Contents

Revised July 2005

Introduction

The E.Z.N.A.® and E-Z 96® 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-Z 96® 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® DNA plate can bind ~12 µg DNA.

Kit Contents

Product Number	D1043-01	D1043-02
Purification times	1 x 96 Preps	5 x 96 Preps
E-Z 96® DNA Plates	1	5
2 ml Collection Plates	1	2
300 µl Collection Plates	1	5
Buffer CP	70 ml	2 x 170 ml
DNA Wash Buffer Concentrate	40 ml	4 x 50 ml
Elution Buffer	15 ml	75 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 22-25ºC. Under cool ambient conditions crystals may form in Buffer CP. Simply warm to 37ºC 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)
- Vacuum source: -100 to -600mbar
- Centrifuge with swinging rotor which is at least capable of 3000 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
- Absolute (96% - 100%) ethanol
- 2ml Deep well Plate (for centrugation protocol)

IMPORTANT	DNA Wash Buffer Concentrate must be diluted with absolute ethanol as follows: D1043-01 Add 160 ml (96-100%) absolute ethanol D1043-02 Add 200 ml (96-100%) absolute ethanol

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 Kit is designed to be simple, fast, and reliable provided that all steps are followed diligently. All 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. Prepare the vacuum manifold as following: 1. Place the waste collection tray inside the vauum manifold base; 2. Place the top plate of the vacuum manifold squarely over the base; 3. Connect the vacuum source to the manifold; 4. Seal the unused wells with sealing film tape; 5. Place the E-Z 96 DNA® plate in the top plate of the manifold, making sure that the plate is sealed securely.

4. Add 3-4 volumes of Buffer CP to 1 volume of PCR sample and mix well by shaking gently or pipetting. For PCR products <200 bp add 6 volumes of Buffer CP.

   Note: **it is not necessary to remove the mineral oil or kerosene. Do not count the volume of volume of the mineral oil.**
5. Tansfer the mixtures into the wells of E-Z 96® DNA Plate. Turn on the vacuum manifold and filter through the mixtures by vacuum.

6. Wash the plate by adding 900 µl of DNA Wash Buffer diluted with absolute ethanol to each well. Turn on the vacuum until all the liquid pass through the membrane.

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

7. Turn off the vacuum source and add another 900µl DNA wash Buffer diluted with absolute ethanol. Turn on the vacuum until all the liquid pass through the membrane.

8. After all the liquid in all wells pass through the membrane, apply maximum vacuum for additional 10 minutes to dry the membrane.

9. Turn off the vacuum source and ventilate the manifold slowly. Take the E-Z 96 DNA? plate off from top plate of the manifold. Tap the E-Z 96 DNA? plate on a stack of absorbent paper until no drops come out. Blot the nozzles of E-Z 96 DNA? plate with absorbent paper.

10. Put the E-Z 96? DNA plate back into vacuum manifold, apply maximum vacuum for additional 10 minutes to dry the membrane.

11. **Elute DNA with 96-well microplate (not provided):** 1. Assemble the vacuum manifold by place waste collection tray) in the base of the manifold; 2. Place a 96-well microplate on the waste tray. 3. Place the top plate of the vacuum manifold squarely over the base; 4. Place the E-Z 96? DNA plate in the top plate of the manifold.

12. Add 80-100µl (depending on desired concentration of final product) sterile deionized water or Elution Buffer (10mMtris-HCl, pH 8.5) directly onto the resin in each well. Leave at room temperature for 1 minute. Turn on the vacuum for 5 minutes to elute DNA. This represents approximately 70-80% 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. Add 3-4 volumes of Buffer CP to 1 volume of PCR sample and mix well by shaking gently or pipetting. For PCR products <200 bp add 6 volumes of Buffer CP.

Note: **it is not necessary to remove the mineral oil or kerosene. Do not count the volume of volume of the mineral oil.**

4. Place the E-Z 96? DNA Plate on top of a 2ml collection plate and transfer the mixture into the wells of the E-Z 96? DNA Plate. Put them into a microplate rotor.

5. Centrifuge at 3000 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 900 µl of DNA wash buffer ***diluted with absolute ethanol*** to each well of the E-Z 96? DNA Plate. Centrifuge at 3000 x g for 5 minutes.

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

8. Discard the flow-through and wash the plate with another 900 µl DNA wash buffer ***diluted with absolute ethanol***. Centrifuge at 3000 x g for 5 minutes.

9. Discard the flow-through and add 500 µl absolute ethanol to each well. Centrifuge at maxi speed (≥3000 x g) for 15 minutes.

10. **(Optional)** 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 80-100 µl water or Elution buffer to each well of the DNA plate. Leave at room temperature for 1 minute.

12. Carefully place the E-Z 96? DNA plate on top of the 96-well 300 µl collection plate(not provided). Centrifuge at 3000 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 = $Absorbance_{260}$ x 50 x (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.

Short Protocol For Experienced Users

1. Determine volume of reaction. Add 3-4 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 900 µl of DNA Wash Buffer each well by vacuum suction.(DNA Wash Buffer should be diluted with ethanol before use).
5. Dry the membrane by 10 minutes further vacuum.
6. Place column into clean E-Z 96® well collection plate and elute DNA with 80-100µl sterile water or Elution Buffer by vacuum suction.

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, then rinse throughly with distilled water. 2ml 96-well collection plates can also be autoclaved after wash.

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.	SPW Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare DNA 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.