omega BIO-TEK

innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Cycle Pure Kit

D6492-00	5 preps	V-spin
D6492-01	100 preps	V-spin
D6492-02	200 preps	V-spin

Manual Date: August 2019
Manual Revision: v5.0

For Research Use Only

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E.Z.N.A.® Cycle Pure Kit

Table of Contents

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Introduction

The E.Z.N.A.® family of products is an innovative system that radically simplifies the extraction and purification of nucleic acids from a variety of sources. The key to this system is the HiBind® matrix that specifically, but reversibly, binds DNA or RNA under optimized conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or a low salt buffer.

The E.Z.N.A.® Cycle Pure Kit is a convenient system for the fast and reliable purification of PCR products. The E.Z.N.A.® Cycle Pure Kit uses HiBind® technology to recover DNA bands from 100 bp to 10 kb free of oligonucleotides, nucleotides, and polymerase with yields exceeding 80%. The binding conditions of the HiBind® DNA Mini Columns are adjusted by the addition of a specially formulated buffer before adding the sample. Following a rapid wash step, DNA is eluted with deionized water or a low salt buffer. Purified DNA can be directly used for most downstream applications include T-A ligations, PCR sequencing, restriction enzyme digestion, or various labeling reactions.

Benefits of the E.Z.N.A.® Cycle Pure Kit

- **Fast -** DNA recovery from enzymatic reactions in less than 10 minutes
- **Reliability -** Optimized buffers that guarantee pure DNA
- **Safety -** No organic extractions
- **Quality -** Purified DNA is suitable for any application

Q-spin column vs. V-spin column

The E.Z.N.A.® Cycle-Pure Kit is available with two different types of columns. V-Spin columns have an attached cap, while Q-spin columns are capless. The columns are otherwise identical in use and application. Either column can be used with either the vacuum or centrifugation protocols. D6492 is the V-Spin version of the Cycle Pure Kit, while D6493 is the Q-Spin version.

Binding Capacity

Each HiBind® DNA Mini Column can bind ~20 µg DNA.

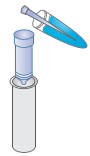
New in this Edition:

August 2019

· D6493 (Q-spin version) has been discontinued and is no longer available for purchase.

Centrifugation Protocol

Add CP Buffer



Transfer Lysate and Bind

Wash

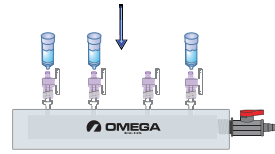
Dry

Elute

Vacuum Protocol

Add CP Buffer

Transfer Lysate and Bind



Wash

Dry

Elute

Kit Contents

Product Number	D6492-00	D6492-01	D6492-02
Preps	5	100	200
HiBind® DNA Mini Columns	5	100	200
2 mL Collection Tubes	5	100	200
CP Buffer	5 mL	80mL	150 mL
Elution Buffer	5 mL	10 mL	20 mL
DNA Wash Buffer	2 mL	2 x 20 mL	3 x 25mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® Cycle Pure Kit components are guaranteed for at least 24 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.

Preparing Reagents

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

Kit	100% Ethanol to be Added
D6492-00	8 mL
D6492-01	80 mL per bottle
D6492-02	100 mL per bottle

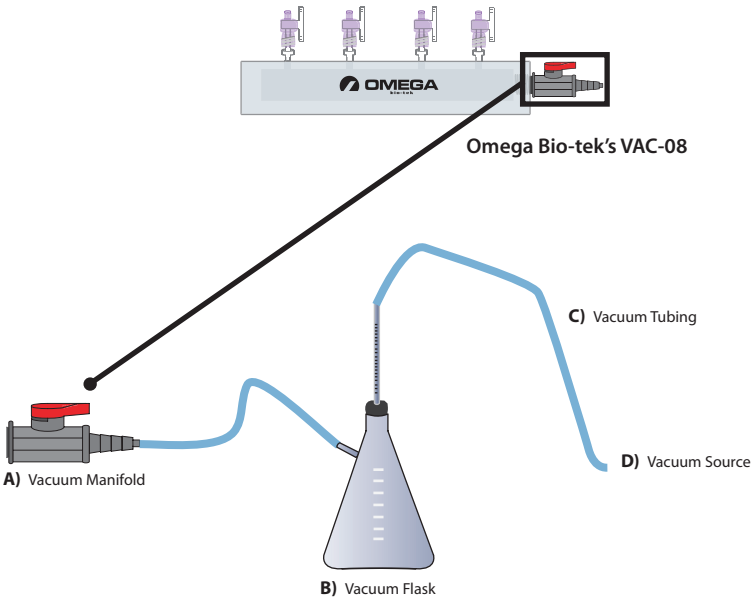
Guidelines for Vacuum Manifold

The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold
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)

Conversion from millibars:	Multiply by:
Millimeters of mercury (mm Hg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Illustrated Vacuum Setup:



E.Z.N.A.® Cycle Pure Kit - Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- Optional: Sterile deionized water or TE Buffer

Before Starting:

- Prepare DNA Wash Buffer according to the "Preparing Reagents" section on Page 4.

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

2. Determine the volume of your PCR reaction.

3. Transfer the sample into a clean 1.5 mL microcentrifuge tube.

4. Add 4-5 volumes CP Buffer. For PCR products smaller than 200 bp, add 6 volumes CP Buffer.

   Note: Volume refers to the size of your PCR reaction. For example, if your PCR reaction is 100 µL and is smaller than 200 bp, you would use 600 µL CP Buffer.

5. Vortex to mix thoroughly. Briefly centrifuge to collect any drops from the inside of the lid.

6. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube (provided).

7. Add the sample from Step 5 to the HiBind® DNA Mini Column.

8. Centrifuge at maximum speed (≥13,000 x *g*) for 1 minute at room temperature.

9. Discard the filtrate and reuse collection tube.

10. Add 700 µL DNA Wash Buffer.

11. Centrifuge at maximum speed for 1 minute.

    Note: DNA Wash Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 4 for instructions.

12. Discard the filtrate and reuse collection tube.

13. Repeat Steps 10-12 for a second DNA Wash Buffer wash step.

14. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column.

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

15. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not provided).

16. Add 30-50 µL Elution Buffer, TE Buffer, or sterile deionized water directly to the center of column matrix.

17. Let sit at room temperature for 2 minutes.

18. Centrifuge at maximum speed for 1 minute.

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

19. Store DNA at -20°C.

E.Z.N.A.® Cycle Pure Kit - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Vacuum Manifold
- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- Optional: Sterile deionized water or TE Buffer

Before Starting:

- Prepare DNA Wash Buffer according to the "Preparing Reagents" section on Page 4.

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

2. Determine the volume of your PCR reaction.

3. Transfer the sample into a clean 1.5 mL microcentrifuge tube.

4. Add 4-5 volumes CP Buffer. For PCR products smaller than 200 bp, add 6 volumes CP Buffer.

   Note: Volume refers to the size of your PCR reaction. For example, if your PCR reaction is 100 µL and is smaller than 200 bp, you would use 600 µL CP Buffer.

5. Vortex to mix thoroughly. Briefly centrifuge to collect any drops from the inside of the lid.

6. Prepare the vacuum manifold according to manufacturer's instructions and connect the HiBind® DNA Mini Column to the manifold.

7. Transfer the entire sample to the HiBind® DNA Mini Column.

8. Switch on vacuum source to draw the sample through the column.

9. Turn off the vacuum.

10. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 4 for instructions.

11. Switch on vacuum source to draw the DNA Wash Buffer through the column.

12. Turn off the vacuum.

13. Repeat Steps 10-12 for a second DNA Wash Buffer wash step.

14. Transfer the HiBind® DNA Mini Column into a 2 mL Collection Tube (provided).

15. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column.

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

16. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not provided).

17. Add 30-50 µL Elution Buffer, TE Buffer, or sterile deionized water directly to the center of column matrix.

18. Let sit at room temperature for 2 minutes.

19. Centrifuge at maximum speed for 1 minute.

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

20. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshot 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

Low DNA Yields	
Not enough CP Buffer added to sample	Add more CP Buffer as indicated. For DNA fragments <200 bp in size, add 6 volumes CP Buffer
Water pH is too low (< 7.5)	Check the pH of the water, adjust the pH of the water to 8.0 using Tris-HCl (2M, pH 8.5)
No DNA eluted	
DNA Wash Buffer was not diluted with 100% ethanol	Prepare DNA Wash Buffer as instructed on the bottle, or refer to page 3
Optical densities do not agree with DNA yield on agarose gel	
Trace contaminants eluted from column will increase A260	Make sure to wash column as instructed in Steps 10-13 of either protocol, rely on agarose gel/ethidium bromide electrophoresis for quantification
DNA sample floats out of well while loading agarose gel	
Ethanol not completely removed from column	Centrifuge as instructed in Step 14 of the centrifugation protocol and Step 15 of the vacuum protocol to completely dry the HiBind® matrix

Ordering Information

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

Product	Part Number
CP Buffer (200 mL)	PDR042
Elution Buffer (100 mL)	PDR048
DNA Wash Buffer (100 mL)	PS010
2 mL Collection Tubes	SS1-1370-00

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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