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

Manual Date: June 2021
Revision Number: v4.1

For Research Use Only

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E-Z 96 Plant DNA DS Kit

Table of Contents

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Introduction

The E-Z 96 Plant DNA DS Kit is designed for efficient recovery of genomic DNA 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 an 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.

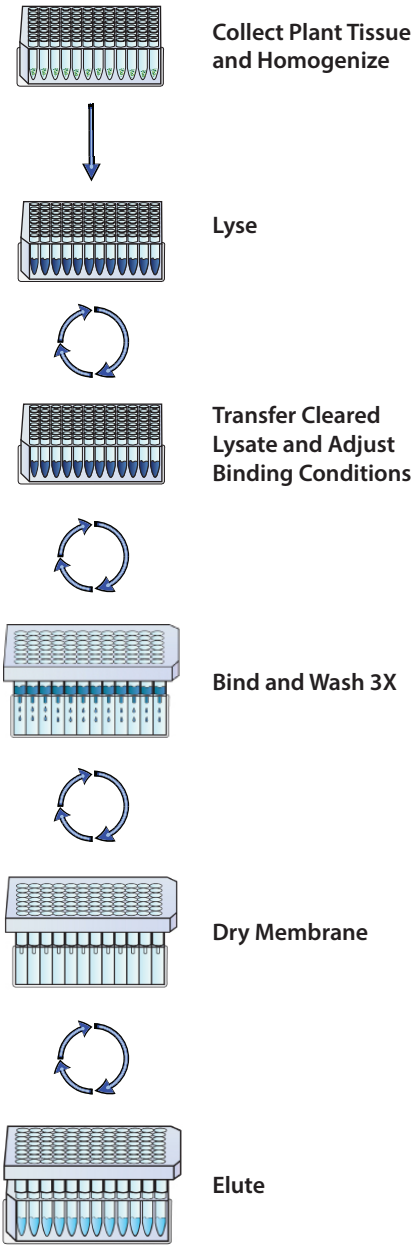
New in this Edition:

July 2019:
- XP2 Buffer has been renamed XP2Binding Buffer. This is a name change only. The formulation has not changed.

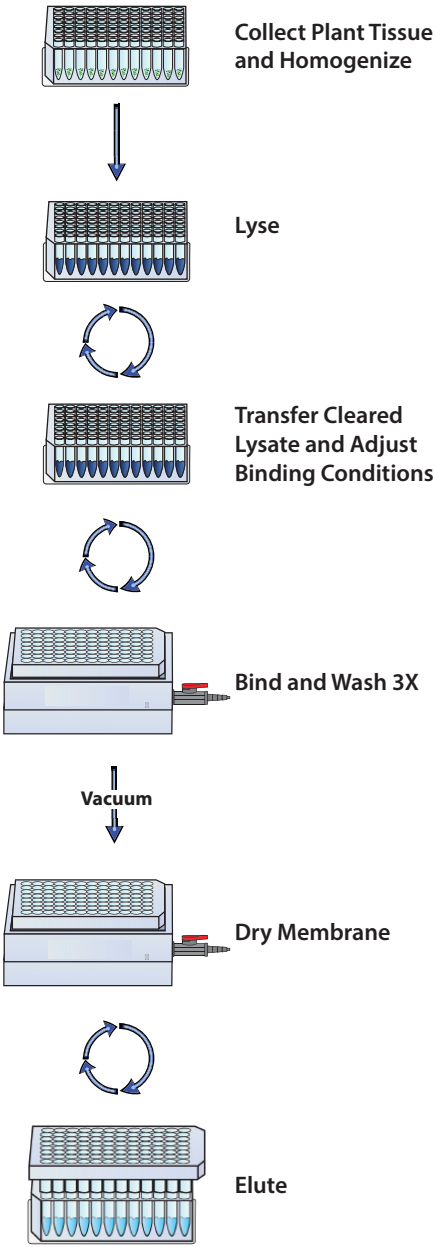
February 2019:
- Minor edits.

September 2018:
- Protocol modifications have been added for use with centrifugation speeds less than 4,000 x *g*.
- 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.

Centrifugation Protocol



Vacuum Protocol



Kit Contents

Product Number	D1411-00	D1411-01
E-Z 96 DNA Plate	1	4
E-Z 96 Homogenizer Plate	1	4
Lysis Plate	1	4
Silicone Mat	2	8
96-well Racked Microtubes	1	4
Caps for Racked Microtubes	12 x 8	50 x 8
96-well Square-well Plate (2.2 mL)	2	8
CSPL Buffer	80 mL	300 mL
XP2 Binding Buffer	60 mL	250 mL
RBB Buffer	60 mL	250 mL
VHB Buffer	44 mL	176 mL
DNA Wash Buffer	50 mL	200 mL
Elution Buffer	60 mL	250 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	200 mL
D1411-01	800 mL

OPTIONAL: Mix Proteinase K Solution and CSPL Buffer as follows. Prepare 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

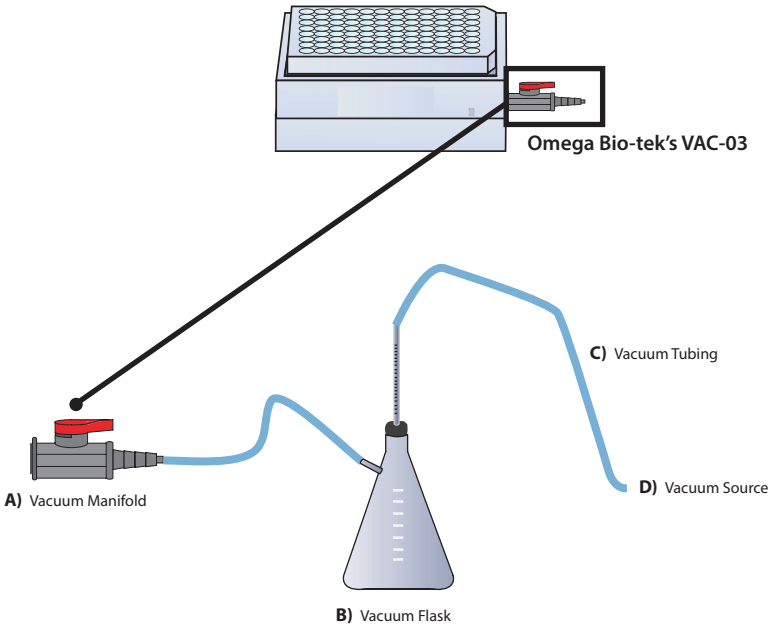
The following is required for use with the Vacuum Protocol:

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

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

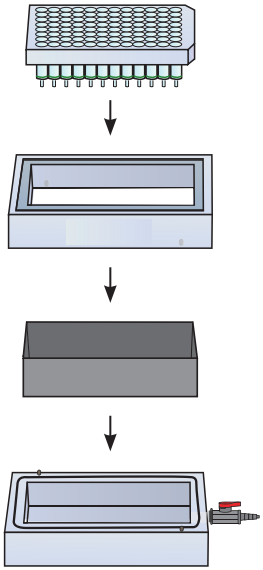
Conversion from millibars:	Multiply by:
Millimeters of Mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of Mercury (inchHg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Illustrated Vacuum Setup

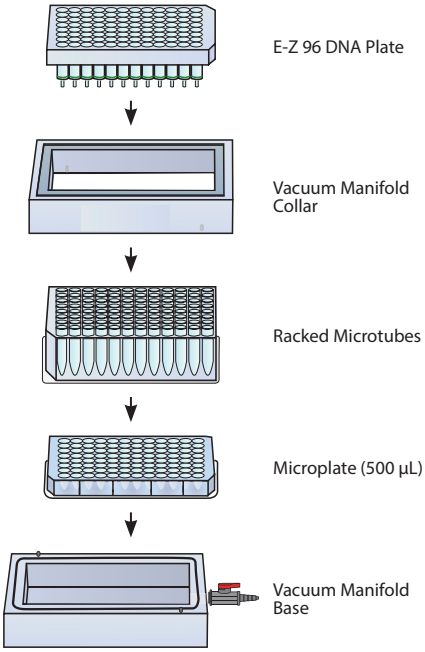


Guidelines for Vacuum Manifold

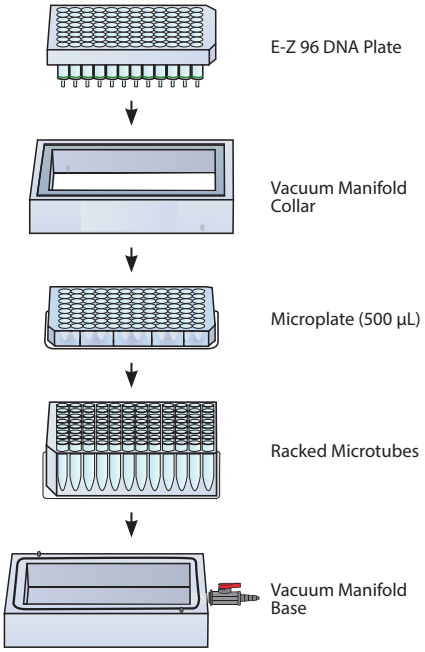
DNA Bind and Wash Setup



Standard Elution Setup



Optional Elution Setup



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 96 Plant DNA DS 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 Lysis Plate.
2. Seal the plate with a Silicone Mat.
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 96 Plant DNA DS Kit Centrifugation Protocol (Page 9) or the Vacuum Protocol (Page 13).

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 Lysis Plate.
2. Complete one of the following options:
   A. Liquid Nitrogen
      1. Freeze samples in liquid nitrogen.
      2. Seal the plate with a Silicone Mat.
      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 96 Plant DNA DS Kit Centrifugation Protocol (Page 9) or the Vacuum Protocol (Page 13).

   OR

   B. Homogenize FRESH tissue in lysis buffer
      1. Add 700 µL CSPL Buffer and 20 µL Proteinase K Solution to each sample.
      2. Seal the plate with a Silicone Mat.
      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 96 Plant DNA DS Kit Centrifugation Protocol (Page 9) or the Vacuum Protocol (Page 13).

E-Z 96 Plant DNA DS Kit - Centrifugation 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 96-well 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*
- Oven or incubator capable of 65°C
- Vortexer
- 100% ethanol
- Liquid nitrogen for freezing/disrupting samples (for fresh/frozen specimens)
- Equipment for disruption of plant tissue
- Optional: 96-well microplate and sealing film for elution and DNA storage

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 up to 10 mg dry powdered tissue or 50 mg fresh (or frozen) tissue to a Lysis Plate (provided) and seal with a Silicone Mat.

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

3. Remove and discard the Silicone Mat.

4. Add 700 µL CSPL Buffer and 20 µL Proteinase K Solution to each sample. Seal the plate with a new Silicone Mat. 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 homogenized 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 2,000-6,000 x *g* for 10 minutes.

7. Remove and discard the Silicone Mat.

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

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

10. Centrifuge at 2,000-6,000 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 Binding Buffer to each sample. Vortex or pipet up and down to mix thoroughly.

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 4,000-6,000 x *g* for 5 minutes or until all the sample has passed through the HiBind® matrix.

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

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 sample.

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

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

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

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

21. Add 700 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.

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

    Note: If the centrifugation speed is less than 4,000 x *g*, increase centrifugation time to 10 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. Select one of the following ethanol removal steps:

    A. Centrifuge at **4,000-6,000 x *g*** for 15 minutes to dry the plate. Continue to Step 26.

    OR

    B. Dry the plate in an oven or incubator set to 65°C for 30 minutes. Let the plate cool for 5 minutes at room temperature after incubation. Continue to Step 26.

    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 150 µL Elution Buffer heated to 65°C to each sample.

28. Let sit at room temperature for 5 minutes.

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

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

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

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

31. Seal the 96-well Racked Microtubes with Caps for Racked Microtubes or the 96-well microplate with sealing film (not provided).

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 and plate adaptor capable of at least 2,000 x *g*
- Oven or incubator capable of 65°C
- Vortexer
- 100% ethanol
- Liquid nitrogen for freezing/disrupting samples (for fresh/frozen samples)
- Equipment for disruption of plant tissue

Before Starting:

- Prepare VHB Buffer and DNA Wash Buffer according to Preparing Reagents section on Page 5.
- Set a 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 up to 10 mg dry powdered tissue or 50 mg fresh (or frozen) tissue to a Lysis Plate (provided) and seal with a Silicone Mat.

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

3. Remove and discard the Silicone Mat.

4. Add 700 µL CSPL Buffer and 20 µL Proteinase K Solution to each sample. Seal the plate with a new Silicone Mat. 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 homogenized 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 2,000-6,000 x *g* for 10 minutes.

7. Remove and discard the Silicone Mat.

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

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

10. Centrifuge at 2,000-6,000 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 Binding Buffer to each sample. Vortex or pipet up and down to mix thoroughly.

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.

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 sample.

    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 sample.

    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 sample.

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. Select one of the following ethanol removal steps:

    A. Centrifuge at **4,000-6,000 x *g*** for 15 minutes to dry plate. Continue to Step 32.

    OR

    B. Dry the plate in an oven or incubator set to 65°C for 30 minutes. Let the plate cool for 5 minutes at room temperature after incubation. Continue to Step 32.

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 150 µL Elution Buffer heated to 65°C to each sample.

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.

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

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 the 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 step, ensure that the plate is completely dried before elution.
No Elution in Wells A1 or H12	Centrifuge Ramp Speed	Decrease the ramp speed of the centrifuge.

Ordering Information

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

Product	Part Number
CSPL Buffer, 1000 mL	CSPL-1000
Elution Buffer, 100 mL	PDR048
96-well Square-well Plate (2.2 mL), 25 plates	EZ9602
E-Z 96 DNA Plates, 10 plates	BD96-01
E-Z 96 Homogenizer Plates, 4 plates	HCR9601-02
Vacuum Manifold	VAC-03

Notices & Disclaimers

For European Union Use.

RBB Buffer contains Triton X-100, 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol (CAS 9002-93-1), a substance included in the European Authorisation list (Annex XIV) of REACH Regulation (EC) No 1907/2006. Substances and mixtures used for the purpose of Scientific Research and Development (SR&D) are exempt from authorization requirements if used below 1 tonne per year in volume.

Scientific Research and Development includes experimental research or analytical activities at a laboratory scale such as synthesis and testing of applications of chemicals, release tests, etc. as well as the use of the substance in monitoring and routine quality control or in vitro diagnostics.

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

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