Contents

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%. Binding conditions are adjusted by the addition of a specially formulated buffer, and the sample is applied to a HiBind® DNA column. 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

- **Fast-**DNA recovery from enzymatic reactions in less than 10 min
- **Reliability-**Optimized buffers that guarantee pure DNA every time
- **Safety**-No organic extractions
- **Quality**-Purified DNA is suitable for most applications

Binding Capacity

Each HiBind® DNA column can bind ~30µg DNA.

Kit Contents

Product Number	D6492-00	D6492-01	D649 2-02
Purification Times	5 preps	5 0 preps	200 preps
HiBind® DNA 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

* Elution Buffer = 10mM Tris-HCl, pH 8.5
* Buffer CP contains chaotropic salts which are irritants. The Equilibration Buffer contains Sodium Hydroxide. Take appropriate laboratory safety measures and wear gloves when handling.
*The volume of the DNA Wash Buffer has been changed, see bottle label for dilution instruction.

Storage and Stability

All E.Z.N.A.™ Cycle-Pure Kit components are guaranteed for at least 24 months from the date of purchase when stored at 22-25°C. Under cool ambient conditions crystals may form in Buffer CP. Simply warm to 37°C to dissolve.

Before Starting

Please read the entire booklet to become familiar with the E.Z.N.A.™ Cycle Pure DNA Kit procedure

- DNA **Wash Buffer** must be diluted with **absolute ethanol** (96-100%) as follows and stored at room temperature.

D6492/D6493-00	Add 6ml of absolute ethanol to bottle
D6492/D6493-01	Add 60ml of absolute ethanol to bottle
D6492/D6493-02	Add 100ml of absolute ethanol to each bottle

E.Z.N.A.™ Cycle-Pure Kit Spin Protocol

It is strongly advised that you familiarize yourself with the entire procedure before beginning this protocol. Omega Bio-Tek, Inc.'s E.Z.N.A. ™ Cycle-Pure Kit is designed to be simple, fast, and reliable provided that all steps are followed diligently.

Materials Supplied by User

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

*All centrifugation steps must be performed at room temperature
*DNA Wash Buffer must be diluted with absolute ethanol prior to use (see page 3, or label instructions)

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 < 200bp add 6 volumes 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 column in a provided 2ml collection tube. Add 100µl Equilibration Buffer to the column. Incubate at room temperature for 4 minutes. Spin at maximum speed for 20 seconds.**

5. **Apply the sample to the HiBind® DNA column and centrifuge at 10,000x g for 1 min at room temperature. Discard the flow-through.**

6. **Wash the HiBind® DNA column by adding 700µl of DNA Wash Buffer diluted with absolute ethanol and centrifuge as above.**
   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. **Discard liquid and repeat Step 7 using 500µl of DNA Wash Buffer.**

8. **Discard liquid and centrifuge the empty HiBind® DNA column for 2 min at maxi speed (≥13,000x g) to dry the column matrix**. This is critical for good yields.

9. **Place HiBind® DNA column into a clean 1.5ml microcentrifuge tube. Add 30-50µl** (depending on desired concentration of final product) **of Elution Buffer (10mM Tris,**

pH8.5) or water directly onto the column matrix and centrifuge for 1 min at ≥13,000x 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.

10. **Yield and quality of DNA: Determine the absorbance of an appropriate dilution of the sample at 260nm and then at 280nm.** The DNA concentration is calculated as follows:

    DNA concentration = A_{260} x 50 x (Dilution Factor) µg/ml

    Fragments greater than 500bp in length can routinely be purified at > 80% yield. Bands ranging from 100bp to 500bp gives yields of 60%- 90%. The ratio of (A_{260}) / (A_{280}) is an indication of nucleic acid purity. Alternatively, yield (as well as quality) can sometimes be best determined by agarose gel/ethidium bromide electrophoresis.

E.Z.N.A.™ Cycle-Pure Vacuum/Spin Protocol

1. **Prepare the sample by following steps 1-3 of the spin protocol on page 4.**

2. **Prepare the vacuum manifold according to manufacturer's instructions.**

3. **Load the PCR reaction/CP solution from step 3 (of spin protocol) to the HiBind® DNA Column by decanting or pipetting and apply vacuum. After the samples have passed through the column switch off the vacuum source.**

4. **Wash the HiBind® DNA Column by adding 700µl of DNA Wash Buffer and turning on the vacuum source.**
   IMPORTANT: DNA Wash Buffer must be diluted with absolute ethanol before use. Refer to label for instructions.

5. **Repeat step 4.**

6. **Assemble the column into a 2ml collection tube and spin for 2 min at maximum speed (≥ 13,000x g) to dry the HiBind® DNA Column.**

7. **Place the column in a clean 1.5ml tube and add 30-50 µl of Elution Buffer(10mM Tris, pH8.5) directly onto the column matrix. Let it sit at room temperature for 1-2 minutes. Centrifuge for 1 min at ≥ 13, 000x g to elute DNA.**

Troubleshooting Guide

Problem	Solution
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 were eluted from the column, thereby increasing A_{260}.	Make Sure to Wash Column as instructed in steps 6 and 7 of the Spin Protocol, and steps 4 and 5 of the Vacuum/Spin Protocol. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantization
DNA sample floats out of well while loading agarose gel	
Ethanol was not completely removed from column following wash steps	Centrifuge column as instructed in step 8 of the spin protocol and step 6 of the Vacuum/Spin protocol.

Ordering Information

Product	Applications	Cat. No.
Cycle-Pure Kit	PCR product purification	D6493-01/02 D6492-01/02
MicroElute ™ Cycle-Pure Kit	PCR product purification - special column for lower elution volume	D6293-01/02
E-Z 96® Cycle-Pure Kit	PCR product purification in a 96-well format	D1043-01/02
Mag-Bind® Cycle-Pure Kit	PCR product purification with magnetic beads	M1322-01/02
MicroElute ™ DNA Cleanup Kit	DNA recovery from enzymatic reactions- special column for lower elution volume	D6296-01/02
Mag-Bind® Oligonucleotide Purification Kit	DNA recovery from enzymatic reactions using magnetic beads	M2514-01/02
E-Z 96® Mag-Bind® Sequencing Dye Removal Kit	Sequencing dye terminator removal with magnetic beads in a 96-well format	M1320-01/02
DNA Probe Purification Kit	DNA Cleanup from enzymatic reactions	D6538-01/02
Gel Extraction Kit	DNA recovery from Agarose Gel	D2501-01/02 D2500-01/02
MicroElute ™ Gel Extraction Kit	DNA recovery from agarose gel- special column for lower elution volume	D6294-01/02
Ultra-Sep® Gel Extraction Kit	Low cost DNA recovery from agarose gel	D2510-01/02
Poly-Gel DNA Extraction Kit	DNA recovery from poly-acrylamide gel	D2561-01/02
Poly-Gel RNA Extraction Kit	RNA recovery from poly-acrylamide gel	R6376-01/02
RNA Probe Purification Kit	RNA Cleanup from enzymatic reactions	R6249-01/02
MicroElute ™ RNA Cleanup Kit	RNA recovery from enzymatic reactions- special column for lower elution volume	R6247-01/02

Product	Size	Product No.
Buffer CP	200ml/500ml	PDR042/PDR043
Elution Buffer	100 ml	PDR048
DNA Wash Buffer	(40 ml; add 60ml ETOH)	PDR044
DNA Wash Buffer	(100 ml; add 150ml ETOH)	PS010
DNA Wash Buffer	(500 ml; add 750 ETOH)	PS011
2ml capless collection tubes	500/BAG	SS1-1370-00
1.5ml DNase/RNase Free Centrifuge Tubes	500/BAG	SS1-1210-00

Please Call, Fax , or e-mail us to place an order.
Tel: 770-931-8400 (US) Fax: 770-931-0230 (US) e-mail: info@omegabiotek.com
Tel: 1-800-832-8896 (Toll free) Fax: 1-888-624-1688 (Toll free)
www.omegabiotek.com