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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.

Gel purification of DNA is a common technique for 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 deionized water (or low salt buffer) and ready for other applications. The product is suitable for ligations, PCR sequencing, restriction digestion, or 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.
- Optional vacuum/spin protocol available for V-Spin column

Binding Capacity

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

Kit Contents

Product Number	D2501-00 D2502-00	D2501-01 D2502-01	D2501-02 D2502-02
Purifications	5	50	200
HiBind® DNA Columns	5	50	200
2 ml Collection Tubes	5	50	200
Binding Buffer	10 ml	60 ml	2 x 120 ml
DNA Elution Buffer	1 ml	10 ml	20 ml
Wash Buffer Concentrate	12 ml	40 ml	3 x 40 ml
Instruction Booklet	1	1	1

Storage and Stability: All E.Z.N.A.®Gel Extraction Kit components are guaranteed for at least to 24 months from the date of purchase when stored at 22-25ºC. Ensure that the bottle of Binding Buffer is capped tightly when not in use.

Materials Supplied By User:

- Water bath equilibrated to 55-65ºC
- 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	Wash Buffer Concentrate must be diluted with absolute ethanol as follows:	
	D2501-00	Add 18 ml 100 % ethanol
	D2501-01 & D2501-02	Add 60 ml 100% ethanol to each bottle

Gel Extraction 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.

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** 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 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.*

3. 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 Binding Buffer equal to 3-4 X the gel volume. Incubate the mixture at 55ºC-65ºC for 7 min or until the gel has completely melted. Mix by shake or vortex the tube in every 2-3 minutes.

   Note: For isolation of DNA fragments <500 bp, add one gel volume of isopropanol once the gel has dissolved in Binding Buffer - i.e. for 0.2 g gel slice, add 0.8 ml Binding Buffer and 0.2 ml isopropanol. For isolation of DNA fragments >4 kb, add 1.5 gel volume of isopropanol once the gel has dissolved in Binding Buffer.

   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.

4. Apply 750 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 10,000 x g for 1 min at room temperature. Discard the liquid. For volumes greater than 800 µl load the column and centrifuge successively, 750 µl at a time. Each HiBind® extraction column has a total capacity of 25-30 µg DNA. If the expected yield is larger, divide the sample into the appropriate number of columns.

5 **Optional: Wash the column by adding 300 µl Binding buffer. Centrifuge at 10,000 x g for 1 min.**

6. Add 750 µl of Wash Buffer diluted with absolute ethanol into the column and wait 2-3 minutes. Centrifuge at 10,000 x g for 1 min at room temperature to wash the column..

7. Discard liquid and repeat step 5 with another 750 µl Wash Buffer.

8. 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.**

9. 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 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.
Note: efficiency of eluting DNA from column is dependent on pH. If eluting DNA with water, make sure that the pH is around 8.0.

10 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:

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

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 ($absorbance_{260}$)/($absorbance_{280}$) 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.

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

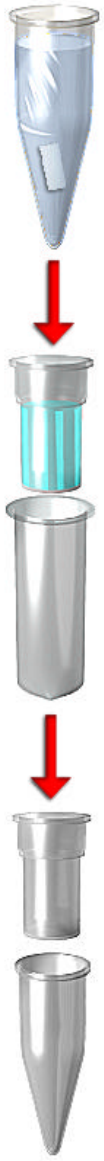
Note: Please read through previous section of this book before using this protocol.

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. **(Optional): Wash the column with 300 µl Binding buffer by vacuum. This step is necessary if downstream application is for auto sequencing.**
6. Wash the column by adding 750 µl DNA wash buffer, draw the wash buffer through the column by turn on the vacuum source. Repeat this step with another 750 µl DNA wash buffer.
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. Stand for 1-2 minute and centrifuge 1 minute to elute DNA.

Trouble Shooting Guide

Problem	Likely Cause	Suggestions
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 55oC to 65ºC 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 55ºC to 65ºC 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 Wash Buffer Concentrate as instructed above.
	Incorrect amount of Binding Buffer added.	Add 4.5-5 X volume of Binding Buffer. For DNA fragments <200 bp add 6 X volume Binding Buffer.
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 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. Excise gel slice containing DNA fragment of interest.
2. Determine volume of gel. Add Binding Buffer to 3-6 X vol gel slice.
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 in 800 µl portions.
5. Centrifuge at maximum speed 1 min at room temperature. Discard liquid.
6. Wash column twice with 750 µl Wash Buffer diluted with ethanol.
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.

Order Information on DNA/RNA Clean-up:

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

* 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.