

The following components are available for purchase separately.  
(Call Toll Free at 1-800-832-8896)

Product	Part Number
BL Buffer (100 mL)	PD062
TL Buffer (100 mL)	PD061
DNA Wash Buffer (100 mL)	PS010
Elution Buffer (100 mL)	PDR048
OB Protease Solution	AC130
AeraSeal Film	AC1201

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