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	50 preps	V-spin
D6492-02	200 preps	V-spin
D6492-03	600 preps	V-spin

Manual Date: April 2022
Manual Revision: v5.1

For Research Use Only

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E.Z.N.A.® Cycle Pure Kit

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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

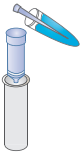
- **Fast -** DNA recovery from enzymatic reactions in less than 10 minutes
- **Reliability -** Optimized buffers that guarantee pure DNA
- **Safety -** No organic extractions
- **Quality -** Purified DNA is suitable for any application

New in this Edition:

April 2022:
- A new 600 prep kit has been added and is now available for purchase.

Centrifugation Protocol

Add CP Buffer



Transfer Lysate and Bind

Wash

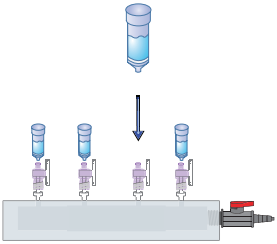
Dry

Elute

Vacuum Protocol

Add CP Buffer

Transfer Lysate and Bind



Wash

Dry

Elute

Kit Contents

Product Number	D6492-00	D6492-01	D6492-02	D6492-03
Preparations	5	50	200	600
HiBind® DNA Mini Columns	5	50	200	600
2 mL Collection Tubes	5	50	200	600
CP Buffer	5 mL	40 mL	150 mL	3 x 120 mL
Elution Buffer	15 mL	30 mL	30 mL	35 mL
DNA Wash Buffer	2.5 mL	25 mL	3 x 25 mL	200 mL
User Manual	✓	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® 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.

Preparing Reagents

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

Kit	100% Ethanol to be Added
D6492-00	10 mL
D6492-01	100 mL
D6492-02	100 mL per bottle
D6492-03	800 mL

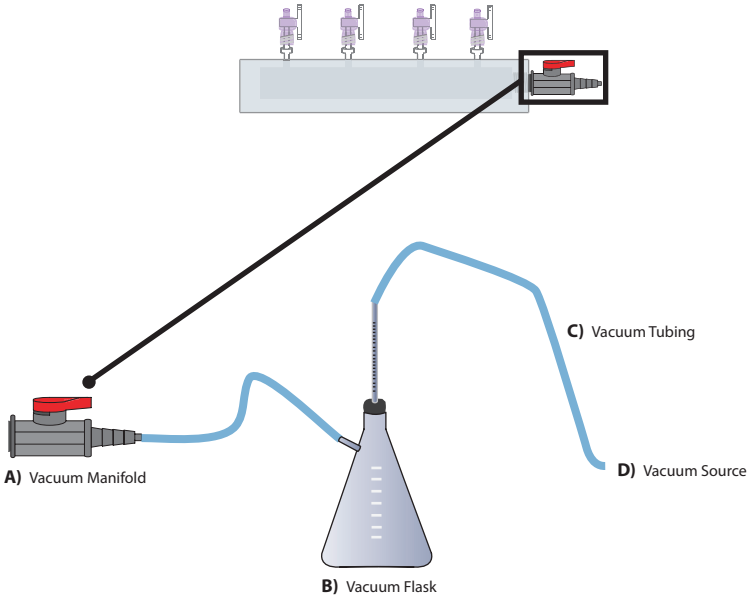
Guidelines for Vacuum Manifold

The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold
Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, 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 (mmHg)	0.75
kilopascals (kPa)	0.1
inches of mercury (inHg)	0.0295
Torrs (Torr)	0.75
atmospheres (atm)	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*g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- Optional: Sterile deionized water or TE Buffer
- For fragments <200 bp, 100% isopropanol

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 (not provided).

4. Add 4-5 volumes CP Buffer. For PCR products smaller than 200 bp, add 5 volumes CP Buffer and 0.4 volumes 100% isopropanol.

   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 500 µL CP Buffer and 40 µL isopropanol.

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.

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

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

9. Discard the filtrate and reuse collection tube.

10. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see the Preparing Reagents section on Page 4 for instructions.

11. Centrifuge at maximum speed for 1 minute.

12. Discard the filtrate and reuse collection tube.

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

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*g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- Optional: Sterile deionized water or TE Buffer
- For fragments <200 bp, 100% isopropanol

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 (not provided).

4. Add 4-5 volumes CP Buffer. For PCR products smaller than 200 bp, add 5 volumes CP Buffer and 0.4 volumes 100% isopropanol.

   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 500 µL CP Buffer and 40 µL isopropanol.

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 100% 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 step.

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

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.

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 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

Low DNA Yields	
Not enough CP Buffer added to sample	Add more CP Buffer as indicated. For DNA fragments <200 bp in size, add 5 volumes CP Buffer and 0.4 volumes isopropanol.
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 4.
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.

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

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innovations in nucleic acid isolation

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