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Introduction

The E.Z.N.A.® 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® 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 Cycle-Pure Kit is a convenient system for fast and reliable purification of 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 HiBind® DNA spin-column. 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.N.A.® 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

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.

Binding Capacity

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

Kit Contents

Product Number	D6492-00 D6493-00 D6494-00	D6492-01 D6493-01 D6494-01	D6492-02 D6493-02 D6494-02
Purification times	5 Preps	100 Preps	200 Preps
HiBind® DNA Columns	5	100	200
2 ml Collection Tubes	5	100	200
Buffer CP	5 ml	80 ml	120 ml
DNA Wash Buffer Concentrate	2 ml	2 x 20 ml	3 x 20 ml
Instruction Booklet	1	1	1

Materials Supplied By User

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

IMPORTANT	DNA Wash Buffer Concentrate must be diluted with absolute ethanol as follows:
	D6492,D6493 & D6494-00 — Add 2 ml ethanol
	D6492,D6493 & D6494-01 — Add 80 ml ethanol to each bottle
	D6492,D6493 & D6494-02 — Add 80 ml ethanol to each bottle
	Store the diluted DNA Wash Buffer at room temperature.

E.Z.N.A. ™ Cycle-Pure Spin Protocol

Please read this booklet thoroughly 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.

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

2. **Determine the volume of the PCR reaction, transfer to a clean 1.5 ml microfuge tube, and add 4-5 volumes of Buffer CP.** For PCR products <200 bp add 6 volumes of Buffer CP. Vortex thoroughly to mix.

3. **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 and re-use the collection tube.

4. **Wash the column by adding 700 µl of DNA Wash Buffer diluted with absolute ethanol. Centrifuge at 10,000 x g for 1 min at room temperature.**

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

5. **Discard liquid and repeat Step 4 with another 700 µl DNA Wash 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**: 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)* µ*g/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.

E.Z.N.A. ™ Cycle-Pure Vacuum/Spin Protocol (V-Spin column only)

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

1. Prepare the sample by following the Protocol Steps 1-2.
2. Prepare the vacuum manifold according to manufacturer's instructions and connect the V-Spin column to the manifold.
3. Load the PCR reaction/Buffer CP solution from Step 2 to the column.
4. Switch on vacuum source to draw the sample through the column; then turn off the vacuum.
5. Wash the column by adding 700 µl DNA Wash Buffer. Draw the DNA Wash Buffer through the column by turning on the vacuum source. Repeat this step with another 700 µl DNA Wash Buffer.
6. Place the column into a 2 ml collection tube and centrifuge 1 minute to dry the column.
7. Place the column in a clean 1.5 ml microcentrifuge tube and add 30-50µl TE or water. Stand for 1-2 minutes and centrifuge 1 minute to elute DNA.

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 vol Buffer CP. For DNA fragments > 4 kb, add 3 volumes of Buffer CP followed by 1 volume distilled water.
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 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 4 volumes of Buffer CP 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 700 µl DNA Wash 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 TE buffer. Centrifuge 1 min.

Ordering Information

Product No.	Product Name	Description
D6492-01/02 D6493-01/02 D6494-01/02	Cycle-Pure Kit	PCR product purification, Q-Column format, V-column format & S-column.
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 if 50 preps and 200 preps. Product number end with"-01" represent 50 preps kit and "-02" represent 200 preps kit.