E.Z.N.A.® Cycle-Pure Kit
Table of Contents

Manual Revision: October 2009

OMEGA bio-tek

Innovations in nucleic acid isolation

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 new 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 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 DNA technology to recover DNA bands from 100bp to 10kb free of oligonucleotides, nucleotides, and polymerase in yields exceeding 80%. The binding conditions of the HiBind DNA 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 is suitable for any downstream applications. No organic extractions or alcohol precipitations signifies a safe and rapid processing of multiple samples in parallel. 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 will be suitable for any application.

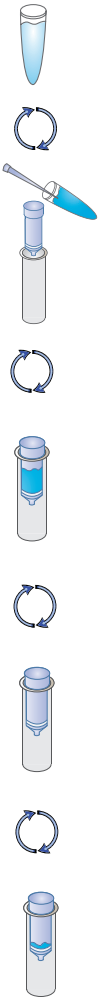
Q-spin column vs. V-spin column

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. The D6492 is the V-Spin version of the Cycle Pure Kit, while the D6493 is the Q-Spin version.

Binding Capacity

Each HiBind DNA Mini column can bind ~30 µg of DNA.

Spin Protocol



Add CP Buffer

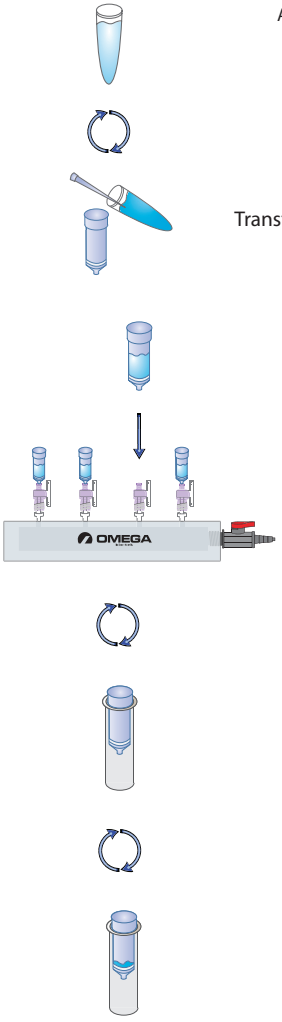
Transfer Lysate & Bind

Wash

Dry

Elute

Vacuum/Spin Protocol



Add CP Buffer

Transfer Lysate & Bind

Wash

Dry

Elute

Kit Contents

Cycle Pure Kit	D6492-00 D6493-00	D6492-01 D6493-01	D6492-02 D6493-02
Preps	5	50	200
HiBind DNA Mini Columns	5	50	200
2 mL Collection Tubes	5	50	200
Buffer CP	5 mL	40 mL	120 mL
Elution Buffer	5 mL	10 mL	20 mL
DNA Wash Buffer	1.5 mL	15 mL	3 x 25 mL
Instruction Booklet	1	1	1

Storage and Stability

All E.Z.N.A.® Cycle-Pure Kit components are guaranteed for at least 12 months from the date of purchase when stored at 22-25°C. If any precipitates form in buffers, warm at 37°C to dissolve.

Preparing Reagents

• Dilute DNA Wash Buffer with absolute ethanol (96-100%) as follows:

Kit	Ethanol Added
D6492-00 D6493-00	Add 6 mL absolute ethanol to each bottle
D6492-01 D6493-01	Add 60 mL absolute ethanol to each bottle
D6492-02 D6493-02	Add 100 mL absolute ethanol to each bottle

Store diluted DNA Wash Buffer at room temperature.

Guideline for Vacuum Manifold

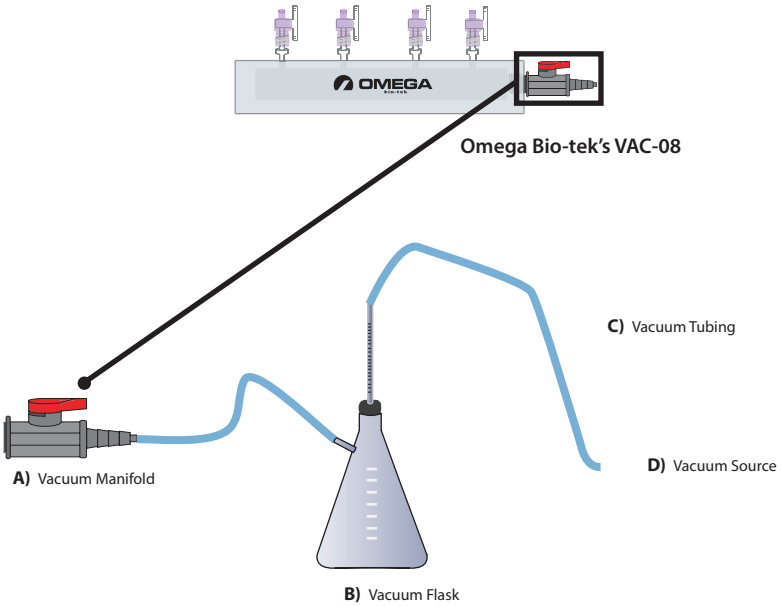
The following is required for use with the Vacuum/Spin Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)
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-**08**	**-200** to **-600**

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 Spin Protocol

User Supplied Equipment:

- **Microcentrifuge capable of at least 13,000 x g**
- **Nuclease-free 1.5 microcentrifuge tubes**
- **Absolute ethanol (~ 96-100%)**
- **Optional: Sterile deionized water (or TE Buffer)**

Things to do before starting:

- **Prepare DNA Wash Buffer according to directions on page 4**

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

2. Determine the volume of the PCR reaction. **Transfer the sample into a clean 1.5ml microcentrifuge tube and add 4-5 volumes of Buffer CP.**
   For PCR products smaller than 200bp, add 6 volumes of Buffer CP.

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

3. Vortex thoroughly to mix. Briefly spin the tube to collect any drops from the inside of the lid.

4. Place a **HiBind DNA Mini Column** into a provided 2 ml collection tube.

5. **Add the mixed sample from step 3** to the HiBind DNA Mini Column and centrifuge at 13,000 x g for 1 minute at room temperature. Discard the flow-through liquid and place the HiBind DNA Mini Column back into the same collection tube.

6. **Add 700µl of DNA Wash Buffer** and centrifuge at 13,000 x g for 1 minute. Discard the flow-through liquid and place the HiBind DNA Mini Column back into the same collection tube.

   IMPORTANT: DNA Wash Buffer must be diluted with absolute ethanol before use. Refer to label for instructions. If refrigerated, DNA Wash Buffer must be brought to room temperature before use.

7. **Add 500µl of DNA Wash Buffer** and centrifuge at 13,000 x g for 1 minute. Discard the flow-through liquid and place the HiBind DNA Mini Column back into the same collection tube.

8. Centrifuge the empty HiBind DNA Mini column for 2 min at maximal speed (≥13,000 x g) to dry the column matrix.

   Note: Do not skip this step, it is critical for the removal of ethanol from the HiBind DNA column.

9. Place the HiBind DNA Mini column into a clean 1.5ml microcentrifuge tube. Depending on the desire concentration of the final product, **add 30-50 µl of Elution Buffer** (10mM Tris, pH8.5) or water directly onto the center of column matrix.
   Let it sit at room temperature for 2 minutes. Centrifuge for 1 min at 13,000 x g to elute the DNA.

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

E.Z.N.A.® Cycle Pure Spin/Vacuum Protocol

User Supplied Equipment:

- **Microcentrifuge capable of at least 13,000 x g**
- **Nuclease-free 1.5 microcentrifuge tubes**
- **Optional: Sterile deionized water (or TE Buffer)**
- **Absolute ethanol (~ 96-100%)**
- **Vacuum Manifold (see page 5)**

Things to do before starting:

- **Prepare DNA Wash Buffer according to directions on page 4**
- **Assemble/Prepare Vacuum Manifold (See page 5)**

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

2. Determine the volume of the PCR reaction. Transfer the sample into a clean 1.5ml microcentrifuge tube and **add 4-5 volumes of Buffer CP.**
   For PCR products smaller than 200bp, add 6 volumes of Buffer CP.

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

3. Vortex thoroughly to mix. Briefly spin the tube to collect any drops from the inside of the lid.

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

5. Place a HiBind DNA Mini column into the leur of the vacuum. Turn on vacuum. **Add the mixed sample from step 3** to the HiBind DNA Mini column by decanting or pipetting. After the sample has passed through the column, switch off the vacuum source.

6. Add **700µl of DNA Wash Buffer** and turn on the vacuum source. After the buffer has passed through the column, switch off the vacuum source.

   IMPORTANT: DNA Wash Buffer must be diluted with absolute ethanol before use. Refer to label for instructions.

7. Add **700µl of DNA Wash Buffer** and turn on the vacuum source. After the buffer has passed through the column, switch off the vacuum source.

8. Place the HiBind DNA Mini column into a 2ml collection tube (provided) and centrifuge for 2 min at maximal speed (≥13,000 x g) to dry the column matrix.

   Important*: *Do not skip this step, it is critical for the removal of ethanol from the HiBind DNA column.

9. Place the HiBind DNA Mini column into a clean 1.5ml microcentrifuge tube. Depending on the desire concentration of the final product, **add 30-50 µl of Elution Buffer** (10mM Tris, pH8.5) or water directly onto the center of column matrix. Let it sit at room temperature for 2 minutes. Centrifuge for 1 min at 13,000 x g to elute the DNA.

   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 solve any problems that may arise. We hope that it will aid in clearing up any questions for you. If for any reason you need further assistance, please contact our technical support staff at our **Toll Free Number (800-832-8896).**

Possible Problems and Suggestions

Low DNA Yields	
Not enough Buffer CP added to sample	Add more Buffer CP as indicated. For DNA fragments < 200bp in size, add up to 6 x volumes of Buffer CP.
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 has not been diluted with absolute ethanol (96-100%)	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 increase A260.	Make sure to wash column as instructed in steps 8 and 9 of either protocol, rely on agarose gel/ethidium bromide electrophoresis for quantization.
DNA sample floats out of well while loading agarose gel.	
Ethanol not completely removed from column.	Centrifuge as instructed in step 10 of the spin protocol and step 8 of the vacuum /spin protocol.

Ordering Information

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

Buffer (Size)	Part Number
Buffer CP (200 mL)	PDR042
Elution Buffer (100 mL)	PDR048
DNA Wash Buffer (100ml)	PS010
2ml capless collection tubes	SS1-1370-00
1.5ml DNase/RNase Free Centrifuge Tubes	SS1-1210-0

Notes: