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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

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.

Binding Capacity

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

Kit Contents

Product Number	D1043-00	D1043-02	D1043-03
Purification times	2 x 96 Preps	5 x 96 Preps	24 x96 Preps
E-Z 96® DNA Plates	2	5	24
Racked Microtubes (1.2 mL)	2	5	24
8-Strip Microtube Caps	24x 8	60 X 8	288 X 8
96 well Collection Plates (2ml)*	2	2	4
Buffer CP	120 ml	2 x 160 ml	3 x 480 ml
DNA Wash Buffer Concentrate	40 ml	100 ml	2 x 200 ml
Elution Buffer	30 mL	60 mL	300 mL
Instruction Booklet	1	1	1

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

Before Starting

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

IMPORTANT	Dilute **DNA Wash Buffer** with **absolute ethanol** as follows: **D1043-00** Add 160 ml absolute ethanol to each bottle **D1043-02** Add 400 ml absolute ethanol to each bottle **D1043-03** Add 800 ml absolute ethanol to each bottle
	Store the diluted DNA Wash Buffer at room temperature !

E-Z 96® Cycle-Pure Vacuum Manifold Protocol

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.
- Absolute (96% - 100%) ethanol
- Necessary accessories such as plate seals and lids
- Protective eye-ware

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

2. Determine the volume of the PCR reaction. **Add 3-5 volumes of Buffer CP to 1 volume of PCR sample and mix. For PCR products <200 bp add 6 volumes of Buffer CP.** Vortex thoroughly to mix**.**

3. Prepare the vacuum manifold according to the instruction of the manufacturer. For Omega Vac-3 manifold, place a 2ml collection plate inside the manifold and place the top plate of manifold squarely over the base**. Apply the samples to the wells of the E-Z 96 DNA Plate.** Seal the unused wells with sealing film tape and place the E-Z 96™ DNA Plate over the top plat of the manifold.

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

5. Wash the plate by adding 800 µl of **DNA 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 of DNA Wash Buffer.

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

6. Discard liquid and vacuum 10 more minutes to dry the resin.

7. Remove the E-Z 96® DNA Plate from the manifold, gently tap the plate on a stack of absorbent paper until no liquid drops come out. This step will ensure the removal of residual DNA Wash Buffer from outlet of nozzles of the E-Z 96® DNA Plate. Residual of ethanol, which is in DNA Wash Buffer, may interfere downstream enzymatic reactions.

8. **Optional:** Place the E-Z 96 DNA Plate into a vacuum oven preset at 70°C for 10 minutes to further dry the plate. (This step will ensure that the DNA plate is completely dried before elution.)

9. Assemble the manifold by placing the racked microtubes inside the based of

manifold. Place the E-Z 96® DNA Plate on top part of the manifold.

10. Place E-Z 96® DNA Plate on top of the vacuum manifold. Add 80-100µl (depending on desired concentration of final product) Elution Buffer (10mM Tris, pH 8.5) 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 Spin Protocol

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

2. Determine the volume of the PCR reaction. **Add 3-5 volumes of Buffer CP to 1 volume of PCR sample and mix. For PCR products <200 bp add 6 volumes of Buffer CP.** Vortex thoroughly to mix**.**

3. Place the E-Z 96® well DNA Plate on top of the 2mL collection plate and transfer samples to the E-Z 96® DNA Plate. Put them into a microplate rotor.

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

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

6. Add 800 µl DNA wash buffer to each well of the E-Z 96® DNA Plate. Centrifuge at 3000-4000 x g for 5 minutes. Repeat this step with another 800 µl DNA wash buffer. Centrifuge at 4000 x g for 10 minutes.

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

7. 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.

8. Add 80-100 µl of Elution Buffer (10mM Tris, pH 8.5) to each well of the DNA plate.

9. Carefully place the E-Z 96® DNA plate on top of the racked microtubes. 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.

10. **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 = $Absorbance_{260}$ × 50 × (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 (Vacuum Protocol)

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 with 800 µl 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 80-100 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.
	pH of water for elution is <7.5	Check the pH of the water, adjust the pH of the water to 8.0 using Tris-HCl (2M, 8.0)
No DNA eluted.	DNA 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.

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
D6538-01/02	DNA Probe Purification Kit	DNA probe purification, Q-column & V-column format
D2561-01/02	Poly-Gel DNA Extraction 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
R6249-01/02	RNA Probe Purification Kit	RNA probe purification, Q-column & V-column format
D6293-01/02	MicroElute Cycle Pure Kit	PCR product purification, MicroElute Spin colume
D6294-01/02	MicroElute Gel Extraction Kit	Agarose gel extraction using MicroElute spin column technology
D6296-01/02	MicroElute DNA clean Up Kit	DNA product purification with MicroElute Spin column.
D6274-01/02	MicroElute RNA Clean Up Kit	RNA product purification with MicroElute Spin column.
M1322-01/02	MagBind Cycle Pure Kit	PCR product purification with magnetic technology
M1320-01/02	MagBind Dye removal Kit	remove Dye terminator with magnetic technology.
S5912-01/02	Ultra-Sep Dye removal Kit	remove Dye terminator with column.

For technical support or to place orders, contact Omega Bio-Tek:
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