Contents

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 DNA Probe Purification Kit is a convenient system for fast and reliable purification of DNA from random-primed or nick-translation labeling reactions. Salts, enzymes and free label (radioisotopic or not) are effectively removed to yield pure DNA probes. Binding conditions are adjusted by addition of a specially formulated buffer, and the sample is applied to a HiBind™ DNA spin-column. Following two rapid wash steps, 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 may directly be used in any hybridization reaction. In addition the kit can be used to purify DNA from other enzymatic reactions.

Benefits

The E.Z.N.A.™ DNA Probe Purification Kit means:

- Speed - DNA recovery from labeling reactions <15 min
- Reliability - optimized buffers guarantee pure DNA
- Safety - No organic extractions
- Quality - DNA suitable for all hybridization protocols
- Convenience - DNA eluted in small volumes unlike gel filtration

New in this edition

New capped V-Spin column (Product# D6538) ensures the elimination of potential contamination and avoid the leaking of readioisotopic during the process.

Binding Capacity

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

Kit Contents

Product Number	D6537-00 D6538-00	D6537-01 D6538-01	D6537-02 D6538-02
Purifications	5	50	200
HiBind™ DNA Columns	5	50	200
2 ml Collection Tubes	5	50	200
Buffer DP	10 ml	40 ml	120 ml
DNA Wash Buffer Concentrate	12 ml	40 ml	3 x 40 ml
Instruction Booklet	1	1	1

Storage and Stability: All E.Z.N.A.™ DNA Probe Purification 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 DP. Simply warm to 37°C to dissolve.

Materials Supplied By User:

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

IMPORTANT	Wash Buffer Concentrate must be diluted with absolute ethanol as follows: D6537-00 D6538-00 — Add 18 ml ethanol D6537-01 D6538-01 — Add 60 ml ethanol D6537-02 D6538-02 — Add 60 ml ethanol/bottle

DNA Probe Purification 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.

For DNA probes to be used in nuclease protection assays, we suggest using the E.Z.N.A.® Poly-Gel DNA Purification Kit (D2561) to ensure probe integrity.

1. **Determine the volume of the labeling reaction, transfer to a clean 1.5 ml microfuge tube, and add 5 volumes of Buffer DP.** Vortex thoroughly to mix.

   Note: Radioisotopic labeling reactions yield sub microgram DNA due to the limiting nucleotide concentrations used. In this case, a carrier such as yeast tRNA should be added to Buffer DP to a final concentration of **2-5 µg/ml** before use to ensure binding to HiBind resin. The mixture is stable at -70°C for 2-3 months, but needs heating to redissolve salts in Buffer DP. Non-radioactive labeling reactions usually use higher dNTP concentrations and yield more product thus obviating the need for carrier.

2. **Apply the sample to an 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.

4. **Wash the column by adding 750 µl of Wash Buffer diluted with absolute ethanol. Centrifuge at 10,000 x g for 1 min at room temperature.**

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

5. ***Optional*: discard liquid and repeat step 5 with another 750 µl 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. Alternatively, elute a second time with the first eluate.

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:

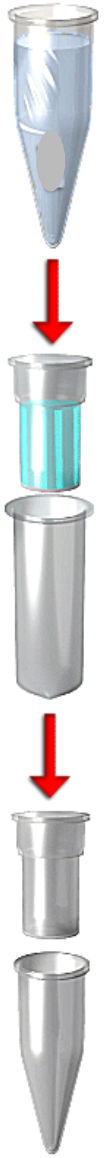
DNA concentration = Absorbance$_{260}$ × 50 × (Dilution Factor) µ*g/ml*

Probes greater than 500 bp in length can routinely be purified at >60% yield with greater than 95% removal of free nucleotides. Fragments ranging from 50 bp to 500 bp give yields of 30%-80%. The ratio of (absorbance$_{260}$)/(absorbance$_{280}$) is an indication of nucleic acid purity. A value greater than 1.8 indicates > 90% nucleic acid. Activity of the probes will also need to be determined prior to setting up a hybridization reaction.

Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Too little Buffer DP added to sample.	Add more Buffer DP as instructed. For DNA fragments ~100 bp in size, add up to 7 x vol Buffer DP.
	Poor labeling reaction.	With radioisotopic labeling reactions, add carrier tRNA to DP before use (page 4, step).
		Optimize labeling reaction.
DNA probe has little activity.	Inefficient labeling reaction.	Optimize labeling reaction.
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 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 7 to dry before proceeding to elution step.

Short Protocol For Experienced Users



1. Determine volume of reaction. Add 5 volumes of Buffer DP. Include rRNA if DNA is labeled with radioisotopic nucleotide(s).
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 750 µl Wash Buffer diluted with ethanol.

   Optional: repeat wash step.
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
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

* 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.

If you have any question regarding this product or interested on other Mmega products, please feel free to fax our customer service specialists at:

US customers: 800 832 8896
All other customers: (770) 441 9600
Or direct your questions via E-mail to info@omegabiotek.com.