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## Introduction

The E.Z.N.A.<sup>®</sup> family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the system is the new HiBind<sup>®</sup> 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 de-ionized water or low salt buffer.

The Cycle-Pure Kit is a convenient system for the rapid and reliable purification of PCR products. The method uses HiBind<sup>®</sup> technology to recover DNA bands ranging in size from 50 bp-40 kb free of oligonucleotides, nucleotides, and polymerase with recoveries exceeding 80% of original volume. Binding conditions are optimized by the addition of a specially formulated buffers, and the sample is subsequently applied to the HiBind<sup>®</sup> DNA spin-column and removed from solution via a brief centrifugation. Following a rapid wash step, purified DNA is eluted with de-ionized water (or low salt buffer) and ready for downstream applications. No organic extractions or alcohol precipitations means safe and rapid processing of multiple samples in parallel. Purified DNA is suitable for T-A ligations, PCR sequencing, restriction digestion, or various labeling reactions. In addition to purifying PCR reactions this kit can be used to purify DNA from any other enzymatic reactions.

## Benefits

The E.Z.N.A.<sup>®</sup> Cycle-Pure Kit means:

- Speed - DNA recovery from enzymatic reactions <15 min
- Reliability - Optimized buffers guarantee pure DNA
- Safety - No organic extractions
- Quality - Purified DNA suitable for any application

## New in this edition

- Newly introduced V-Spin column (Catalog Number D6492) features an attached cap and a standard outlet luer. The attached cap ensures the elimination of potential contamination.
- Optional vacuum/spin protocol is available for V-Spin column.

## Binding Capacity

Each HiBind<sup>®</sup> DNA column can bind ~30 µg DNA.

## Kit Contents

Product Number	D6494-01 D6493-01 D6492-01	D6494-02 D6493-02 D6492-02
Preps	50	200
HiBind® DNA Columns	50	200
2 ml Collection Tubes	50	200
XP1 Buffer (New CP)	20 ml	80 ml
SPW Buffer	20 ml	3 x 20 ml
Instruction Booklet	1	1

**Storage and Stability:** All E.Z.N.A.® Cycle-Pure Kit components are guaranteed for 24 months from the date of purchase when stored at 22-25°C. Under cool ambient conditions crystals may form in Buffer XP1. Simply incubate at 37°C to re-solubilize.

### Materials Supplied By User:

- Microcentrifuge capable of at least 10,000 x g.
- Nuclease-free 1.5 ml centrifuge tubes.
- Sterile de-ionized water (or TE buffer)
- Absolute (or 95%) ethanol
- Protective eye-wear

<b>IMPORTANT</b>	SPW Buffer Concentrate must be diluted with absolute ethanol as follows:	
	D6492, D6493, D6494-01/02	Add 80 ml ethanol to each bottle

## Cycle-Pure Protocol

Please read this protocol thoroughly prior to using this product to ensure that you are familiar with the entire procedure. E.Z.N.A.® Kits are designed to be simple, fast, and reliable provided that all steps are followed diligently. All centrifugation steps must be performed at room temperature unless otherwise specified.

- 1. Perform agarose gel/ethidium bromide electrophoresis to determine the size and concentration of your PCR product.**
  - 2. Determine the volume of the PCR reaction, transfer to a clean 1.5 ml microfuge tube, and add equal volumes of Buffer XP1.** For PCR products <200 bp add 2 volumes of Buffer XP1. Vortex thoroughly to mix
  - (For Vacuum Protocol see step 3 page 5).* **Apply the sample to a HiBind® DNA spin-column assembled in a clean 2 ml collection tube (provided) and centrifuge in a microcentrifuge at 10,000 x g for 1 min at room temperature.** Discard the liquid.
  - Wash the column by adding 700 µl of SPW Buffer diluted with absolute ethanol. Centrifuge at 10,000 x g for 1 min at room temperature.**
- Note:** SPW Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions.
- 5. Discard liquid and repeat Step 5 with another 700 µl SPW Buffer.**
  - 6. Discard liquid and centrifuge the empty column for 1 min 10,000 x g to dry the column matrix. This is critical for good DNA yields.**
  - 7. Place column into a clean 1.5 ml microcentrifuge tube. Add 30-50 µl (depending on desired concentration of final product) sterile deionized water (or TE buffer) directly onto the column matrix and centrifuge 1 min at 10,000 x g to elute DNA.** This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

- 8. Yield and quality of DNA:** DNA Yield and quality can be determined by measuring the absorbance of the sample under UV light at 260nm and 280 nm. To determine the DNA concentration use the following calculation:

$$\text{DNA concentration} = \text{Absorbance}_{260} \times 50 \times (\text{Dilution Factor}) \mu\text{g/ml}$$

To determine the quality of your sample, determine the ratio of absorbance at 260 and 280nm ( $\text{absorbance}_{260}/\text{absorbance}_{280}$ ). The ratio of is an indication of the samples purity, a value greater than 1.8 indicates > 90% nucleic acid. Alternatively, yield and quality can also be determined by agarose gel/ethidium bromide electrophoresis.

**Vacuum/Spin Protocol for Cycle-Pure Kit (V-Spin column only)**

Note: Please read through previous section of this book before using this protocol.

3. Prepare the vacuum manifold according to manufacturer's instructions and insert the V-Spin column in to the manifold.
4. Load the PCR Reaction/XP1 solution from Step 2 to the column.
5. Apply vacuum to draw the sample through the column. Ensure that vacuum is turned off immediately as sample has passed through as to not over dry the column.
6. Add 700 µl DNA SPW buffer to the column and draw it through the column by turning on the vacuum source. Repeat this step with an additional 700 µl DNA SPW buffer.
7. Transfer the column to a 2 ml collection tube and centrifuge for 1 minute to dry the column.
8. Place the column in a clean 1.5 ml microcentrifuge tube and add 30-50µl Elution Buffer or Dnase free de-ionized water. Incubate for 1 minutes and centrifuge at 10 000 x g for 1 minute to elute DNA.

**Troubleshooting Guide**

Problem	Likely Cause	Suggestions
Low DNA yields	Too little Buffer XP1 added to sample.	Add more Buffer XP1 as indicated. For DNA fragments <200 bp in size, add up to 3 vol Buffer XP1. For DNA fragments > 4 kb, add 3 volumes of Buffer XP1 followed by 1 volume distilled water.
No DNA eluted.	S P W B u f f e r Concentrate not diluted with absolute ethanol.	Prepare SPW Buffer Concentrate as instructed above.
Optical densities do not agree with DNA yield on agarose gel.	Trace contaminants eluted from column increase $A_{260}$ .	Make sure to wash column as instructed in Steps 4 and 5. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantitation.
DNA sample floats out of well while loading agarose gel	Ethanol not completely removed from column following wash steps.	Centrifuge column as instructed in Step 6 to dry before proceeding to elution step.

## Short Protocol For Experienced Users

1. Determine volume of reaction. Add equal volumes of Buffer XP1 to PCR reaction.
2. Apply solution to HiBind® DNA column assembled in 2ml collection tube.
3. Centrifuge at maximum speed 1 min at room temperature. Discard liquid.
4. Wash column twice with 750 µl SPW Buffer diluted with ethanol.
5. Centrifuge empty column 1 min at max speed to dry.
6. Place column into clean 1.5 ml tube and elute DNA with 30-50 µl sterile water or Elution Buffer. Centrifuge 1 min.

## Ordering Information

Product No.	Product Name	Description
D6493-01/02 D6492-01/02	Cycle-Pure Kit	PCR product purification, Q-Column format & V-column format.
D1043-01/02	E-Z 96 Cycle-Pure Kit	96 well format PCR purification
D6537-01/02 D6538-01/02	DNA Probe Purification Kit	DNA probe purification, Q-column & V-column format
D2561-01/02	Poly-Gel DNA Purification Kit	Isolate DNA from polyacrylamide gel
D2501-01/02 D2500-01/02	Gel Extraction Kit	Agarose gel extraction using spin column technology
D2510-01/02	Ultra-Sep Gel Purification Kit	Agarose gel extraction using silica beads.
R6376-01/02	Poly-Gel RNA Purification Kit	Isolate RNA from polyacrylamide gel
R6537-01/02 R6538-01/02	RNA Probe Purification Kit	RNA probe purification, Q-column & V-column format

\* All OBI products available with size of 50 preps and 200 preps. Product number end with "-01" represent 50 preps kit and "-02" represent 200 preps kit.