omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E-Z 96 Cycle-Pure Kit

D1043-01	1 x 96 preps
D1043-02	5 x 96 preps

Manual Date: July 2019
Revision Number: v6.0

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 Cycle-Pure Kit

Table of Contents

Manual Date: July 2019
Revision Number: v6.0

omega BIO-TEK
innovations in nucleic acid isolation

Introduction

The E.Z.N.A.® and E-Z 96 family of products are innovative systems that radically simplify extraction and purification of nucleic acids from a variety of sources. Key to these systems is the HiBind® matrix that specifically, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

The E-Z 96 Cycle-Pure Kit is a convenient system for fast and reliable purification of up to 96 PCR products. The method uses HiBind® technology to recover DNA fragments from 100 bp to 10 kb, free of oligonucleotides, nucleotides, and polymerase in yields exceeding 80%. Binding conditions are adjusted by the addition of a specially formulated buffer, and the sample is transferred to an E-Z 96 DNA Plate. Following two rapid wash steps, DNA is eluted with deionized water (or low salt buffer) and is ready for other applications. The product is suitable for T-A ligations, PCR sequencing, restriction digestion, or various labeling reactions. In addition, the kit can be used to purify DNA from any other enzymatic reaction.

Please read this booklet thoroughly to ensure that you are familiar with the entire procedure. The E-Z 96 Cycle-Pure Kit is designed to be simple, fast, and reliable provided that all steps are followed diligently.

New in this Edition:

Protocol for fragments less than 200 bp has been updated.

Determine the absorbance of an appropriate dilution (20- to 50- fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

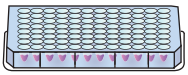
DNA concentration = Absorbance 260 × 50 × (Dilution Factor) µg/mL

A value greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations.

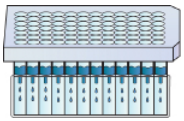
Binding Capacity

Each well of the E-Z 96 DNA Plate can bind ~12 µg DNA.

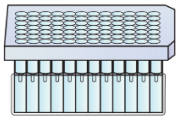
Centrifugation Protocol



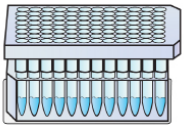
Add CP Buffer and Mix



Bind and Wash 2x

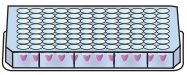


Dry Membrane

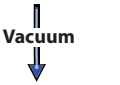


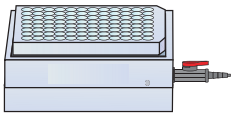
Elute

Vacuum Protocol

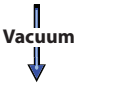


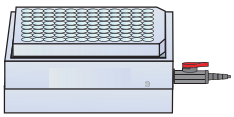
Add CP Buffer and Mix



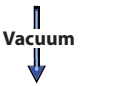


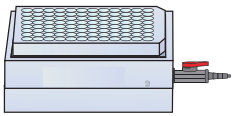
Bind and Wash 2x





Dry Membrane





Elute

Kit Contents

Product Number	D1043-01	D1043-02
Preparations	1 x 96	5 x 96
E-Z 96 DNA Plates	1	5
96-well Microplate (500 µL)	1	5
96-well Square-well Plates (2.2 mL)*	1	2
CP Buffer	60 mL	2 x 160 mL
DNA Wash Buffer	50 mL	200 mL
Elution Buffer	30 mL	60 mL
User Manual	✓	✓

* 96-well Square-well Plates are reusable, see Page 6 for cleaning instructions.

Storage and Stability

All of the E-Z 96 Cycle-Pure Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in CP Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

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

Kit	100% Ethanol to be Added
D1043-01	200 mL
D1043-02	800 mL

Cleaning of 96-well Square-well Plates

The 96-well Square-well Plates supplied with this kit are reusable. To avoid cross-contamination, rinse the plates thoroughly with tap water after each use. Soak the plates in 0.5M HCl for 5 minutes then wash thoroughly with distilled water. The 96-well Square-well Plates also can be autoclaved following washing.

Guidelines for Vacuum Manifold

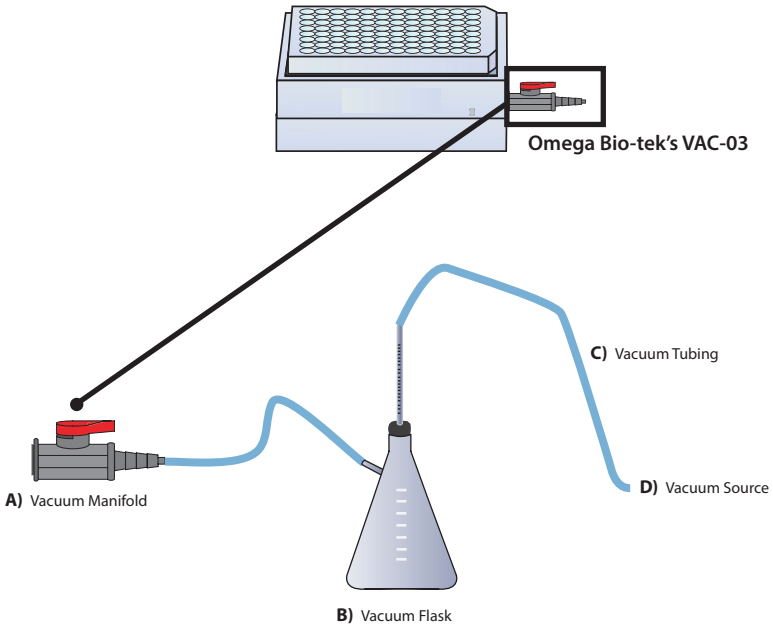
The following is required for use with the Vacuum Protocol:

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

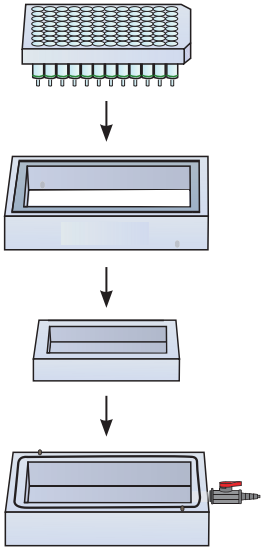
Conversion from millibars:	Multiply by:
Millimeters of Mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of Mercury (inchHg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Illustrated Vacuum Setup



Guidelines for Vacuum Manifold

DNA Bind & Wash Setup



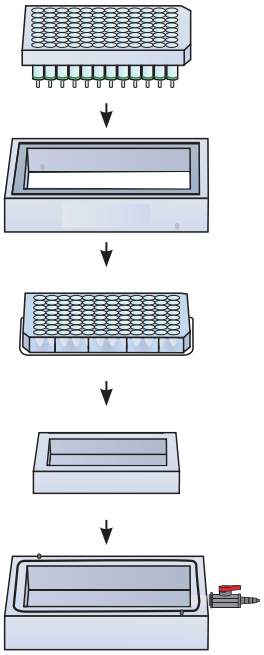
E-Z 96 DNA Plate

Vacuum Manifold Collar

Waste Collection/96-well Square-well Plate

Vacuum Manifold Base

Standard Elution Setup



E-Z 96 DNA Plate

Vacuum Manifold Collar

96-well Microplate (500 µL)

Waste Collection

Vacuum Manifold Base

E-Z 96 Cycle-Pure Kit Protocol - Vacuum Protocol

All steps must be performed at room temperature.

Materials and Equipment to be Supplied by User:

- Vacuum manifold which can fit the 96-well Square-well Plates (Recommend Omega Cat# VAC-03)
- 100% ethanol
- Nuclease-free 96-well deep-well plates
- Sealing film
- For fragments <200 bp, 100% isopropanol

Before Starting:

- Prepare the DNA Wash Buffer according to the instructions in the Preparing Reagents section on Page 6.

1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product.

2. Determine the volume of each PCR reaction.

3. Transfer each sample to nuclease-free 96-well deep-well plates.

4. Add 3-5 volumes CP Buffer to 1 volume PCR sample. Vortex to mix thoroughly.

   Note: For PCR products <200 bp, add 5 volumes CP Buffer and 0.4 volumes 100% isopropanol. Volume refers to the size of your PCR reaction. For example, if your PCR reaction is 100 µL and the PCR product is smaller than 200 bp, you would use 500 µL CP Buffer and 40 µL 100% isopropanol.

5. Prepare the vacuum manifold according to manufacturer's instructions. For Omega's VAC-03 manifold, set up the manifold as follows (refer to Page 8 for illustrations):
   a. Place a 96-well Square-well Plate inside the Vacuum Manifold Base.
   b. Place the Vacuum Manifold Collar squarely over the base.
   c. Place the E-Z 96 DNA Plate over the Vacuum Manifold Collar.
   d. Transfer the samples to the E-Z 96 DNA Plate.
   e. Seal the unused wells with sealing film.

6. Switch on vacuum source to draw the sample through the plate.

7. Turn off the vacuum.

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

9. Add 800 µL DNA Wash Buffer to each sample.

   Note: DNA Wash Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 6 for instructions.

10. Switch on vacuum source to draw the buffer through the plate.

11. Repeat Steps 7-10 for a second DNA Wash Buffer wash step.

12. Continue to apply the vacuum for 10 minutes to dry the HiBind® matrix.

13. Turn off the vacuum.

14. Remove the E-Z 96 DNA Plate from the manifold and gently tap the plate on a stack of absorbent paper towels until no liquid spots are visible on the towels.

    Note: This step will ensure the removal of residual DNA Wash Buffer from the tips of the E-Z 96 DNA Plate. This step is critical for removal of trace ethanol that may interfere with downstream applications.

15. Assemble the manifold as follows (refer to Page 8 for illustrations):
    a. Place the Waste Collection container into the Vacuum Manifold Base.
    b. Place a 96-well Microplate (provided) on top of the Waste Collection container.
    c. Place the Vacuum Manifold Collar squarely over the base.
    d. Place the E-Z 96 DNA Plate over the Vacuum Manifold Collar.

16. Add 80-100 µL Elution Buffer directly onto the center of the HiBind® matrix in each well of the E-Z 96 DNA Plate.

17. Switch on vacuum source for 5 minutes.

18. Store DNA at -20°C.

    Note: This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

E-Z 96 Cycle-Pure Kit Protocol - Centrifugation Protocol

All steps must be performed at room temperature.

Materials and Equipment to be Supplied by User:

- Centrifuge with swing bucket rotor capable of 4,000 x *g*
- 100% ethanol
- Nuclease-free 96-well deep-well plates
- Sealing film
- For fragments <200 bp, 100% isopropanol

Before Starting:

- Prepare the DNA Wash Buffer according to the instructions in the Preparing Reagents section on Page 6.

1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product.

2. Determine the volume of each PCR reaction.

3. Transfer each sample to nuclease-free 96-well deep-well plate.

4. Add 3-5 volumes CP Buffer to 1 volume PCR sample. Vortex to mix thoroughly.

   Note: For PCR products <200 bp, add 5 volumes CP Buffer and 0.4 volumes 100% isopropanol. Volume refers to the size of your PCR reaction. For example, if your PCR reaction is 100 µL and the PCR product is smaller than 200 bp, you would use 500 µL CP Buffer and 40 µL 100% isopropanol.

5. Place the E-Z 96 DNA Plate on top of the 96-well Square-well Plate (provided).

6. Transfer the samples to the E-Z 96 DNA Plate.

7. Centrifuge at 3,000-4,000 x *g* for 5 minutes.

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

9. Add 800 µL DNA Wash Buffer to each sample.

   Note: DNA Wash Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 6 for instructions.

10. Centrifuge at 3,000-4,000 x *g* for 5 minutes.

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

12. Repeat Steps 9-11 for a second DNA Wash Buffer wash step.

13. Centrifuge the empty E-Z 96 DNA Plate at 4,000 x *g* for 10 minutes to dry the HiBind® matrix.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

14. Place the E-Z 96 DNA Plate on top of a 96-well Microplate (provided).

15. Add 80-100 µL Elution Buffer directly onto the center of the HiBind® matrix in each well of the E-Z 96 DNA Plate.

16. Centrifuge at 4,000 x *g* for 5 minutes.

17. Store DNA at -20°C.

    Note: This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

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.

Problem	Cause	Solution
Low DNA yields	Too little CP Buffer added to sample.	Add more CP Buffer as indicated. For DNA fragments <200 bp in size, add up to 6X CP Buffer. For DNA fragments > 4 kb, add 3 volumes CP Buffer followed by 1 volume distilled water.
	pH of water for elution is <7.5	Check the pH of the water, adjust the pH of the water to 8.0 using Tris-HCl (2M, pH 8.5).
Problem	**Cause**	**Solution**
Optical densities do not agree with DNA yield on agarose gel	Trace contaminants eluted from column increase A_{260}	Make sure to wash plate as instructed in Steps 9-11 of either protocol, rely on agarose gel/ethidium bromide electrophoresis for quantification
Problem	**Cause**	**Solution**
DNA sample floats out of well while loading agarose gel	Ethanol not completely removed from plate	Centrifuge as instructed in Step 13 of the centrifugation protocol and Step 12 of the vacuum protocol to completely dry the HiBind® matrix.

Ordering Information

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

Product	Part Number
Vacuum Manifold	VAC-03
CP Buffer (200 mL)	PDR042
Elution Buffer (100 mL)	PDR048
DNA Wash Buffer (40 mL)	PDR044
E-Z 96 DNA Plates (10 plates)	BD96-01

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