omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E-Z 384 Plant DNA HT Kit

D1341-00	1 x 384 preps
D1341-01	4 x 384 preps

Manual Date: December 2021
Revision Number: v3.0

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 384 Plant DNA HT Kit

Table of Contents

Manual Date: December 2021
Revision Number: v3.0

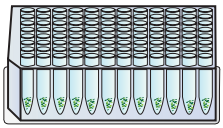
omega BIO-TEK
innovations in nucleic acid isolation

Introduction

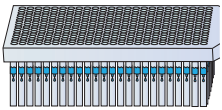
The E-Z 384 Plant DNA HT Kit enables rapid and high throughput isolation of genomic DNA from a wide variety of plant tissue samples. Up to 768 fresh, frozen, or dried plant tissue samples rich in polyphenols, polysaccharides or having a lower DNA content can be processed in approximately 1.5 hours when processing two 384-plates in parallel 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.

The purification procedure relies on the well-established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with a unique binding system to provide high quality DNA. The system eliminates the need for chloroform extractions traditionally associated with CTAB-based lysis methods. Samples are lysed in a high salt buffer containing CTAB and centrifuged to pellet the debris. The purification of the DNA from the cleared lysates follows the "bind-wash-elute" routine using an E-Z 384 DNA Plate. The eluted DNA is inhibitor-free and ready for downstream applications such as genotyping, plant breeding, population level analyses, next generation sequencing, PCR, and more.

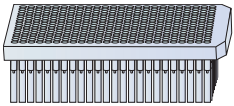
Centrifugation Protocol



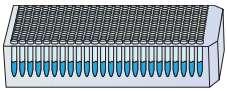
Collect Plant Tissue and Homogenize and Lyse (96-well Plate)



Bind to E-Z 384 DNA Plate and Wash 3X

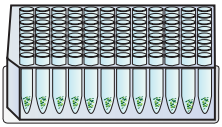


Dry Membrane

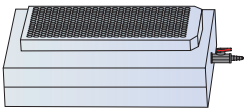


Elute

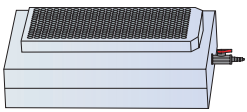
Vacuum Protocol



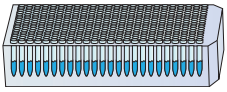
Collect Plant Tissue and Homogenize and Lyse (96-well Plate)



Bind to E-Z 384 DNA Plate and Wash 3X



Dry Membrane



Elute

Kit Contents

Product Number	D1341-00	D1341-01
Purifications	1 x 384	4 x 384
E-Z 384 DNA Plate	1	4
96-well Square-well Plate (2.2 mL)	1	4
CSPL Buffer	2 x 100 mL	2 x 360 mL
VHB Buffer	2 x 22 mL	2 x 44 mL
DNA Wash Buffer	25 mL	100 mL
Elution Buffer	60 mL	250 mL
User Manual	✓	✓

Storage and Stability

All components of the E-Z 384 Plant DNA HT Kit are guaranteed for at least 12 months from date of purchase when stored as follows. All components should be stored at recommended temperatures. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

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

Kit	100% Ethanol to be Added
D1341-00	28 mL to each bottle
D1341-01	56 mL to each bottle

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

Kit	100% Ethanol to be Added
D1341-00	100 mL
D1341-01	400 mL

Guidelines for Vacuum Manifold

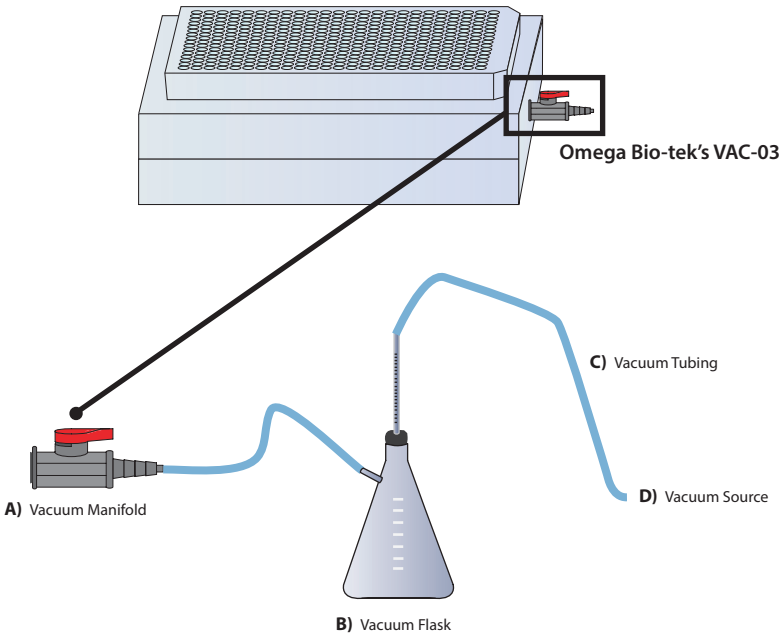
The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-03)
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

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

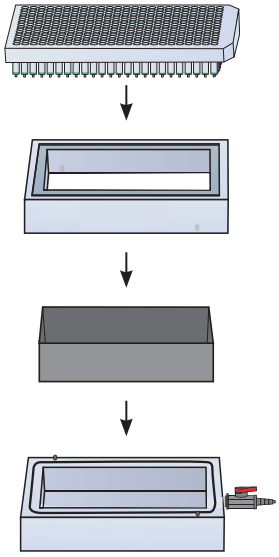
Conversion from millibars:	Multiply by:
Millimeters of Mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of Mercury (inchHg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Illustrated Vacuum Setup



Guidelines for Vacuum Manifold

DNA Bind and Wash Setup



E-Z 384 DNA Plate

Vacuum Manifold Collar

Waste Collection Tray

Vacuum Manifold Base

Disruption of Plant Tissue With Commercial Homogenizers

Proceed with one of the following tissue disruption techniques after completing Step 1 in either the E-Z 384 Plant DNA HT Kit Centrifugation Protocol (Page 9) or the Vacuum Protocol (Page 13).

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 96-well deep-well plate (not provided).
2. Seal the plate with a 96-well sealing mat (not provided).
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.
5. Continue to Step 3 in either the E-Z 384 Plant DNA HT Kit Centrifugation Protocol (Page 9) or the Vacuum Protocol (Page 14).

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 96-well deep-well plate (not provided).
2. Complete one of the following options:
   A. Liquid Nitrogen
      1. Freeze samples in liquid nitrogen.
      2. Seal the plate with a 96-well sealing mat (not provided).
      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.
      5. Continue to Step 3 in either the E-Z 384 Plant DNA HT Kit Centrifugation Protocol (Page 9) or the Vacuum Protocol (Page 14).

   OR

   B. Homogenize fresh plant tissue in lysis buffer
      1. Add 400 µL CSPL Buffer to each sample.
      2. Seal the plate with a 96-well sealing mat (not provided).
      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.
      5. Continue to Step 5 in either the E-Z 384 Plant DNA HT Kit Centrifugation Protocol (Page 10) or the Vacuum Protocol (Page 14).

E-Z 384 Plant DNA HT Kit - Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge equipped with swing-bucket rotor and plate adapter capable of at least 2,000*g*
- Oven or incubator capable of 65°C
- Vortexer
- 96-well deep-well plate capable of 2 mL (Recommend VWR, Cat# 73520-476)
- 96-well sealing mats (Recommend VWR, Cat# 44200-004)
- 384-well elution plate (Recommend VWR, Cat# 10036-134)
- 100% ethanol
- 100% isopropanol
- Equipment for disruption of plant tissue
- Sealing film (Recommend Omega Bio-tek, Cat# AC1200)
- Optional: Liquid nitrogen for freezing/disrupting samples (for fresh/frozen samples)

Before Starting:

- Prepare VHB Buffer and DNA Wash Buffer according to "Preparing Reagents" section on Page 5.
- Set an oven or incubator to 65°C.
- Heat Elution Buffer to 65°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.***

1. Transfer 2 leaf punches to a 96-well deep-well plate (not provided) and seal with a 96-well sealing mat (not provided).

   Note: (1) Do not use more than 30 mg fresh or 10 mg dry plant sample per well. After initial testing, more or less can be used depending on results. Water content (and buffer absorption) of samples affect optimal starting amounts. (2) Set up 4 x 96-well deep-well plates for processing up to 384 samples.

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 CSPL Buffer with fresh tissue, skip to Step 5 after homogenization is complete.

3. Remove and discard the 96-well sealing mat.

4. Add 400 µL CSPL Buffer. Seal the plate with a new 96-well sealing mat. Vortex to mix thoroughly.

   Note: Ensure that all the samples are completely homogenized and that there are no clumps in the solution. Clumps will result in lower yields.

Optional: Proteinase K can be added to CSPL Buffer for slightly increased yields. Add 20 µL Proteinase K (20mg/mL) to 400 µL CSPL Buffer, if the Proteinase K treatment is desired.

5. Incubate at 65°C for 30 minutes. Mix samples twice during incubation by briefly vortexing the plate.

6. Centrifuge at 2,000*g* for 10 minutes.

7. Remove and discard the 96-well sealing mat.

8. Transfer 300 µL supernatant without disturbing the pellet to fresh 96-well deep-well plate (not provided).

   Note: Prepare lysate in up to 4 x 96-well plates for processing up to 384 samples.

Optional: If RNA-free genomic DNA is desired, add 5 µL of RNase A (25 mg/mL) and incubate at room temperature for 5 minutes.

9. Add 300 µL 100% isopropanol. Vortex or pipet up and down to mix thoroughly.

10. Place an E-Z 384 DNA Plate onto a new 96-well Square-well Plate.

11. Carefully transfer 85 µL sample to the E-Z 384 DNA Plate. Be careful not to spill sample liquid onto the rims of the wells during the transfer.

    Note: Transfer lysate from up to 4 x 96-well plates to one 384-well plate.

12. Centrifuge at 2,000*g* for 2 minutes or until all the sample has passed through the 384-well DNA Plate.

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

Optional: For increased DNA yield, repeat Steps 11-13 with additional lysate from Step 10.

14. Add 85 µL VHB Buffer to each sample.

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

15. Centrifuge at 2,000*g* for 2 minutes.

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

17. Add 85 µL DNA Wash Buffer to each sample.

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

18. Centrifuge at 2,000*g* for 2 minutes.

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

20. Repeat Steps 17-19 for a second DNA Wash Buffer wash step.

21. Select one of the following ethanol removal steps:

    A. Centrifuge at 2,000*g* for 10 minutes to dry the plate. Continue to Step 23.

    OR

    B. Dry the plate in an oven or incubator set to 65°C for 10 minutes. Let the plate cool for 5 minutes at room temperature after incubation. Continue to Step 23.

    Note: It is important to dry the 384-well plate before elution. Residual ethanol may interfere with downstream applications.

22. Transfer the E-Z 384 DNA Plate to a 384-well elution plate (not provided).

23. Add 50-85 µL Elution Buffer heated to 65°C to each sample.

24. Let sit at room temperature for 5 minutes.

25. Centrifuge at 2,000*g* for 2 minutes.

    Note: (1) Adjusting the acceleration speed to a low setting is critical for optimal elution recovery. (2) The estimated retention volume is ~10-20 µL per well.

Optional: Repeat Steps 23-25 for a second elution step. To maintain higher DNA concentration, the second elution may be performed with the first eluate.

26. Seal the 384-well Elution Plate with sealing film (not provided).

27. Store DNA at -20°C.

E-Z 384 Plant DNA HT 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 and plate adapter capable of at least 2,000*g*
- Oven or incubator capable of 65°C
- Vortexer
- 96-well deep-well plate capable of 2 mL (Recommend VWR, Cat# 73520-476)
- 96-well sealing mats (Recommend VWR, Cat# 44200-004)
- 384-well elution plate (Recommend VWR, Cat# 10036-134)
- 100% ethanol
- 100% isopropanol
- Equipment for disruption of plant tissue
- Sealing film (Recommend Omega Bio-tek, Cat# AC1200)
- Optional: Liquid nitrogen for freezing/disrupting samples (for fresh/frozen samples)

Before Starting:

- Prepare VHB Buffer and DNA Wash Buffer according to "Preparing Reagents" section on Page 5.
- Set an oven or incubator to 65°C.
- Heat Elution Buffer to 65°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.***

1. Transfer 2 leaf punches to a 96-well deep-well plate (not provided) and seal with a 96-well sealing mat (not provided).

   Note: (1) Do not use more than 30 mg fresh or 10 mg dry plant sample per well. After initial testing, more or less can be used depending on results. Water content (and buffer absorption) of samples affect optimal starting amounts. (2) Set up 4 x 96-well deep-well plates for processing up to 384 samples.

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 CSPL Buffer with fresh tissue, skip to Step 5 after homogenization is complete.

3. Remove and discard the 96-well sealing mat.

4. Add 400 µL CSPL Buffer to each sample. Seal the plate with a new 96-well sealing mat. Vortex to mix thoroughly.

   Note: Ensure that all the samples are completely homogenized and that there are no clumps in the solution. Clumps will result in lower yields.

Optional: Proteinase K can be added to CSPL Buffer for slightly increased yields. Add 20 µL Proteinase K (20mg/mL) to 400 µL CSPL Buffer, if the Proteinase K treatment is desired.

5. Incubate at 65°C for 30 minutes. Mix samples twice during incubation by briefly vortexing the plate.

6. Centrifuge at 2,000*g* for 10 minutes.

7. Remove and discard the 96-well sealing mat.

8. Transfer 300 µL supernatant without disturbing the pellet to fresh 96-well deep-well plate (not provided).

   Note: Prepare lysate in up to 4 x 96-well plates for processing up to 384 samples.

Optional: If RNA-free genomic DNA is desired, add 5 µL of RNase A (25 mg/mL) and incubate at room temperature for 5 minutes.

9. Add 300 µL 100% isopropanol. Vortex or pipet up and down to mix thoroughly.

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

11. Place an E-Z 384 DNA Plate on top of the vacuum manifold collar. Place the waste collection tray inside the vacuum manifold base.

12. Carefully transfer 85 µL sample to the E-Z 384 DNA Plate. Be careful not to spill sample liquid onto the rims of the wells during the transfer.

    Note: Transfer lysate from up to 4 x 96-well plates to one 384-well plate.

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

14. Turn off the vacuum.

Optional: For increased DNA yield, repeat Steps 12-14 with additional lysate from Step 10.

15. Add 85 µL VHB Buffer to each sample.

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

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

17. Turn off the vacuum.

18. Add 85 µL DNA Wash Buffer to each sample.

    Note: DNA 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 DNA Wash Buffer through the plate.

20. Turn off the vacuum.

21. Repeat Steps 18-20 for a second DNA Wash Buffer wash step.

22. Add 85 µL 100% ethanol to each sample.

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

24. Turn off the vacuum.

25. Remove the waste collection plate and discard the filtrate.

26. Dry the plate in an oven or incubator set to 65°C for 10 minutes. Let the plate cool at room temperature for 5 minutes after incubation.

27. Transfer the E-Z 384 DNA Plate to a 384-well elution plate (not provided).

28. Add 50-85 µL Elution Buffer heated to 65°C to each sample.

29. Let sit at room temperature for 5 minutes.

30. Centrifuge at 2,000*g* for 5 minutes.

    Note: (1) Adjusting the acceleration speed to a low setting is critical for optimal elution recovery. (2) The estimated retention volume is ~10-20 µL per well.

Optional: Repeat Steps 28-30 for a second elution step. To maintain higher DNA concentration, the second elution may be performed with the first eluate.

31. Seal the 384-well elution plate with sealing film (not provided).

32. 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 or use less starting material.
	Sample not vacuuming properly	Stop vacuum protocol and follow centrifugation protocol
Problem	**Cause**	**Solution**
Low DNA yield	Incomplete disruption of starting material	Completely homogenize the sample.
	Poor lysis of tissue	Decrease the amount of starting material or increase the amount of CSPL Buffer. Proteinase K can be added to CSPL Buffer for slightly increased yields. Add 20 µL Proteinase K (20mg/mL) to 400 µL CSPL Buffer, if the Proteinase K treatment is desired.
	DNA remains bound to column	Cover plate with sealing film (not provided) after addition of Elution Buffer and incubate for 5 minutes at 65°C. Or perform optional second elution step with additional Elution Buffer or using first eluate.
	Insufficient sample amount transferred after supernatant removal	If 300 µL lysis buffer cannot be transferred after clearing by centrifugation, increase volume of CSPL Buffer. For example, if only 250 µL could be recovered then increase amount by 50 µL (300 µL desired amount - 250 µL = 50 µL additional lysis buffer amount required).

Troubleshooting Guide

Problem	Cause	Solution
Problems in downstream applications	Salt carryover	Repeat wash step with DNA Wash Buffer.
	Ethanol carryover	Ensure that the plate is completely dried before elution.
Uneven elution volume across plate	Centrifuge ramp speed	Decrease the ramp speed of the centrifuge. If the centrifuge has an acceleration speed scale 1-10, set to 3.

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


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

omega BIO-TEK

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