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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 & PCR 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 & PCR Clean Up Kit uses HiBind® technology to recover DNA bands 100 bp - 20 kb from all grades of agarose gel and PCR products in yields exceeding 80%. The DNA band of interest is excised from the gel, dissolved in XP5 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 & PCR Clean Up Kit

- **Speed -** DNA recovery from agarose gel or PCR products< 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
- **High Concentrations** -10-30 µl elution volume

Storage and Stability

All E.Z.N.A.® Gel & PCR Clean Up 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 XP5 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 XS column can bind up to 9 µg of DNA.

Kit Contents

Product Number	D2000-00	D2000-01	D2000-02
Purification Times	5 preps	100 preps	200 preps
HiBind? DNA XS Columns	5	100	200
2 ml Collection Tubes	5	100	200
XP5 Buffer	5 ml	80 ml	160 ml
Elution Buffer	5 ml	10 ml	20 ml
SPW Wash Buffer	2 ml	2 x 20 ml	3 x 25 ml
Instruction Booklet	1	1	1

*XP5 Buffer contains chaotropic salts which are irritants. Take appropriate laboratory safety measures and wear gloves when handling.

Before Starting

Materials Supplied by User

- Water bath equilibrated to 50-55°C
- Microcentrifuge capable of at least 10,000 x g
- Nuclease-free 1.5 microcentrifuge tubes
- Sterile deionized water (or TE Buffer)
- Absolute ethanol (~ 96-100%)
- Protective eye-ware
- 5 M Sodium Acetate, pH 5.2

Important	**SPW Wash Buffer** must be diluted with absolute ethanol (96-100%) as follows and stored at room temperature:	
	D2000-00	Add 8 ml of absolute ethanol
	D2000-01	Add 80 ml of absolute ethanol to each bottle
	D2000-02	Add 100 ml of absolute ethanol to each bottle

E.Z.N.A.? Gel & PCR Clean Up Spin Protocol

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 & PCR Clean Up Kit is designed to be simple, fast, and reliable provided that all steps are followed diligently.

NOTE: The yellow color of XP5 Buffer signifies a pH ≤ 7.5.

Gel Clean Up Protocol

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 XP5 Buffer. Incubate the mixture at 50°C-55°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/XP5 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 ?l 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 XS column in a provided 2 ml collection tube**.

5. **Apply 700 µl of the DNA/agarose solution to the HiBind? DNA XS column, and centrifuge at 10, 000 x g for 1 min at room temperature.**

6. **Discard liquid and place the HiBind? DNA XS column back into the same collection tube.** For volumes greater than 700 µl, load the column and centrifuge successively, 700 ?l at a time. Each HiBind? DNA XS column has

a total capacity of 9 µg DNA. If the expected yield is larger, divide the sample into the appropriate number of columns.

7. **Add 300 µl of XP5 Buffer into the HiBind? DNA XS column. Centrifuge at 10,000 x g for 1 min at room temperature to wash the column. Discard the flow-through and re-use the collection tube.**

8. **Wash the HiBind? DNA column by adding 700 µl of SPW Wash Buffer diluted with absolute ethanol. Centrifuge at 10,000 x g for 1 min 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 XS 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 XS column.

11. Place a HiBind? DNA XS column into a clean 1.5 ml microcentrifuge tube. **Add 10-30 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 XS column is dependent of pH. If eluting DNA with deionized water, make sure that the pH is around 8.0.

12. **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:

DNA concentration = A_{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 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.

PCR Clean Up Protocol

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 1 volumes of XP5 Buffer.** Vortex thoroughly to mix.

3. Follow the Gel Clean Up protocol from Step 4 on page 4.

E.Z.N.A.? Gel & PCR Clean Up 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 gel sample and dissolve the gel by following steps 1 - 3 of the spin protocol on page 4 and PCR sample by follow steps 1-2 of the spin protocol on page 6.

2. Prepare the vacuum manifold according to manufacturer? s instructions.

3. **Load the DNA/agarose solution from step 3 to the HiBind? DNA XS 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? DNA XS column by adding 300 µl of XP5 Buffer and turning on the vacuum source.**

5. **Wash the sample by adding 700 µl of SPW Wash Buffer diluted with absolute ethanol. Repeat this step with another 700 µl of SPW Wash Buffer.**

6. Assemble the HiBind® DNA XS column into a provided 2 ml collection tube and spin at maximal speed (≥13,000 x g) for 2 min to dry the HiBind® DNA XS column.

7. Place the HiBind® DNA XS column in a clean 1.5 ml microcentrifuge tube and add 30-50 µl of Elution Buffer(10mM Tris-HCl, pH 8.5). Let it sit at room temperature for 1-2 minutes. Centrifuge at maximal speed (≥ 13,000 x g) for 1 min to elute DNA.

Troubleshooting Guide

Please use this guide to solve any problems that may arise. We hope that it will aid in clearing up any questions for you, if for any reason you need further assistance the scientists at Omega Bio-Tek, Inc. We are always happy to answer any questions you may have about either the information and procedures of this manual or molecular biology applications (see page 8 for contact information).

Low DNA yields	
a) Too little XP5 Buffer added to gel	Volume of agarose gel slice determined incorrectly. Add enough XP5 Buffer as instructed.
b) Agarose gel does not completely dissolve	Make sure water bath is set to 50°C to 55°C and allow gel to completely melt. Add more Binding Buffer if necessary.
c) Inappropriate elution buffer	Check pH of the water or use 10mM Tris-HCl, pH 8.5 to elute DNA.
d) 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.
Column clogged	
e) Agarose gel not completely dissolved in XP5 Buffer.	Make sure water bath is set to 50-55 °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.
No DNA eluted	
f) SPW Wash Buffer not diluted with absolute ethanol	Prepare SPW Wash Buffer Concentrate as instructed on page 3 or as indicated on bottle.
g) Incorrect amount of XP5 Buffer added	Measure the gel accurately and use 0.1 ml of XP5 Buffer per 0.1 g of gel.
Optical densities do not agree with DNA yield on agarose gel	
h) 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.
DNA sample floats out of well while loading agarose gel.	
i) Ethanol not completely removed from column.	Centrifuge as instructed in step 10 of the spin protocol and step 6 of the vacuum /spin protocol.

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