

E-Z 96 Plant DNA DS Kit

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|----------|--------------|
| D1411-01 | 1 x 96 preps |
| D1411-02 | 4 x 96 preps |

August 2015

E-Z 96 Plant DNA DS Kit

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Manual Revision: August 2015



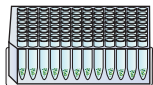
Introduction

The E-Z 96 Plant DNA DS Mini Kit is designed for efficient recovery of genomic DNA up to 30 kb in size from fresh, frozen, or dried plant tissue samples rich in polysaccharides, polyphenols, or those having a lower DNA content. Up to 50 mg wet tissue can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of the HiBind® matrix with the speed and versatility of spin column technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant tissue lysates. Purified DNA is suitable for PCR, restriction digestion, and hybridization applications.

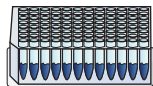
This procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the unique binding system to increase yields and provide high-quality DNA. The system eliminates the need for chloroform extractions traditionally associated with CTAB-based lysis methods. Samples are homogenized and lysed in a high salt buffer containing CTAB, binding conditions are adjusted, and DNA is purified using a E-Z 96 DNA Plate . Salts, proteins, and other contaminants are removed to yield high-quality genomic DNA suitable for downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization applications.

Centrifugation Protocol

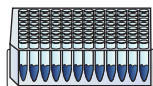
Vacuum Protocol



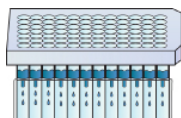
Collect Plant Tissue and Homogenize



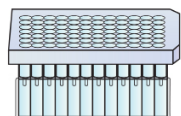
Lyse



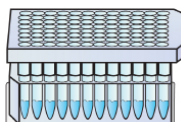
Transfer Cleared Lysate and Adjust Binding Conditions



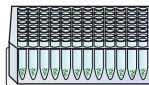
Bind and Wash 3X



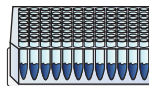
Dry Membrane



Elute



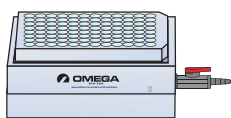
Collect Plant Tissue and Homogenize



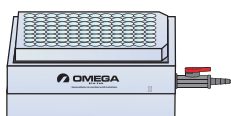
Lyse



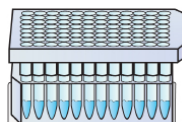
Transfer Cleared Lysate and Adjust Binding Conditions



Bind and Wash 3X



Dry Membrane



Elute

Kit Contents

| Product Number | D1411-00 | D1411-01 |
|------------------------------------|-------------|----------|
| E-Z 96 DNA Plate | 1 | 4 |
| 1.2 mL HTS Plate | 1 | 4 |
| Caps for Racked Microtubes | 40 x 8 | 160 x 8 |
| 96-well Square-well Plate (2.2 mL) | 2 | 8 |
| 96-well Racked Microtubes | 1 | 4 |
| E-Z 96 Homogenizer Plates | 1 | 4 |
| CSPL Buffer | 80 mL | 300 mL |
| Proteinase K Solution | 2.2 mL | 8.8 mL |
| XP2 Buffer | 60 mL | 250 mL |
| RBB Buffer | 60 mL | 250 mL |
| VHB Buffer | 44 mL | 176 mL |
| DNA Wash Buffer | 40 mL | 160 mL |
| Elution Buffer | 15 mL | 50 mL |
| RNase A | 550 μ L | 2.2 mL |
| User Manual | ✓ | ✓ |

Storage and Stability

All components of the E-Z 96 Plant DNA DS Kit are guaranteed for at least 12 months from date of purchase when stored as follows. Store RNase A at 2-8° C. All other components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in VHB Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

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

| Kit | 100% Ethanol to be Added |
|----------|--------------------------|
| D1411-00 | 56 mL |
| D1411-01 | 224 mL |

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

| Kit | 100% Ethanol to be Added |
|----------|--------------------------|
| D1411-00 | 160 mL |
| D1411-01 | 640 mL |

Guidelines for Vacuum Manifold

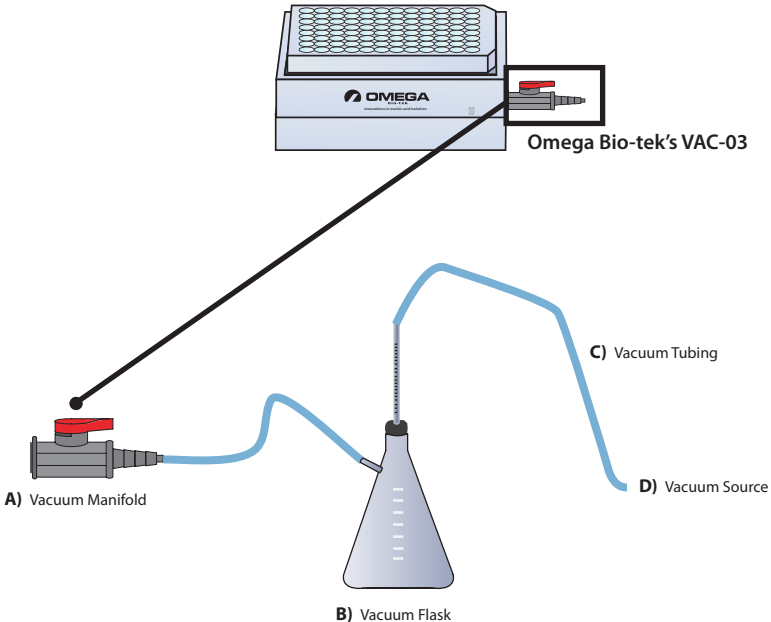
The following is required for use with the Vacuum Protocol:

- 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 |

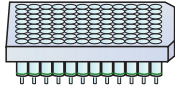
| Conversion from millibars: | Multiply by: |
|-------------------------------|--------------|
| Millimeters of Mercury (mmHg) | 0.75 |
| Kilopascals (kPa) | 0.1 |
| Inches of Mercury (inchHg) | 0.0295 |
| Torrs (Torr) | 0.75 |
| Atmospheres (atmos) | 0.000987 |
| Pounds per Square Inch (psi) | 0.0145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

Illustrated Vacuum Setup



Guidelines for Vacuum Manifold

DNA Bind and Wash Setup



E-Z 96 DNA Plate



Vacuum Manifold Top

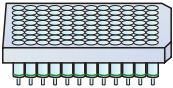


Waste Collection

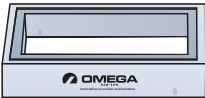


Vacuum Manifold Base

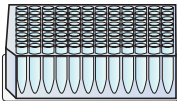
Standard Elution Setup



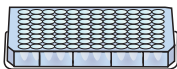
E-Z 96 DNA Plate



Vacuum Manifold Top



Racked Microtubes

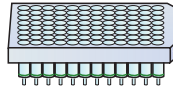


Microplate (300 µL)



Vacuum Manifold Base

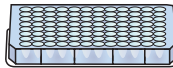
Optional Elution Setup



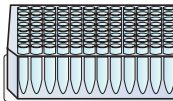
E-Z 96 DNA Plate



Vacuum Manifold Top



Microplate (300 µL)



Racked Microtubes



Vacuum Manifold Base

Disruption of Plant Tissues

Disrupt Samples With Commercial Homogenizers

A) Dry Specimens

Dried/lyophilized plant tissue can be effectively disrupted and homogenized by rapid agitation in the presence of beads.

1. Add one 3-4 mm stainless steel bead to each well of a 1.2 mL HTS plate.
2. Close the individual tubes with Caps for Racked Microtubes
3. Place the racks or plates into the clamps of the homogenizer.
4. Homogenize for 60-90 seconds at 30 Hz. Tissue samples are disrupted and simultaneously homogenized with the shearing and crushing action of the beads.

B) Fresh/Frozen Specimens

Fresh, frozen, and dried plant tissue can be effectively disrupted and homogenized by rapid agitation in the presence of beads.

For Fresh, Frozen and Lyophilized/Dried Tissue

1. Add one 3-4 mm stainless steel bead to each well of a 1.2 mL HTS plate.
2. Close the individual tubes with Caps for Racked Microtubes.
3. Freeze samples in liquid nitrogen.

Alternative to liquid nitrogen: Sample can be homogenized in presence of 700 μ L CSPL Buffer and 20 μ L proteinase K solution for fresh samples. Skip to step 4 of the E-Z 96 Plant DS Protocol if homogenized in presence of lysis buffer. Complete steps 4 and 5 of disruption of plant tissue(below):

4. Place the racks or plates into the clamps of the homogenizer.
5. Homogenize for 60-90 seconds at 30 Hz. Tissue samples are disrupted and simultaneously homogenized with the shearing and crushing action of the beads.

E-Z 96 Plant DNA DS Kit Protocols

E-Z 96 Plant DNA DS Kit Protocol - Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge equipped with swing-bucket rotor capable of at least 3,000 x *g*
- Water baths, ovens, or incubators capable of 65°C
- Vortexer
- 100% ethanol
- Liquid nitrogen for freezing/disrupting samples (for fresh/frozen specimens)
- Equipment for disrupting plant tissue

Before Starting:

- Prepare VHB Buffer, and DNA Wash Buffer according to Preparing Reagents section on Page 5
- Set a water bath, oven, or incubator to 65°C
- Heat Elution Buffer to 65°C

1. Transfer up to 10 mg dry powdered tissue or 50 mg fresh (or frozen) tissue to a 96-well Round-well Plate(provided) and seal with Caps for Round-well Plate.

Note: No more than 50 mg (wet weight) or 10 mg (dry weight) starting material is recommended. More or less can be used depending on results. Water content (and buffer absorption) of samples affect optimal starting amounts.

2. Homogenize plant tissue following one of the methods described in the Disruption of Plant Tissue section on Page 8. If homogenizing in the presence of lysis buffer with fresh tissue skip to step 4 after homogenization is complete.

3. Add 700 μ L CSPL Buffer and 20 μ L Proteinase K Solution to each sample. Seal the wells with Caps. Vortex to mix thoroughly.

Note: CSPL Buffer can be mixed with Proteinase K before use. Please see the Preparing Reagents section on Page 5 for instructions. Ensure that all the samples are completely suspended and that there are no clumps in the solution. Clumps will result in low yields.

4. Incubate at 65°C for 30 minutes. Mix samples twice during incubation by briefly

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shaking the plate side to side.

5. Centrifuge at 3,000-6,000 x *g* for 10 minutes.
6. Remove and discard the caps.
7. Place the E-Z 96 Homogenizer Plate on to a 96-well Square-well Plate (provided).
8. Transfer 550 μ L cleared supernatant to the E-Z 96 Homogenizer Plate.
9. Centrifuge at 3,000-6,000 x *g* for 5 minutes.
10. Add 5 μ L Rnase A. Let sit at room temperature for 5 minutes.
11. Add 525 μ L RBB Buffer and 525 μ L XP2 Buffer. Mix thoroughly by pipetting up and down or vortexing.
12. Place the E-Z 96 DNA Plate on to a new 96-well Square-well Plate (provided).
13. Carefully transfer 750 μ L sample to the E-Z 96 DNA Plate. Be careful not to spill sample liquid onto the rims of the wells during the transfer.
14. Centrifuge at 3,000-5,000 x *g* for 5 minutes or until all the sample has passed through the HiBind® membrane.
15. Discard the filtrate and reuse the 96-well Square-well Plate.
16. Carefully transfer another 750 μ L sample to the E-Z 96 DNA Plate. Be careful not to spill sample liquid onto the rims of the wells during the transfer.
17. Centrifuge at 3,000-5,000 x *g* for 5 minutes or until all the sample has passed through the HiBind® membrane.

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18. Discard the filtrate and reuse the 96-well Square-well Plate.

19. Add 500 μ L VHB Wash Buffer to each well of the E-Z 96 DNA Plate.

Note: VHB Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

20. Centrifuge at 3,000-5,000 $\times g$ for 5 minutes.

21. Discard the filtrate and reuse the 96-well Square-well Plate.

22. Add 700 μ L DNA Wash Buffer to each well of the E-Z 96 DNA Plate.

Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

23. Centrifuge at 3,000-5,000 $\times g$ for 5 minutes.

24. Discard the filtrate and reuse the 96-well Square-well Plate.

25. Repeat Steps 22-24 for a second DNA Wash Buffer wash step.

26. Centrifuge at 3,000-5,000 $\times g$ for 15 minutes to dry the plate.

Note: It is important to dry the plate membrane before elution. Residual ethanol may interfere with downstream applications.

27. Transfer the E-Z 96 DNA Plate to new 96-well Racked Microtubes (provided) or a 96-well microplate (not provided).

28. Add 100 μ L Elution Buffer heated at 65°C to each well.

29. Incubate at 65°C for 5 minutes.

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30. Centrifuge at 5,000 x *g* for 5 minutes.

31. Repeat Steps 28-30 for a second elution step.

Note: To maintain higher DNA concentration, second elution may be performed with first eluate.

32. Seal the 96-well Racked Microtubes with Caps for Racked Microtubes.

33. Store DNA at -20°C.

E-Z 96 Plant DNA DS Kit Protocols

E-Z 96 Plant DNA DS Kit - Vacuum Protocol

The following protocol is based on using Omega Bio-tek's vacuum manifold (Cat# VAC-03).

Materials and Equipment to be Supplied by User:

- Vacuum manifold and vacuum source
- Centrifuge equipped with swing-bucket rotor capable of at least 3,000 x *g*
- Water bath, oven, or incubator capable of 80°C
- Vortexer
- 100% ethanol
- Liquid nitrogen for freezing/disrupting samples (For Fresh/Frozen Specimens)
- Equipment for disrupting plant tissue
- Ice, freezer, or 96-well cryorack at -20°C
- Sealing film

Before Starting:

- Prepare HBC Buffer and DNA Wash Buffer according to Preparing Reagents section on Page 5
- Set a water bath, oven, or incubator to 65°C
- Heat Elution Buffer to 65°C

1. Transfer up to 10 mg dry powdered tissue or 50 mg fresh (or frozen) tissue to a 96-well Round-well Plate(provided) and seal with Caps for Round-well Plate.

Note: No more than 50 mg (wet weight) or 10 mg (dry weight) starting material is recommended. More or less can be used depending on results. Water content (and buffer absorption) of samples affect optimal starting amounts.

2. Homogenize plant tissue following one of the methods described in the Disruption of Plant Tissue section on Pages 8. If homogenizing in the presence of lysis buffer with fresh tissue skip to step 4 after homogenization is complete.
3. Add 700 μ L CSPL Buffer and 20 μ L Proteinase K Solution to each sample. Seal the wells with Caps. Vortex to mix thoroughly.

Note: CSPL Buffer can be mixed with Proteinase K before use. Please see the Preparing Reagents section on Page 5 for instructions. Ensure that all the samples are completely suspended and that there are no clumps in the solution. Clumps will result in low yields.

E-Z 96 Plant DNA DS Kit Protocols

4. Incubate at 65°C for 30 minutes. Mix samples twice during incubation by briefly shaking the plate side to side.
5. Centrifuge at 3,000-6,000 $\times g$ for 10 minutes.
6. Remove and discard the caps.
7. Place the E-Z 96 Homogenizer Plate on to a 96-well Square-well Plate (provided).
8. Transfer 550 μL cleared supernatant to the E-Z 96 Homogenizer Plate.
9. Centrifuge at 3,000-6,000 $\times g$ for 5 minutes.
10. Add 5 μL Rnase A. Let sit at room temperature for 5 minutes.
11. Add 525 μL RBB Buffer and 525 μL XP2 Buffer. Mix thoroughly by pipetting up and down or vortexing.
12. Prepare the vacuum manifold according to manufacturer's instructions.
13. Place an E-Z 96 DNA Plate on the top part of the vacuum manifold. Place the waste collection tray inside the base of the manifold. Seal any unused wells with sealing film (not provided).
14. Transfer 750 μL sample to the E-Z 96 DNA Plate.
15. Turn on the vacuum source to draw the sample through the plate.
16. Turn off the vacuum.
17. Repeat Steps 14-16 until all the sample has been transferred to the E-Z 96 DNA Plate.

E-Z 96 Plant DNA DS Kit Protocols

18. Add 500 μ L VHB Wash Buffer to each well.

Note: VHB Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

19. Turn on the vacuum source to draw the VHB Wash Buffer through the plate.

20. Turn off the vacuum.

21. Add 700 μ L DNA Wash Buffer to each well.

Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

22. Turn on the vacuum source to draw the DNA Wash Buffer through the plate.

23. Turn off the vacuum.

24. Repeat Steps 21-23 for a second DNA Wash Buffer wash step.

25. Add 400 μ L 100% ethanol to each well.

26. Turn on the vacuum source to draw the ethanol through the plate.

27. Turn off the vacuum.

28. Continue to apply the vacuum for 10 minutes after all liquid has passed through the E-Z 96 DNA Plate.

29. Turn off the vacuum.

30. Discard and remove the filtrate and waste collection plate.

E-Z 96 Plant DNA DS Kit Protocols

31. Place the E-Z 96 DNA Plate upside down on a stack of paper towels and tap several times to remove any residual ethanol.

Note: It is very important to completely dry the E-Z 96 DNA Plate before elution. If a swing bucket centrifuge with a 96-well plate adaptor is available, centrifuge at 5,000 $\times g$ for 5 minutes to dry the plate. Or if an oven/incubator is available, dry the plate at 65°C for 10 minutes.

32. Place the 96-well Racked Microtubes inside the base of the manifold.
33. Place the E-Z 96 DNA Plate on top of the manifold.
34. Add 100 μL Elution Buffer heated to 65°C to each well.
35. Let sit at room temperature for 5 minutes.
36. Turn on the vacuum source to draw the Elution Buffer through the plate.
37. Turn off the vacuum.

Optional: Repeat Steps 34-37 for a second elution step.

Note: 100 μL Elution Buffer is sufficient to elute up to 85% of the DNA from each well of the E-Z 96 DNA Plate. A second elution step with same 100 μL elute containing DNA, reheated to 65°C, will increase yield by up to 10-15%. Total DNA yields vary depending on type and quantity of sample.

38. Seal the 96-well Racked Microtubes with Caps for Racked Microtubes (provided).
39. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Possible Problems and Suggestions

| Problem | Cause | Solution |
|-------------------------------------|---|---|
| Clogged well | Sample too viscous | Do not exceed suggested amount of starting material. |
| Problem | Cause | Solution |
| Low DNA yield | Incomplete disruption of starting material | Completely homogenize sample. |
| | Poor lysis of tissue | Decrease the amount of starting material or increase the amount of CSPL Buffer |
| | DNA remains bound to column | Increase elution volume to 200 μL and incubate the plate at 65°C for 5 minutes before centrifugation. |
| | DNA washed off | Dilute SPW Wash Buffer by adding appropriate volume of 100% ethanol prior to use (Page 5). |
| | Insufficient sample amount transferred after supernatant removal | If 550 μL lysis buffer cannot be transferred after clearing lysate by centrifugation. Increase volume of CSPL Buffer. If only 350 μL could be recovered then increase amount by 200 μL (550 μL Desired amount - 350 μL = 200 μL additional lysis buffer amount required). |
| Problem | Cause | Solution |
| Problems in downstream applications | Salt carryover | Repeat wash step with SPW Wash Buffer. |
| | Ethanol carryover | Following the second wash spin, ensure that the plate is completely dried before elution. |

Ordering Information

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

| Product | Part Number |
|--|-------------|
| Elution Buffer (100 mL) | PDR048 |
| Sealing Film | AC1200 |
| AeraSeal Film | AC1201 |
| 96-well Square-well Plate (2.2 mL) | EZ9602 |
| E-Z 96 DNA Plates (10) | BD96-01 |
| E-Z 96 Homogenizer Plates (4 x 96) | HCR9601-02 |
| E-Z 96 Lysate Clearance Plates (10 x 96) | FL9601 |
| Vacuum Manifold | VAC-03 |

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

