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Revised July 2002

Introduction

The E.Z.N.A.® and E-Z96® family of products are innovative systems that radically simplify extraction and purification of nucleic acids from a variety of sources. Key to the 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 low salt buffer.

The E-Z96® Cycle-Pure Kit is a convenient system for fast and reliable purification of up to 96 PCR products. The method uses HiBind® technology to recover DNA bands 50 bp-40 kb free of oligonucleotides, nucleotides, and polymerase in yields exceeding 80%. Binding conditions are adjusted by addition of a specially formulated buffer, and the sample is applied to a E-Z 96® DNA Plate. Following a rapid wash step, DNA is eluted with deionized water (or low salt buffer) and ready for other applications. No organic extractions or alcohol precipitations means safe and rapid processing of multiple samples in parallel. The product is suitable for T-A ligations, PCR sequencing, restriction digestion, or various labeling reactions. In addition the kit can be used to purify DNA from any other enzymatic reaction.

Benefits

The E-Z 96® Cycle-Pure Kit means:

- Speed - Up to 96 DNA product can be recovery from enzymatic reactions <25 min
- Reliability - Optimized buffers guarantee pure DNA
- Safety - No organic extractions
- Quality - Purified DNA suitable for any application

Binding Capacity

Each well on the E-Z 96® plate can bind ~12 µg DNA.

Kit Contents

Product Number	D1043-01	D1043-02
Purifications	1 x 96	5 x 96
E-Z 96® well DNA Plates	1	5
96 well Collection Plates (300µl)	1	5
96 well Collection Plates (2ml)*	1	2
Buffer CP	50 ml	2 x 125 ml
DNA Wash Buffer Concentrate	2 x40 ml	3 x 100 ml
Instruction Booklet	1	1

* 2ml collection plate can be cleaned and reused. See page 6 for details.

Storage and Stability: All E-Z 96® 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.

Materials Supplied By User:

- Vacuum manifold which can fit the 96-well plate and the 96-well collection plate. (Omega Product # VAC-03 preferred) (for vacuum protocol)
- Centrifuge with swinging rotor which is capable of 4000 x g (such as Eppendof 5810 with MTP rotor or Beckman Allegra 6 with PTS-2000 rotor.) (For centrifugation protocol)
- Protective eye-ware.
- Sterile deionized water (or TE buffer)
- Absolute (96% - 100%) ethanol
- Necessary accessories such as plate seals and lids
- Protective eye-ware

IMPORTANT	Wash Buffer Concentrate must be diluted with absolute ethanol as follows:	
	D1043-01	Add 60 ml ethanol
	D1043-02	Add 150 ml ethanol to each bottle

E-Z 96® Cycle-Pure Protocol (Vacuum Manifold)

Please read this booklet thoroughly to ensure that you are familiar with the entire procedure. E-Z 96® Cycle-Pure 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.

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

2. Determine the volume of the PCR reaction, and put the E-Z 96® DNA plate into vacuum manifold.

3. Transfer sample to the E-Z 96® well DNA Plate, and add 3-5 volumes of Buffer CP. For PCR products <200 bp add 6 volumes of Buffer CP. Vortex thoroughly to mix

4. Turn on the vacuum manifold and filter through the mixtures by vacuum. Discard the pass through liquid.

   Note: Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions.

5. Wash the plate by adding 800 µl of Wash Buffer diluted with absolute ethanol. Vacuum through for 5 min at room temperature. Discard liquid , reuse the collection plate and repeat step 5 with another 800 µl Wash Buffer.
   Note: Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions.

6. Discard liquid and vacuum 5 more minutes to dry the resin.

7. Place the DNA Plate into a vacuum oven preset at 70C for 10 Minutes. (This step will ensure that the DNA plate is completely dried before elution.

8. Assemble the vacuum manifold by place a new 300 µl 96-well collection plate (provided). If Omega manifold (Vacuum-03) is used in this procedure, a used E-Z 96® DNA Plate or a 800 µl plate should be placed under the 300 µl collection plate to give the collection plate a proper position.

9. Place E-Z® 96 DNA Plate on top of the vacuum manifold. Add 30-50 µl (depending on desired concentration of final product) sterile deionized water (or TE buffer) directly onto the resin in each well and turn on the vacuum for 5 minutes 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.

E-Z 96® Cycle-Pure Protocol (Centrifugation)

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

2. Determine the volume of the PCR reaction.

3. Transfer samples to the E-Z 96® DNA Plate, and add 3-5 volumes of Buffer CP. For PCR products <200 bp add 6 volumes of Buffer CP. Mix by shaking gently or by pipetting.

4. Place the E-Z 96® well DNA Plate on top of the 2ml collection plate and put them into a microplate rotor.

5. Centrifuge at 3000-4000 x g for 5 minutes.

6. Discard the flow-through by invert the 2ml collection plate to a waste container. Reuse the collection plate.

7. Add 700 µl DNA wash buffer to each well of the E-Z 96® DNA Plate. Centrifuge at 3000-4000 x g for 5 minutes.

8. Discard the flow-through and wash the plate with another 700 µl DNA wash buffer.

9. Add 500 µl absolute ethanol to each well. Centrifuge at 4000 x g for 5 minutes.

10. Remove the DNA plate and place it into a vacuum oven or incubator which was preset to 70°C for 10 minutes. This step will ensure that the DNA plate is completely dried before DNA elution.

11. Add 30-50 µl water or TE buffer to each well of the DNA plate.

12. Carefully place the E-Z 96® DNA plate on top of the 96-well 300 µl collection plate(supplied). Centrifuge at 4000 x g for 5 minutes to elute DNA. This represents approximately 75%-80% of bound DNA. An optional second elution will yield any residual DNA, though at lower concentration.

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

    DNA concentration = $Absorbance_{260} \times 50 \times$ (Dilution Factor) µg/ml

    Fragments greater than 500 bp in length can routinely be purified at >80% yield. Bands ranging from 50 bp to 500 bp give yields of 60%-90%. The ratio of $(absorbance_{260})/(absorbance_{280})$ is an indication of nucleic acid purity. A value greater than 1.8 indicates > 90% nucleic acid. Alternatively, yield (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis.

Cleaning of 2ml 96-Well Plates:

The 2ml 96-well collection plates are reusable. To avoid cross-contamination, rinse the plates throughly with tap water after each user. Rinse with 0.5M Hcl for 5minutes and water throughly with distill water. 2ml 96-well collection plates can also be autoclaved after wash

Short Protocol For Experienced Users

1. Determine volume of reaction. Add 4 volumes of Buffer CP to PCR reaction.

2. Apply solution to E-Z 96® DNA plate assembled vacuum manifold.

3. Turn on the vacuum and filter through the mixtures by vacuum suction.

4. Wash plate twice with 500 µl each well by vacuum suction.(Wash Buffer should be diluted with ethanol before use).

5. Dry the membrane by 5 minutes further vacuum.

6. Place column into clean E-Z 96® well collection plate and elute DNA with 30-50 µl sterile water or TE buffer by vacuum suction.

Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Too little Buffer CP added to sample.	Add more Buffer CP as indicated. For DNA fragments <200 bp in size, add up to 6 x vol Buffer CP. For DNA fragments > 4 kb, add 3 volumes of Buffer CP followed by 1 volume distilled water.
No DNA eluted.	Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare Wash 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 quantization.
DNA sample floats out of well while loading agarose gel	Ethanol not completely removed from column following wash steps.	Vacuum or centrifuge the plate as instructed and incubate the plate before proceeding to elution step.

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

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