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## Introduction

The E.Z.N.A.™ family of products is an innovative system that radically simplifies the extraction and purification of nucleic acids from a variety of sources. The key to this 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 a low salt buffer.

Gel purification of DNA is a common technique for the isolation of specific fragments from reaction mixtures. However, most methods either fail to completely remove agarose (which can lead to problems in downstream manipulations), shear the DNA, or result in very low yields. The E.Z.N.A.™ Gel Extraction Kit uses HiBind® technology to recover DNA bands 50 bp -40 kb from all grades of agarose gel in yields exceeding 85%. The DNA band of interest is excised from the gel, dissolved in Binding Buffer, and applied to a HiBind® DNA spin-column. Following a rapid wash step, DNA is eluted with Elution Buffer and is ready for other applications. The product is suitable for ligations, PCR, sequencing, restriction digestion, or various labeling reactions.

### Benefits of the E.Z.N.A.™ Gel Extraction Kit

- **Fast** DNA recovery from agarose gel < 10 minutes.
- **Reliability** with optimized buffers that guarantee pure DNA.
- **Safety** due to no organic extractions
- **Quality** ensures that purified DNA will be suitable for any application.

### Storage and Stability

All E.Z.N.A.™ Gel Extraction Kit components are guaranteed for at least 24 months from the date of purchase when stored at 15-25°C. Please ensure that the bottle of Binding Buffer is tightly capped when not in use. If any precipitates form in buffers, warm at 37°C to dissolve.

### Binding Capacity

Each HiBind® DNA column can bind ~ 25 µg of DNA.

## Kit Contents

Product Number	D2500-00 D2501-00	D2500-01 D2501-01	D2500-02 D2501-02
Purification Times	5 preps	50 preps	200 preps
HiBind® DNA Mini Columns	5	50	200
2 ml Collection Tubes	5	50	200
Binding Buffer* (XP2)	5 ml	40 ml	150 ml
Elution Buffer *	1 ml	10 ml	20 ml
SPW Wash Buffer Concentrate	5 ml	25 ml	3 x 25 ml
Instruction Booklet	1	1	1

\*Binding Buffer contains chaotropic salts which are irritants. Take appropriate laboratory safety measures and wear gloves when handling.

\* Elution Buffer = 10mM Tris-HCl, pH 8.5

## Before Starting

It is strongly advised that you familiarize yourself with the entire procedure before beginning this protocol. Omega Bio-Tek, Inc.'s E.Z.N.A.™ Gel Extraction Kit is designed to be simple, fast, and reliable provided that all steps are followed diligently.

<b>Important</b>	<b>SPW Wash Buffer Concentrate</b> must be diluted with absolute ethanol (~96-100%) as follows and store at room temperature:
	<b>D2500/2501-00</b> Add 20 ml of absolute ethanol
	<b>D2500/2501-01</b> Add 100 ml of absolute ethanol
	<b>D2500/2501-02</b> Add 100 ml of absolute ethanol per bottle

## E.Z.N.A.™ Gel Extraction Spin Protocol

### Materials Supplied by User

- Water bath equilibrated to 55-60°C
- Microcentrifuge capable of at least 10,000 xg
- Nuclease-free 1.5 microcentrifuge tubes
- Sterile deionized water (or TE Buffer)
- Absolute ethanol (~ 96-100%)
- Protective eye-ware
- 5M Sodium Acetate, pH 5.2

**NOTE:** The yellow color of Binding Buffer (XP2) signifies a pH of  $\leq 7.5$ .

1. Perform agarose gel/ethidium bromide electrophoresis to fractionate DNA fragments. Any type or grade of agarose may be used. It is strongly recommended however, that fresh TAE buffer, or TBE buffer be used as running buffer. Do not reuse running buffer as its pH will increase and reduce yields.
2. When adequate separation of bands has occurred, CAREFULLY excise the DNA fragment of interest using a wide, clean, sharp scalpel. Minimize the size of the gel slice by removing extra agarose.
3. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 ml microcentrifuge tube. Assuming a density of 1g/ml of gel, the volume of gel is derived as follows: a gel slice of mass 0.3g will have a volume of 0.3 ml. Add an equal volume of Binding Buffer (XP2). Incubate the mixture at 55°C-60°C for 7 min or until the gel has completely melted. Mix by shaking or vortexing the tube in increments of 2-3 minutes.
 

**IMPORTANT:** Monitor the pH of the Gel/Binding Buffer mixture after the gel has completely dissolved. DNA yields will significantly decrease when pH > 8.0. If the color of the mixture becomes orange or red, add 5 µl of 5M sodium acetate, pH 5.2 to bring the pH down. After this adjustment, the color of the Gel/Binding Buffer mixture should be light yellow.

4. Place a HiBind® DNA Mini Column in a provided 2 ml collection tube.
  5. Apply 700µl of the DNA/agarose solution to the HiBind® DNA Mini Column, and centrifuge at 10,000 x g for 1 min at room temperature.
  6. Discard liquid and place the HiBind® DNA Mini Column back into the same collection tube. For volumes greater than 700 µl, load the column and centrifuge successively, 700 µl at a time. Each HiBind® DNA Mini Column has a total capacity of 25 µg DNA. If the expected yield is larger, divide the sample into the appropriate number of columns.
  7. Add 300 µl of Binding Buffer (XP2) into the HiBind® DNA Mini Column. Centrifuge at 10,000 x g for 30 to 60 seconds at room temperature to wash the HiBind® DNA Mini Column. Discard the flow-through and re-use the collection tube.
  8. Wash the HiBind® DNA Mini Column by adding 700 µl of SPW Wash Buffer diluted with absolute ethanol. Centrifuge at 10,000 x g for 30 to 60 seconds at room temp.
- NOTE: SPW Wash Buffer Concentrate must be diluted with absolute ethanol before use. See label for directions. If refrigerated, SPW Wash Buffer must be brought to room temperature before use.
9. OPTIONAL: repeat step 8 with another 700 µl of SPW Wash Buffer diluted with absolute ethanol.
- NOTE: Perform the second wash step for any salt sensitive downstream applications
10. Discard liquid and centrifuge the empty HiBind® DNA Mini Column for 2 min at maximal speed ( ≥ 13,000 x g) to dry the column matrix. Do not skip this step, it is critical for the removal of ethanol from the HiBind® DNA column.
  12. Place a HiBind® DNA Mini Column into a clean 1.5 ml microcentrifuge

tube. Add 30-50 µl (depending on desired concentration of final product) of Elution Buffer (10 mM Tris-HCl, pH 8.5) directly onto the column matrix and incubate at room temperature for 1 minute. Centrifuge for 1 min at maximal speed ( ≥ 13,000 x g) to elute DNA. This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

NOTE: The efficiency of eluting DNA from the HiBind® DNA Mini Column is dependent of pH. If eluting DNA with deionized water, make sure that the pH is around 8.5.

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:

$$\text{DNA concentration} = A_{260} \times 50 \times (\text{Dilution Factor}) \mu\text{g/ml}$$

Fragments greater than 500 bp in length can routinely be purified at > 80% yield. Bands ranging from 50 bp to 500bp gives yields of 55%-80%. The ratio of ( $A_{260}$ ) / ( $A_{280}$ ) is an indication of nucleic acid purity. A value greater than 1.8 indicates > 90% nucleic acid purity. Alternatively, yield (as well as quality) can sometimes be best determined by agarose gel/ethidium bromide electrophoresis.

## E.Z.N.A.<sup>™</sup> Gel Extraction Vacuum/Spin Protocol

Please read through previous sections of this book before using this protocol.

Switch off vacuum between steps for consistency.

1. Prepare the sample and dissolve the gel by following steps 1 - 3 of the spin protocol on page 4.
2. Prepare the vacuum manifold according to manufacturer's instructions.
3. Load the DNA/agarose solution from step 3 to the HiBind<sup>®</sup> DNA Mini Column by decanting or pipetting, and apply vacuum. After the samples have passed through the column, switch off the vacuum source.
4. Wash the HiBind<sup>®</sup> DNA Mini Column by adding 300  $\mu$ l of Binding Buffer (XP2) and turning on the vacuum source.
5. Wash the sample by adding 700  $\mu$ l of SPW Wash Buffer diluted with absolute ethanol. Repeat this step with another 700  $\mu$ l of SPW Wash Buffer.
6. Assemble the HiBind<sup>®</sup> DNA Mini Column into a provided 2 ml collection tube and spin at maximal speed ( $\geq 13,000$  x g) for 2 min to dry the HiBind<sup>®</sup> DNA Mini Column.
7. Place the HiBind<sup>®</sup> DNA Mini Column in a clean 1.5 ml microcentrifuge tube and add 30-50  $\mu$ l of Elution Buffer (10mM Tris-HCl, pH 8.5). Let it sit at room temperature for 1-2 minutes. Centrifuge at maximal speed ( $\geq 13,000$  x g) for 1 min to elute DNA.

## Protocol for Purification of DNA From Enzymatic Reactions

1. Determine the volume of enzymatic reaction. Transfer the sample into a clean 1.5 ml microcentrifuge tube, and add an equal volume of Binding Buffer.
2. Vortex thoroughly to mix sample and briefly spin the tube to collect any drops from the inside of the lid.

3. Place a HiBind<sup>®</sup> DNA column in a provided 2 ml collection tube. Apply the sample to the HiBind<sup>®</sup> DNA column, and centrifuge at 10, 000 x g for 30 to 60 seconds at room temperature.
4. Discard liquid and place the HiBind<sup>®</sup> DNA column back into the same collection tube.
5. Add 300  $\mu$ l of Binding Buffer into the Column and centrifuge as above. Discard the flow-through and reuse the collection tube.
6. Wash the HiBind<sup>®</sup> DNA column by adding 700  $\mu$ l of SPW Wash Buffer diluted with absolute ethanol and centrifuge as above.  
  
IMPORTANT: SPW Wash Buffer must be diluted with absolute ethanol before use. Refer to label for instructions. If refrigerated, SPW Wash Buffer must be brought to room temperature before use.
7. Discard liquid and repeat step 6 using 700  $\mu$ l of SPW Wash Buffer.
8. Discard liquid and centrifuge the empty HiBind<sup>®</sup> DNA column for 2 min at maxi speed ( $\geq 13,000$  x g) to dry the column matrix. *This is critical for good yields.*
9. Place HiBind<sup>®</sup> DNA column into a clean 1.5 ml microcentrifuge tube. Add 30-50  $\mu$ l (depending on desired concentration of final product) of Elution Buffer (10mM Tris, pH8.5) or water directly onto the column matrix and centrifuge for 1 min at  $\geq 13,000$  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.

## Short Protocol For Experienced Users

	1.	<b>Excise gel slice containing DNA fragment of interest.</b>
	2.	<b>Determine volume of gel. Add an equal volume of Binding Buffer (XP2).</b>
	3.	<b>Incubate at 55-60°C for 7 min or until gel melts completely.</b>
	4.	<b>Apply solution to HiBind® DNA Mini Column assembled in a 2 ml collection tube.</b>
	5.	<b>Centrifuge at maximal speed for 1 min at room temperature. Discard liquid.</b>
	6.	<b>Wash HiBind® DNA Mini Column with 300 µl Binding Buffer (XP2).</b>
	7.	<b>Wash HiBind® DNA Mini Column twice with 700 µl of SPW Wash Buffer.</b>
	8.	<b>Centrifuge empty column for 2 min at max speed to dry.</b>

## Troubleshooting Guide

Problem	Solution
<b>Low DNA yields</b>	
Too little Binding Buffer added to gel	Volume of agarose gel slice determined incorrectly. Add enough Binding Buffer as instructed.
Agarose gel does not completely dissolve	Make sure water bath is set to 55° C to 60° C and allow gel to completely melt. Add more Binding Buffer if necessary.
Inappropriate elution buffer	Check pH of the water or use 10mM Tris-HCl, pH 8.5 to elute DNA.
TAE running buffer is not fresh	With overuse, TAE Buffer loses its buffering capacity, and its pH increases. This raises the pH of the agarose/DNA Binding Buffer solution which interferes with DNA binding to the HiBind® matrix. Adjust pH by adding 5 µl of 5M sodium acetate pH 5.2 to the gel slice at the adsorption step. Use freshly prepared TAE buffer for gel purification in order to prevent the contamination of isolated DNA and improve yields.
<b>Column clogged</b>	
Agarose gel not completely dissolved in Binding Buffer.	Make sure water bath is set to 55-60° C and allow gel to completely melt. For large agarose slices (>0.3 ml) it is recommended that the gel be diced into smaller fragments to aid melting.
<b>No DNA eluted</b>	
SPW Wash Buffer not diluted with absolute ethanol	Prepare SPW Wash Buffer Concentrate as instructed on page 3 or as indicated on bottle.

Incorrect amount of Binding Buffer added	Measure the gel accurately and use 0.1 ml of Binding Buffer per 0.1 g of gel.
<b>Optical densities do not agree with DNA yield on agarose gel</b>	
Trace contaminants eluted from column increase $A_{260}$ .	Make sure to wash column as instructed in steps 8 and 9 of the spin protocol, and step 5 of the vacuum/spin protocol. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantization.
<b>DNA sample floats out of well while loading agarose gel.</b>	
Ethanol not completely removed from column.	Centrifuge as instructed in step 10 of the spin protocol and step 6 of the vacuum /spin protocol.

## Ordering Information

Product	Applications	Cat. No.
Cycle-Pure Kit	PCR product purification	D6493-01/02 D6492-01/02
Gel Extraction Kit	DNA recovery from Agarose Gel	D2501-01/02 D2500-01/02
Poly-Gel DNA Extraction Kit	DNA recovery from poly-acrylamide gel	D2561-01/02
Poly-Gel RNA Extraction Kit	RNA recovery from poly-acrylamide gel	R6376-01/02
DNA Probe Purification Kit	DNA Cleanup from enzymatic reactions	D6538-01/02
RNA Probe Purification Kit	RNA Cleanup from enzymatic reactions	R6249-01/02
MicroElute® Cycle-Pure Kit	PCR product purification - special column for lower elution volume	D6293-01/02
MicroElute® Gel Extraction Kit	DNA recovery from agarose gel- special column for lower elution volume	D6294-01/02
MicroElute® DNA Cleanup Kit	DNA recovery from enzymatic reactions- special column for lower elution volume	D6296-01/02

MicroElute® RNA Cleanup Kit	RNA recovery from enzymatic reactions- special column for lower elution volume	R6247-01/02
E-Z 96® Cycle-Pure Kit	PCR product purification in a 96-well format	D1043-01/02
Ultra-Sep® Gel Extraction Kit	Low cost DNA recovery from agarose gel	D2510-01/02
Mag-Bind® Cycle-Pure Kit	PCR product purification with magnetic beads	M1322-01/02
Mag-Bind® Oligonucleotide Purification Kit	DNA recovery from enzymatic reactions using magnetic beads	M2514-01/02
E-Z 96® Mag-Bind® Sequencing Dye Removal Kit	Sequencing dye terminator removal with magnetic beads in a 96-well format	M1320-01/02

Product	Size	Product No.
Binding Buffer	200 ml	PDR040
Binding Buffer	500 ml	PDR041
SPW Wash Buffer	(25 ml; add 100ml ETOH)	PDR045
2ml capless collection tubes	500/BAG	SS1-1370-00
1.5ml DNase/RNase Free Centrifuge Tubes	500/BAG	SS1-1210-0

Please Call, Fax, or e-mail us to place an order. We will be happy to answer any questions for you.  
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Tel: 1-800-832-8896 (Toll free) Fax: 1-888-624-1688 (Toll free) Visit our web: [www.omegabiotek.com](http://www.omegabiotek.com)