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Introduction

The E.Z.N.A.[®] family of products are an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. The key to the system is Omega Bio-Tek's proprietary HiBind[®] matrix that avidly, but reversibly, binds nucleic acids under specific conditions, the removal of interfering compounds. Nucleic acids are subsequently eluted with de-ionized water or low salt buffer.

Gel purification of DNA is a routine technique for the isolation of DNA separated via agarose electrophoresis. Unfortunately, most methods either fail to completely remove agarose (leads to problems in downstream applications), shear DNA, or are inefficient in retaining DNA. The E.Z.N.A.[®] Gel Extraction Kit uses HiBind[®] technology to recover DNA bands (50 bp-40 kb) from all concentrations and types agarose based gels. The DNA fragment of interest is excised from the gel, and dissolved in Binding Buffer. The sample is then applied to a HiBind[®] DNA spin-column and washed for impurities. DNA subsequently eluted with de-ionized water or low salt buffer and in a form ready for downstream applications. The product is suitable for ligation, sequencing, restriction digestion, and various labeling reactions.

Benefits

The E.Z.N.A.[®] Gel Extraction Kit means:

- Speed - DNA recovery from agarose in <15 min
- Reliability - Optimized buffers guarantee pure DNA
- Safety - No organic extractions
- Quality - Purified DNA suitable for any application

New in this edition

- pH indicator has been introduced.
- New DNA elution is supplied.
- New SPW Buffer has been introduced

Binding Capacity

Each HiBind[®] DNA column can bind ~25 µg DNA.

Kit Contents

Product Number	D2500-01 D2501-01 D2502-01	D2500-02 D2501-02 D2502-02
Purifications	50	200
HiBind® DNA Columns	50	200
2 ml Collection Tubes	50	200
Binding Buffer (XP2)	30 ml	120 ml
DNA Elution Buffer	10 ml	20 ml
SPW Buffer	25 ml	3 x 25 ml
Instruction Booklet	1	1

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 22-25°C. Ensure that the bottle of Binding Buffer is capped tightly when not in use.

Materials Supplied By User:

- Water bath equilibrated to 55-65°C
- Microcentrifuge capable of at least 10,000 x g.
- Sterile 1.5 ml centrifuge bottles.
- Sterile deionized water (or TE buffer)
- Absolute (or 95%) ethanol.
- Protective eye-ware.
- 5M Sodium acetate, PH 5.2.

IMPORTANT

SPW Buffer Concentrate must be diluted with absolute ethanol as follows:

D2500-01 & D2501-01& D2502-01	Add 20 ml 100 % ethanol
D2500-02 & D2501-02& D2502-02	Add 100 ml 100% ethanol to each bottle

Gel Extraction Protocol

Please read this booklet in its entirety prior to commencing 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.

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.

1. **Carefully excise the DNA fragment of interest using a UV light box ensuring that as much agarose gel as possible has been removed. Avoid more than 30 seconds exposure of UV light to the DNA. Always use protective eye-ware when working with UV light.**
2. **Determine the approximate volume of the gel slice by weighing it in a clean 1.5 ml microfuge tube.** Assuming a density of 1 g/ml of gel, the volume of gel is derived as follows: a gel slice of mass 0.2 g will have a volume of 0.2 ml. **Add equal volume of Binding Buffer. Incubate the mixture at 55°C-60°C for 7 min or until the gel has completely melted. Mix every 2-3 minutes. Centrifuge the tube briefly to collect all the liquid to the bottom of the tube.**

Important: Monitor the pH of the Gel/Binding buffer mixture after the gel completely dissolved. DNA yield will significantly decreased when pH > 8.0. If the color of the mixture become 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.

3. **Apply a maximum of 700 µl of the DNA/agarose solution to a HiBind® DNA spin-column assembled in a clean 2 ml collection tube (provided) and centrifuge in a microcentrifuge at minimum 8000 x g for 1 minute at room temperature (assume room temperature throughout unless otherwise indicated).** Discard the liquid. For volumes greater than 700 µl repeat this procedure until all the solution has passed through the column. Each HiBind® extraction column has a total capacity of a maximum of 30 µg DNA. If the expected yield is larger, divide the sample into the appropriate number of columns.
4. **Discard flow through and add 300µl Binding Buffer and centrifuge at 10,000 x g for 1 minute.** Discard the flow-through and reuse the collection tube.
5. **Add 700 µl of SPW Buffer to the column and incubate 3 minutes. Centrifuge at 10,000 x g for 1 min at room temperature to wash the column.**
6. **(Optional)** Discard liquid and repeat step 6 with another 700 µl SPW Buffer.
7. **Discard liquid and centrifuge the empty column at 10,000 x g for 1 minute to dry the column matrix.** This is critical for high DNA yields.
8. **Place column into a clean 1.5 ml microcentrifuge tube. Add 30-50 µl**

(depending on desired concentration of final product) DNA Elution Buffer (Supplied) or sterile deionized water directly onto the column matrix and centrifuge at 10,000 x g for 1 minute 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: efficiency of eluting DNA from column is dependent on pH. If eluting DNA with water, ensure that the pH is around 8.0.

Yield and quality of DNA: To determine the yield and quality of your sample take an absorbance of an appropriate dilution of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

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

Fragments greater than 500 bp in length can routinely be purified at >80% yield. Bands ranging from 50 bp to 500 bp give yields of 55%-80%. The ratio of $(\text{absorbance}_{260})/(\text{absorbance}_{280})$ 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.

Vacuum/Spin Protocol for Gel Extraction (V-Spin column only)

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

1. **Prepare the gel sample and dissolve the gel by following the Gel Extraction Protocol step 1-3.**
2. **Prepare the vacuum manifold according to manufacturer's instruction and connect the V-Spin column to the manifold.**
3. **Load the dissolved DNA/agarose solution from step 3 to the column.**
4. **Switch on vacuum source to draw the sample through the column and turn off the vacuum.**
5. **Wash the column by adding 300µl Binding buffer, and use vacuum to draw the Binding buffer through the column.**
6. **Add 700 µl SPW buffer to the HiBind® DNA spin-column, and draw the SPW buffer through the column via the vacuum source. Repeat this step.**
7. **Assemble the column into a 2 ml collection tube and transfer the column to a micro centrifuge. Spin 1 minute to dry the column.**
8. **Place the column in a clean 1.5 ml microcentrifuge tube and add 30-50µl TE or water. Incubate for 1 minute and centrifuge at 10,000 x g for 1 minute to elute DNA.**

Trouble Shooting Guide

Problem	Potential Cause	Solutions
Low DNA yields	Too little Binding Buffer added to gel.	Volume of agarose gel slice determined incorrectly. Add enough Binding Buffer as instructed.
	Agarose gel not completely dissolved in Binding Buffer.	Make sure water bath is set to 55°C to 65°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 9.0 to elute DNA.
	TAE running buffer not fresh.	With overuse, TAE loses its buffering capacity and increases in pH. This raises the pH of the agarose/ DNA/ Binding Buffer solution which interferes with DNA binding to HiBind® matrix. Adjust pH by adding 5 ul of 5M sodium acetate pH 5.2 to the gel slice at the adsorption step. Use freshly prepared TAE buffer for gel purification (and prevent contamination of isolated DNA in addition to improving yields).
Column clogged	Agarose gel not completely dissolved in Binding Buffer.	Make sure water bath is set to 55°C to 65°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	Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare SPW Wash Buffer Concentrate as instructed above.
	Incorrect amount of Binding Buffer added.	Measure the gel accurately and use 0.1ml of Binding Buffer per 0.1 g gel.
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 5 and 6. 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.	Centrifuge column as instructed in step 7 to dry before proceeding to elution step.

Short Protocol For Experienced Users



1. Excise gel slice containing DNA fragment of interest.
2. Determine volume of gel. Add equal volume of Binding Buffer.
3. Incubate at 55°C-65°C 7 min or until gel melts completely.
4. Apply solution to HiBind® extraction column assembled in 2ml collection tube.
5. Centrifuge at maximum speed 1 min at room temperature. Discard liquid.
6. Wash column with 300µl Binding Buffer.
6. Wash column twice with 700 µl SPW Buffer.
7. Centrifuge empty column 1 min at max speed to dry.
8. Place column into clean 1.5 ml tube and elute DNA with 30-50 µl sterile water or TE buffer. Centrifuge 1 min

Product Information for Additional DNA/RNA Clean-Up Applications:

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
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