omega BIO-TEK

innovations in nucleic acid isolation

# Product Manual

## E-Z 96 Tissue DNA Kit

| | |
|---|---|
| D1196-00 | 1 x 96 preps |
| D1196-01 | 4 x 96 preps |

**Manual Date: May 2019**
**Revision Number: v11.0**

**For Research Use Only**


Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

omega-bio-tek
omegabiotek
omegabiotek

# E-Z 96 Tissue DNA Kit
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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**

May 2019:
- OB Protease Solution has been renamed Proteinase K Solution. This is a name change only. The formulation has not changed.
- D1196-02 has been discontinued and is no longer available to purchase.

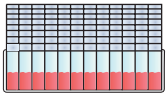
November 2018:
- D1196-02 has been added back to the manual.

October 2018:
- Centrifugation speeds and recommended drying times have been modified to improve overall extraction quality.

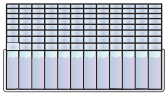
August 2017:
- 1.2 mL HTS Plate has been replaced with Lysis Plate.
- The number of Caps for Racked Microtubes has been reduced and Silicone Mats are now included for use with the Lysis Plate.

March 2016:
- 96-well Round-well Plates (1.2 mL) have been replaced with 1.2 mL HTS Plates.
- Caps for Round-well Plates have been replaced with Caps for Racked Microtubes.

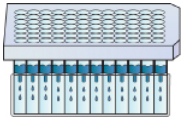
## Centrifugation Protocol



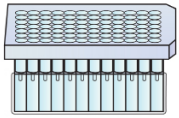
Lyse the Samples



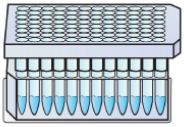
Adjust the Binding Conditions



Bind and Wash 3x

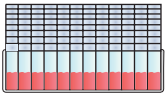


Dry Membrane

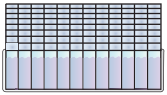


Elute

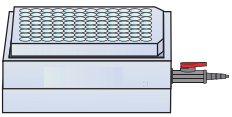
## Vacuum Protocol



Lyse the Samples

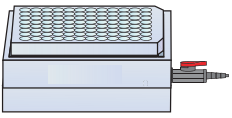


Adjust the Binding Conditions

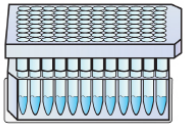


Bind and Wash 3x

Vacuum



Dry Membrane



Elute

# Kit Contents

| Product Number | D1196-00 | D1196-01 |
|---|---|---|
| E-Z 96 DNA Plate | 1 | 4 |
| Lysis Plate | 1 | 4 |
| 96-well Square-well Plate (2.2 mL) | 1 | 4 |
| 96-well Racked Microtubes (1.2 mL) | 1 | 4 |
| Caps for Racked Microtubes | 12 x 8 | 50 x 8 |
| TL Buffer | 25 mL | 100 mL |
| BL Buffer | 30 mL | 110 mL |
| HBC Buffer | 40 mL | 200 mL |
| DNA Wash Buffer | 25 mL | 200 mL |
| Elution Buffer | 60 mL | 250 mL |
| Proteinase K Solution | 3 mL | 12 mL |
| AeraSeal™ Film | 5 | 20 |
| Silicone Mat | 2 | 8 |
| 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. Proteinase K Solution can be stored at room temperature for up to 12 months. For long-term storage, store Proteinase K 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 HBC Buffer with 100% isopropanol as follows and store at room temperature.

| Kit | 100% Isopropanol to be Added |
|---|---|
| D1196-00 | 16 mL |
| D1196-01 | 78 mL |

- 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 | 800 mL |

# Guideline for Vacuum Manifold

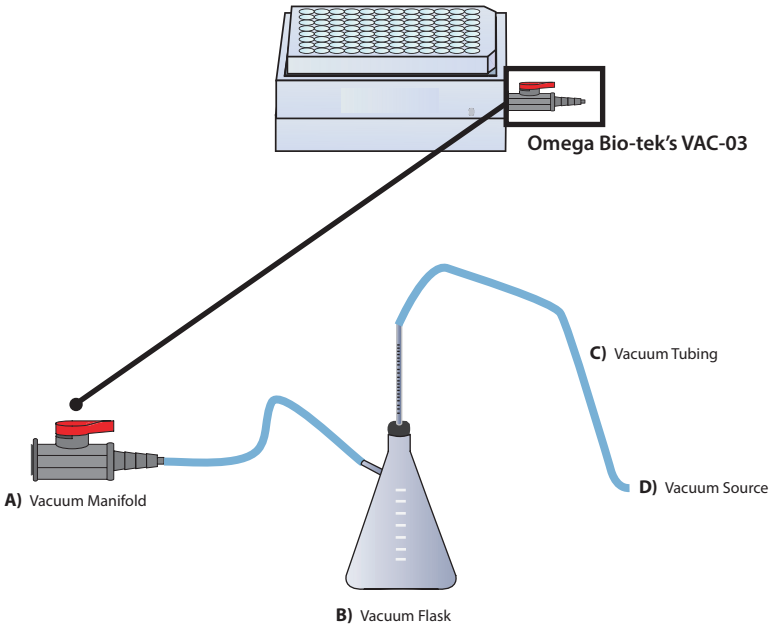
**The following is required for use with the Vacuum Protocol:**

**A)** Vacuum Manifold (We recommend Omega Bio-tek's VAC-03)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, 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 |

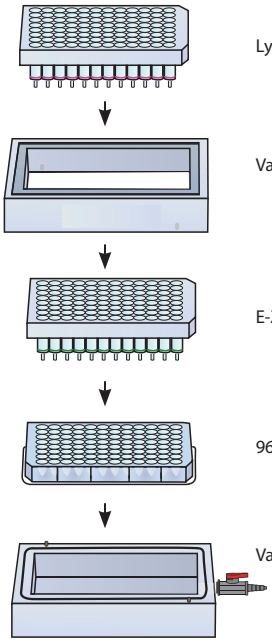
| Conversion from millibars: | Multiply by: |
|---|---|
| Millimeters of Mercury (mmHg) | 0.75 |
| Kilopascals (kPa) | 0.1 |
| Inches of Mercury (inchHg) | 0.0295 |
| Torrs (Torr) | 0.75 |
| Atmospheres (atmos) | 0.000987 |
| Pounds per Square Inch (psi) | 0.0145 |

**Illustrated Vacuum Setup**

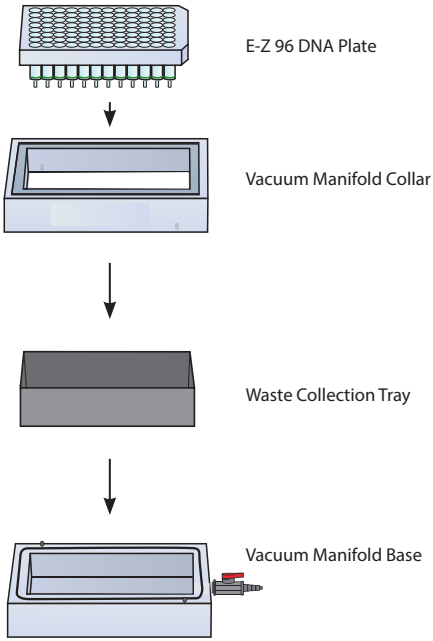


# Guideline for Vacuum Manifold

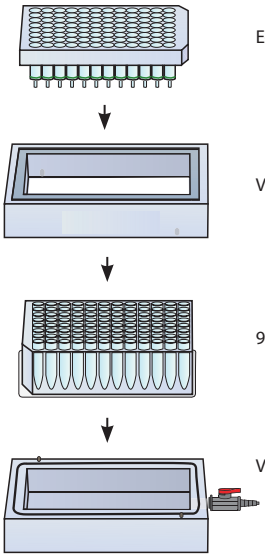
## Lysate Clearance Setup



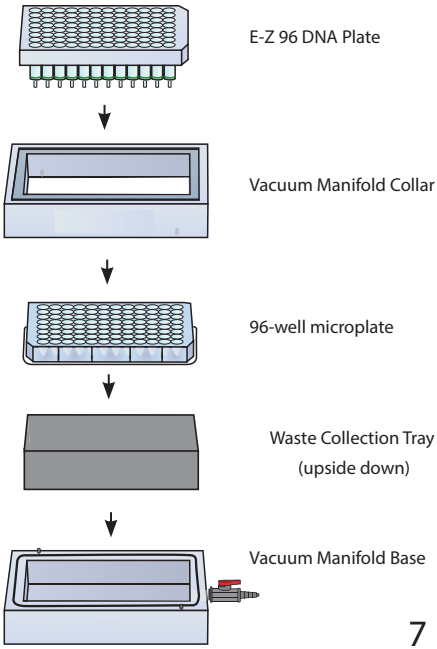

## DNA Bind Setup




## Standard Elution Setup - Racked Microtubes




## Alternative Elution Setup - Microplate



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

Omega Bio-tek recommends centrifugation with speeds greater than 4,000 x *g* for optimal DNA recovery and yield. Higher centrifugation speed increases the consistency across the E-Z 96 DNA Plate and lowers the retention volume of the HiBind® matrix.

**A**lthough 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 Lysis Plate (provided) 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 equipped with swing-bucket rotor and plate adaptor capable of at least 2,000 x *g*
- Water baths, incubators, or heat blocks capable of 70°C
- 100% ethanol
- 100% isopropanol
- Multichannel pipette with tips
- Vortexer
- Optional: RNase A (25 mg/mL)
- Optional: Liquid nitrogen for freezing/disrupting samples
- Optional: 3M NaOH
- Optional: 1M Dithiothreitol (DTT)

**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
- ***Important: The ramp or acceleration speed for centrifuge should be set to a low acceleration. If the centrifuge has a scale of 1-10, set to 3.***
- Optional: Add 40 µL 1M DTT per 1 mL TL Buffer before use.

1. Mince 20 mg tissue and place into the Lysis 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 µL TL Buffer and 25 µL Proteinase K Solution to each sample. Seal with a Silicone Mat (provided). 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 µL. (Additional reagent can be purchased separately). TL Buffer and Proteinase K Solution can be made as a mastermix. A**dding 40 µL 1M DTT per 1 mL TL Buffer before use helps digest hair and other particles completely.**

3. Briefly centrifuge the Lysis Plate at 2,000-3,000 x *g* to collect any residual solution from the Silicone Mat.

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. Vortex or shake the plate vigorously from side to side (do not shake up and down to avoid leaking around the mat). Hold the mat 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 (25 mg/mL, not provided) to each sample and let sit at room temperature for 5 minutes.

6. Add 1 volume BL Buffer and 1 volume 100% ethanol to each sample. A white precipitate may form at this step; it will not interfere with DNA isolation. Seal the Lysis Plate with a new Silicone Mat. Mix the sample by shaking or vortex the plate vigorously (side to side) for 1 minute.

   **Note:** BL Buffer and 100% ethanol can be made as mastermix and added to the sample.

7. Briefly spin the Lysis Plate at 2,000-3,000 x *g* to collect any residual solution from the Silicone Mat.

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

**Optional Plate 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 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.

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

11. Centrifuge at 2,000-6,000 x *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 the next step.

12. Remove and discard the AeraSeal Film.

13. Add 500 µL HBC Buffer to each well. Seal the plate with new AeraSeal Film.

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

14. Centrifuge at 4,000-6,000 x *g* for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate.

    **Note:** If the centrifugation speed is less than 4,000 x *g*, repeat centrifugation for another 5 minutes.

15. Remove and discard the AeraSeal Film.

16. Add 600 µL 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 4,000-6,000 x *g* for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate.

    **Note:** If the centrifugation speed is less than 4,000 x *g*, repeat centrifugation for another 5 minutes.

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

19. Select one of the following ethanol removal steps. If the maximum speed of the centrifuge is less than 4,000 x *g*, then use option B.

    A. Centrifuge the empty plate at **4,000-6,000 x *g*** for 15 minutes. Discard the filtrate and the 96-well Square-well Plate. Continue to Step 20.

    **OR**

    B. Discard the filtrate and the 96-well Square-well Plate. Dry the empty plate in an incubator or oven set at 70°C for 30 minutes. Let the plate cool for 5 minutes at room temperature after incubation. Continue to Step 20.

    **Note:** This step is critical for removing residual ethanol that might otherwise interfere with downstream applications.

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 150 µL Elution Buffer heated to 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 4,000-6,000 x *g* for 5 minutes.

    **Note:** If centrifugation speed is less than 4,000 x *g*, increase the centrifugation time to 10 minutes. Adjusting the acceleration speed is critical for optimal elution recovery.

**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. To maintain higher DNA concentration, the second elution may be performed with the first eluate, 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 - Cultured Cells Protocol

Omega Bio-tek recommends centrifugation with speeds greater than 4,000 x *g* for optimal DNA recovery and yield. Higher centrifugation speed increases the consistency across the E-Z 96 DNA Plate and lowers the retention volume of the HiBind® matrix.

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

- Centrifuge equipped with a swing-bucket rotor and plate adaptor capable of at least 2,000 x *g*
- Water baths, incubators, or heat blocks capable of 70°C
- 100% ethanol
- 100% isopropanol
- Multichannel pipette with tips
- Ice bucket
- Vortexer
- Trypsin
- PBS
- Optional: RNase A (25 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
- Chill PBS on ice
- ***Important: The ramp speed or acceleration of the centrifuge should be set to a low acceleration. If the centrifuge has a scale of 1-10, set to 3.***

1. Harvest and resuspend the cells using one of the following protocols.

   A. For cells grown in suspension, do not use more than 5 x $10^6$ cells.
      1. Centrifuge cells at 300 x *g* for 5 minutes.
      2. Resuspend cells with 200 µL cold (4°C) PBS and transfer to a Lysis Plate (provided).
      3. Add 25 µL Proteinase K Solution and 225 µL BL Buffer. Seal the Lysis Plate with a Silicone Mat.
      4. Incubate at 60°C for 10 minutes.
      5. Continue the protocol with Step 2.

B. For cells grown in a monolayer, do not use more than 5 x $10^6$ cells.
   1. Release the cells with trypsin.
   2. Centrifuge at 300 x *g* for 5 minutes.
   3. Resuspend cells with 200 µL cold (4°C) PBS and transfer to a Lysis Plate (provided).
   4. Add 25 µL Proteinase K Solution and 225 µL BL Buffer. Seal the Lysis Plate with a Silicone Mat.
   5. Incubate at 60°C for 10 minutes.
   6. Continue the protocol with Step 2.

2. Vortex or shake the plate vigorously from side to side (do not shake up and down to avoid leaking around the mat). Hold the mat 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 (25 mg/mL, not provided) to each sample and let sit at room temperature for 5 minutes.

3. Add one volume 100% ethanol (about 225 µL) to each sample. A white precipitate may form at this step; it will not interfere with DNA isolation. Seal the plate with a new Silicone Mat. Mix the sample by shaking or vortex the plate vigorously (side to side) for 1 minute.

4. Briefly centrifuge the plate at 2,000-3,000 x *g* to collect any residual solution from the Silicone Mat.

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

**Optional Plate 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.

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.

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

8. Centrifuge at 2,000-6,000 x *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 the next step.

9. Remove and discard the AeraSeal Film.

10. Add 500 µL HBC Buffer to each well. Seal the plate with new AeraSeal Film.

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

11. Centrifuge at 4,000-6,000 x *g* for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

    **Note:** If the centrifugation speed is less than 4,000 x *g*, increase centrifugation time to 10 minutes.

12. Remove and discard the AeraSeal Film.

13. Add 600 µL 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 4,000-6,000 x *g* for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

    **Note:** If the centrifugaiton speed is less than 4,000 x *g*, increase centrifugation time to 10 minutes.

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

16. Select one of the following ethanol removal steps. If the maximum speed of the centrifuge is less than 4,000 x *g*, then use option B.

    A. Centrifuge the empty plate at **4,000-6,000 x *g*** for 15 minutes. Discard the filtrate and the 96-well Square-well Plate. Continue to Step 17.

    **OR**

    B. Discard the filtrate and the 96-well Square-well Plate. Dry the empty plate in an incubator or oven set at 70°C for 30 minutes. Let the plate cool for 5 minutes at room temperature after incubation. Continue to Step 17.

    **Note:** This step is critical for removing residual ethanol that might otherwise interfere with downstream applications.

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

18. Remove and discard the AeraSeal Film.

19. Add 150 µL Elution Buffer heated to 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 4,000-6,000 x *g* for 5 minutes.

    **Note:** If the centrifugation speed is less than 4,000 x *g*, increase the centrifugation time to 10 minutes. Adjusting the acceleration speed is critical for optimal elution recovery.

**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. To maintain higher DNA concentration, the second elution may be performed with the first eluate, 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 - Bacteria Protocol

Omega Bio-tek recommends centrifugation with speeds greater than 4,000 x *g* for optimal DNA recovery and yield. Higher centrifugation speed increases the consistency across the E-Z 96 DNA Plate and lowers the retention volume of the HiBind® matrix.

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

- Centrifuge equipped with swing-bucket rotor and plate adaptor capable of at least 2,000 x *g*
- 100% ethanol
- 100% 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 (25 mg/mL)
- Vortexer
- Deep-well culture plate with mat or caps
- Optional: 3M NaOH

**Before Starting:**

- Prepare reagents according to "Preparing Reagent" section on Page 5
- Set a shaking water bath to 56°C
- Set a water bath, incubator, or heat block to 30°C
- Heat Elution Buffer to 70°C
- ***Important: The ramp speed or acceleration of the centrifuge should be set to a low acceleration. If the centrifuge has a scale of 1-10, set to 3.***

1. Collect and resuspend the bacteria in a deep-well culture plate (not provided) using one of the following protocols depending on baterial type.

   A. For 2 mL gram-positive bacterial culture:
      1. Centrifuge bacteria at 4,000 x *g* for 10 minutes.
      2. Remove and discard the supernatant.
      3. Add 180 µL TE Buffer and resuspend the pellet.
      4. Add 18 µL lysozyme (50 mg/mL) and vortex to mix.
      5. Incubate at 30°C for 10 minutes.
      6. Centrifuge digested cells at 4,000 x *g* for 5 minutes.
      7. Aspirate and discard supernatant leaving ~20 µL residual liquid.
      8. Vortex to resuspend pellet.
      9. Continue the protocol with Step 2.

B. For 2 mL gram-negative bacterial culture:
   1. Centrifuge bacteria at 4,000 x *g* for 10 minutes.
   2. Aspirate and discard the supernatant.
   3. Add 20 µL TE Buffer and resuspend the pellet.
   4. Continue the protocol with Step 2.

2. Add 200 µL TL Buffer and 25 µL Proteinase K Solution to each sample. Seal with a mat or caps compatible with the deep-well culture plate used (not provided). Vortex to mix thoroughly.

   **Note:** TL Buffer and Proteinase K Solution can be made as a mastermix.

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 a shaking water bath is not available, incubate and shake or briefly vortex the samples every 20-30 minutes.

4. Add 5 µL RNase A (25 mg/mL, not provided). Vortex to mix.

5. Let sit at room temperature for 5 minutes.

6. Add 225 µL BL Buffer and 225 µL 100% ethanol. A precipitate may form at this point but does not interfere with DNA isolation. Vortex for 30 seconds to mix thoroughly.

   **Note:** BL Buffer and 100% ethanol can be made as a mastermix.

**Optional Plate 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.

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.

10. Centrifuge at 2,000-6,000 x *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 the next step.

11. Remove and discard the AeraSeal Film.

12. Add 500 µL HBC Buffer to each well. Seal the plate with new AeraSeal Film.

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

13. Centrifuge at 4,000-6,000 x *g* for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

    **Note:** If the centrifugation speed is less than 4,000 x *g*, increase the centrifugation time to 10 minutes.

14. Remove and discard the AeraSeal Film.

15. Add 600 µL 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 4,000-6,000 x *g* for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

    **Note:** If the centrifugation speed is less than 4,000 x *g*, increase the centrifugation time to 10 minutes.

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

18. Select one of the following ethanol removal steps. If the maximum speed of the centrifuge is less than 4,000 x *g*, then use option B.

    A. Centrifuge the empty plate at **4,000-6,000 x *g*** for 15 minutes. Discard the filtrate and the 96-well Square-well Plate. Continue to Step 19.

    **OR**

    B. Discard the filtrate and the 96-well Square-well Plate. Dry the empty plate in an incubator or oven set at 70°C for 30 minutes. Let the plate cool for 5 minutes at room temperature after incubation. Continue to Step 19.

    **Note:** This step is critical for removing residual ethanol that might otherwise interfere with downstream applications.

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

20. Remove and discard the AeraSeal Film.

21. Add 150 µL Elution Buffer heated to 70°C to each well. Seal the E-Z 96 DNA Plate with new AeraSeal Film.

22. Let sit at room temperature for 2-5 minutes.

23. Centrifuge at 4,000-6,000 x *g* for 5 minutes.

    **Note:** If the centrifugation speed is less than 4,000 x *g*, increase the centrifugation time to 10 minutes. Adjusting the acceleration speed is critical for optimal elution recovery.

**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. To maintain higher DNA concentration, the second elution may be performed with the first eluate, 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 - Blood Protocol

Omega Bio-tek recommends centrifugation with speeds greater than 4,000 x *g* for optimal DNA recovery and yield. Higher centrifugation speed increases the consistency across the E-Z 96 DNA Plate and lowers the retention volume of the HiBind® matrix.

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

- Centrifuge equipped with a swing-bucket rotor and plate adaptor capable of at least 2,000 x *g*
- Water baths, incubators, or heat blocks capable of 70° C
- 100% ethanol
- 100% isopropanol
- Multichannel pipette with tips
- Vortexer
- Optional: RNase A (25 mg/mL)
- Optional: 3M NaOH
- Optional: Nuclease-free water

**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
- ***Important: The ramp speed or acceleration of the centrifuge should be set to a low acceleration. If the centrifuge has a scale of 1-10, set to 3.***

1. Add 250 µL whole blood, serum, or body fluid to each well of the Lysis Plate. (Up to 6 x $10^6$ lymphocytes can be used in each well.)

   **Note:** For sample volumes smaller or larger than 250 µL, adjust the sample volume to 250 µL with nuclease-free water

2. Add 250 µL BL Buffer and 25 µL Proteinase K Solution to each sample. Seal the plate with a Silicone Mat. Vortex or shake the plate vigorously from side to side for 1 minute (do not shake up and down to avoid leaking around the mat). Hold the mat to ensure the plate is sealed properly.

**Optional**: Add 20 µL RNase A solution (25 mg/mL, not provided) to each sample and let sit at room temperature for 5 minutes.

3. 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.

4. Centrifuge briefly at 2,000-3,000 x *g* to collect any solution from the Silicone Mat. Remove the mat and add 250 µL 100% ethanol to each well.

5. Seal the Lysis Plate using a new Silicone Mat.

6. Mix the samples by vortexing or vigorously shaking the plate (side to side) for 1 minute. Centrifuge briefly at 2,000-3,000 x *g* to collect any liquid from the mat.

**Optional Plate 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.

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.

10. Centrifuge at 2,000-6,000 x *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 the next step.

11. Remove and discard the AeraSeal Film.

12. Add 500 µL HBC Buffer to each well. Seal the plate with new AeraSeal Film.

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

13. Centrifuge at 4,000-6,000 x *g* for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

    **Note:** If the centrifugation speed is less than 4,000 x *g*, repeat centrifugation for another 5 minutes.

14. Remove and discard the AeraSeal Film.

15. Add 600 µL 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 4,000-6,000 x *g* for 5 minutes. Discard the filtrate and reuse the 96-well Square-well Plate in the next step.

    **Note:** If the centrifugation speed is less than 4,000 x *g*, repeat centrifugation for another 5 minutes.

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

18. Select one of the following ethanol removal steps. If the maximum speed of the centrifuge is less than 4,000 x *g*, then use option B.

    A. Centrifuge the empty plate at **4,000-6,000 x *g*** for 15 minutes. Discard the filtrate and the 96-well Square-well Plate. Continue to Step 19.

    **OR**

    B. Discard the filtrate and the 96-well Square-well Plate. Dry the plate in an incubator or oven set at 70°C for 30 minutes. Let the plate cool for 5 minutes at room temperature after incubation. Continue to Step 19.

    **Note:** This step is critical for removing trace residual ethanol that might otherwise interfere with downstream applications.

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

20. Remove and discard the AeraSeal Film.

21. Add 150 µL Elution Buffer heated to 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 4,000-6,000 x *g* for 5 minutes.

    **Note:** If the centrifugation speed is less than 4,000 x *g*, increase the centrifugation time to 10 minutes. Adjusting the acceleration speed is critical for optimal elution recovery.

**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. To maintain higher DNA concentration, the second elution may be performed with the first eluate, 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 - 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.

**Note:** If a full plate is not being extracted, seal the empty wells with AeraSeal Film to maintain vacuum pressure.

**Important:** This method is not recommended for extraction with Blood Protocol.

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

- Vacuum Manifold
- 96-well microplate for Lysate Clearance Setup (refer to Page 7 for details)
- Optional: 96-well microplate for alternative elution setup (refer to Alternative Elution Setup on Page 7 for details)

1. Prepare the lysate using one of the previous protocols, Tissue and Mouse Tail Protocol Steps 1-7, Cultured Cells Protocol Steps 1-4, Bacteria Protocol Steps 1-6, or Blood Protocol Steps 1-6.

2. Assemble the plate on the vacuum manifold according the manufacturer's instructions.

**Optional Plate Equilibration Protocol:** Assemble the Vacuum Manifold according the the Guidelines for DNA Bind Setup on Page 7. Set the Waste Collection Tray inside the Vacuum Manifold Base, place the Vacuum Manifold Collar ontop of the Waste COllection Tray, and finally place the E-Z 96 DNA Plate on top of the Vacuum Manifold Collar. Add 100 µL 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. Turn off the vacuum source.

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 µL HBC Buffer to each well.

   **Note:** HBC Buffer must be diluted with 100% 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.

9. Add 600 µL 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 Buffer wash step.

13. Add 600 µL 100% 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 set to 70°C for 20 minutes to further dry the plate. It is critical to completely dry the plate before elution.

17. Place the 96-well Racked Microtubes inside the base of the manifold.

    **Note:** If eluting in a 96-well microplate (Alternative Elution Setup, Page 7) and using Omega Bio-tek's VAC-03 vacuum manifold, place the included waste collection tray upside down inside the Vacuum Manifold base, place the 96-well microplate on top of the waste collection tray, place the Vacuum Manifold collar on top of the 96-well microplate, and finally place the E-Z 96 DNA Plate on top of the Vacuum Manifold collar.

18. Place the E-Z 96 DNA Plate on top of the manifold.

19. Add 150 µL 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. If 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 Proteinase K 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 Proteinase K Solution, TL Buffer, BL Buffer, and 100% 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 µL with 10 mM Tris-HCl. |
| | Improper washing | DNA Wash Buffer must be diluted with 100% ethanol as instructed on Page 5 before use. |
| | Centrifugation speed less than 4,000 x *g* | Adjust the ramp or acceleration speed as recommended and repeat the centrifugtion step. |
| Problem | Cause | Solution |
| Washing leaves colored residue in sample | 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 100% ethanol before use. |

| Problem | Cause | 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 Proteinase K Solution. Ensure that no visible pieces of tissue remain. |
| | Samples are rich in protein. | After transferring to the plate, wash with 300 µL of a 1:1 mixture of BL Buffer and ethanol and then with HBC Buffer and DNA Wash Buffer. |
| Problem | Cause | Solution |
| 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 the DNA Wash Buffer | Dilute DNA Wash Buffer with the indicated volume of 100% ethanol before use. |
| | Isopropanol was not added to the HBC Buffer | Dilute HBC Buffer with the indicated volume of 100% isopropanol before use. |
| | Poor elution | Repeat elution or increase elution volume (see note on Page 12). 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 |
| Proteinase K Solution (10 mL) | AC116 |
| AeraSeal Film (100/pk) | AC1201 |

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PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.

**Notes:**

**Notes:**

For more purification solutions, visit **www.omegabiotek.com**

## AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

## SAMPLE TYPES


Blood / Plasma

Plasmid


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FFPE


Fecal Matter

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innovations in nucleic acid isolation

Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

omega-bio-tek
omegabiotek
omegabiotek