OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E-Z 96® Plant DNA DS Kit

D1411-00	1 x 96 preps
D1411-01	4 x 96 preps

August 2015

For research use only.Not intended for diagnostic testing.

E-Z 96® Plant DNA DS Kit

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Manual Revision: August 2015

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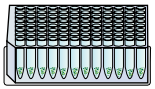
Introduction

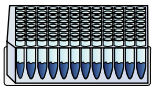
The E-Z 96® Plant DNA DS Kit is designed for efficient recovery of genomic DNA up to 30 kb in size from fresh, frozen, or dried plant tissue samples rich in polysaccharides, polyphenols, or those having a lower DNA content. Up to 50 mg wet tissue can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of the HiBind® matrix with the speed and versatility of silica plate 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 a 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.

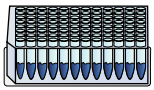
Centrifugation Protocol



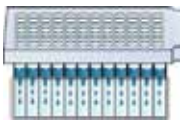
Collect Plant Tissue and Homogenize



Lyse

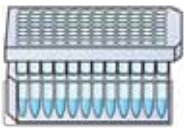


Transfer Cleared Lysate and Adjust Binding Conditions



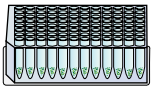
Bind and Wash 3X

Dry Membrane

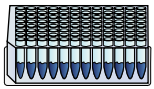


Elute

Vacuum Protocol



Collect Plant Tissue and Homogenize



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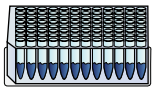
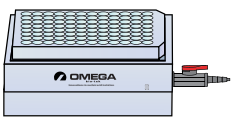
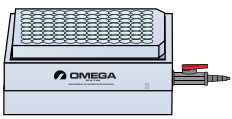


Transfer Cleared Lysate and Adjust Binding Conditions

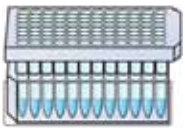


Bind and Wash 3X

Vacuum



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
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	25 mL	100 mL
Proteinase K Solution	2.2 mL	8.8 mL
RNase A	550 µL	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. 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. 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.

- 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

OPTIONAL: Mix Proteinase K Solution and CSPL Buffer as follows. Make only what is needed and use immediately. Use the following table as a guide.

Preps	CSPL Buffer to be Added	Proteinase K Solution to be Added
1	0.7 mL	20 µL
96*	70 mL	2 mL
192*	140 mL	4 mL
288*	210 mL	6 mL
384*	280 mL	8 mL

* Overage (rounded to nearest 100) is included in the calculations.

Guidelines for Vacuum Manifold

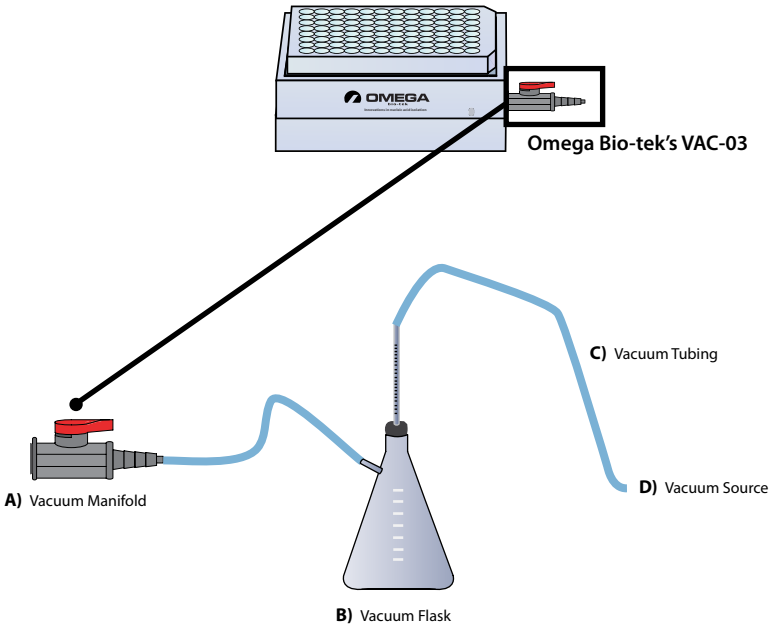
The following is required for use with the Vacuum Protocol:

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

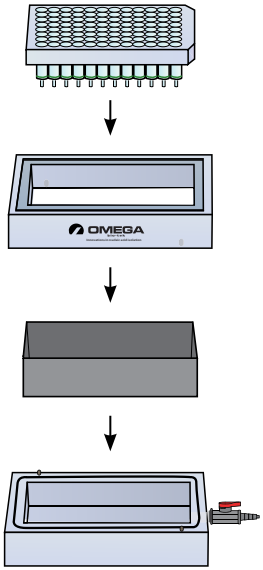
Conversion from millibars:	Multiply by:
Millimeters of Mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of Mercury (inchHg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Illustrated Vacuum Setup

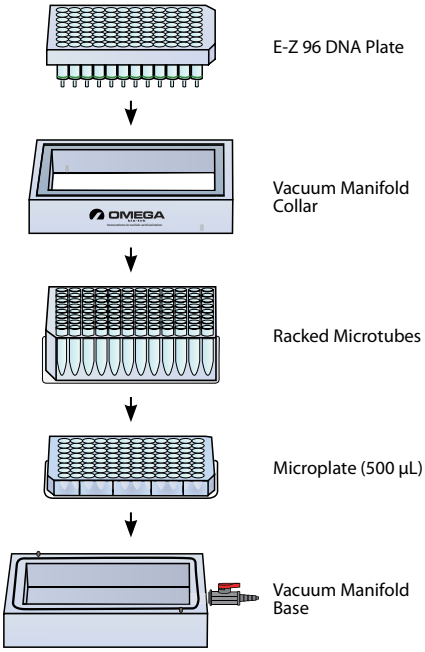


Guidelines for Vacuum Manifold

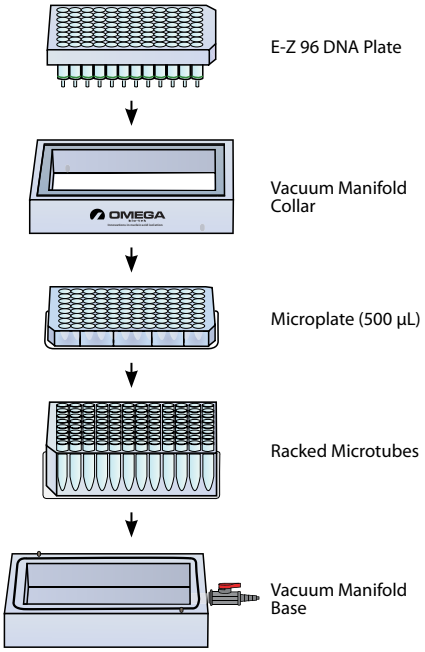
DNA Bind and Wash Setup



Standard Elution Setup



Optional Elution Setup



Disrupt Samples With Commercial Homogenizers

A) Dried/Lyophilized Samples

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. Seal the wells with Caps for Racked Microtubes.
3. Place the plate 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 Samples

Fresh and frozen 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. Seal the wells with Caps for Racked Microtubes.
3. Freeze samples in liquid nitrogen.

Alternative to liquid nitrogen: Sample can be homogenized in presence of 700 µL CSPL Buffer and 20 µL Proteinase K Solution for fresh samples. Skip to Step 4 of the E-Z 96 Plant DS Protocol if homogenized in presenece 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 Protocol - Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge equipped with swing-bucket rotor capable of at least 5,000 x *g*
- Water bath, oven, or incubator 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 1.2 mL HTS Plate (provided) and seal with Caps for Racked Microtubes.

   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. Remove and discard the caps.

4. Add 700 µL CSPL Buffer and 20 µL Proteinase K Solution to each sample. Seal the wells with new Caps for Racked Microtubes. Vortex to mix thoroughly.

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

5. Incubate at 65°C for 30 minutes. Mix samples twice during incubation by briefly shaking the plate side-to-side.

6. Centrifuge at 3,000-6,000 x *g* for 10 minutes.

7. Remove and discard the caps.

8. Place a E-Z 96 Homogenizer Plate on to a 96-well Square-well Plate (provided).

9. Transfer 550 µL cleared supernatant to the E-Z 96 Homogenizer Plate.

10. Centrifuge at 3,000-6,000 x *g* for 5 minutes. Discard the E-Z 96 Homogenizer Plate.

11. Add 5 µL RNase A to each sample. Let sit at room temperature for 5 minutes.

12. Add 525 µL RBB Buffer and 525 µL XP2 Buffer to each sample. Mix thoroughly by pipetting up and down or vortexing.

13. Place a E-Z 96 DNA Plate on to a new 96-well Square-well Plate (provided).

14. Carefully transfer 750 µL sample to the E-Z 96 DNA Plate. Be careful not to spill sample liquid onto the rims of the wells during the transfer.

15. Centrifuge at 3,000-5,000 x *g* for 5 minutes or until all the sample has passed through the HiBind® matrix.

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

17. Repeat Steps 14-16 until all the sample has been transferred to the E-Z 96 DNA Plate.

18. Add 500 µL VHB Buffer to each well of the E-Z 96 DNA Plate.

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

19. Centrifuge at 3,000-5,000 x *g* for 5 minutes.

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

21. Add 700 µL 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.

22. Centrifuge at 3,000-5,000 x *g* for 5 minutes.

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

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

25. Centrifuge at 3,000-5,000 x *g* for 15 minutes to dry the plate.

    Note: It is important to dry the HiBind® matrix before elution. Residual ethanol may interfere with downstream applications.

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

27. Add 100 µL Elution Buffer heated to 65°C to each well of the E-Z 96 DNA Plate.

28. Incubate at 65°C for 5 minutes.

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

30. Repeat Steps 27-29 for a second elution step.

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

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

32. Store DNA at -20°C.

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 5,000 x *g*
- Water bath, oven, or incubator capable of 65°C
- Vortexer
- 100% ethanol
- Liquid nitrogen for freezing/disrupting samples (for fresh/frozen samples)
- Equipment for disrupting plant tissue
- Sealing film

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 1.2 mL HTS Plate (provided) and seal with Caps for Racked Microtubes.

   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. Remove and discard the caps.

4. Add 700 µL CSPL Buffer and 20 µL Proteinase K Solution to each sample. Seal the wells with new Caps for Racked Microtubes. Vortex to mix thoroughly.

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

5. Incubate at 65°C for 30 minutes. Mix samples twice during incubation by briefly shaking the plate side-to-side.

6. Centrifuge at 3,000-6,000 x *g* for 10 minutes.

7. Remove and discard the caps.

8. Place a E-Z 96 Homogenizer Plate on to a 96-well Square-well Plate (provided).

9. Transfer 550 µL cleared supernatant to the E-Z 96 Homogenizer Plate.

10. Centrifuge at 3,000-6,000 x *g* for 5 minutes.

11. Add 5 µL RNase A to each sample. Let sit at room temperature for 5 minutes.

12. Add 525 µL RBB Buffer and 525 µL XP2 Buffer to each sample. Mix thoroughly by pipetting up and down or vortexing.

13. Prepare the vacuum manifold according to manufacturer's instructions.

14. Place an E-Z 96 DNA Plate on top of the vacuum manifold collar. Place the waste collection tray inside the vacuum manifold base. Seal any unused wells with sealing film (not provided).

15. Transfer 750 µL sample to the E-Z 96 DNA Plate.

16. Turn on the vacuum source to draw the samples through the plate.

17. Turn off the vacuum.

18. Repeat Steps 15-17 until all the sample has been transferred to the E-Z 96 DNA Plate.

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

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

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

21. Turn off the vacuum.

22. Add 700 µL 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. Turn on the vacuum source to draw the DNA Wash Buffer through the plate.

24. Turn off the vacuum.

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

26. Add 400 µL 100% ethanol to each well of the E-Z 96 DNA Plate.

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

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. Remove the waste collection plate and discard the filtrate.

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 x *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 vacuum manifold base.

33. Place the E-Z 96 DNA Plate on top of the vacuum manifold collar.

34. Add 100 µL Elution Buffer heated to 65°C to each well of the E-Z 96 DNA Plate.

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.

38. Repeat Steps 34-37 for a second elution step.

39. Seal the 96-well Racked Microtubes with Caps for Racked Microtubes (provided).

40. 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 DNA 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 DNA 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