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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 Omega Bio-Tek's (OBI) proprietary HiBind™ matrix that avidly, 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 The E.Z.N.A.[®] Fastfilter Plasmid Midiprep Kit combines the power of HiBind™ technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality DNA.. Omega Bio-Tek' s HiBind™ Midi-columns facilitate the binding, washing, and elution steps thus enabling multiple samples to be simultaneously processed. This kit also include a special filter cartridge, which replaces the centrifugation step following alkaline lysis. Following lysis the DNA is bound to the silica membrane and contaminants are removed with a simple wash step. Yields vary according to plasmid copy number, *E.coli* strain, and conditions of growth, but up to 200 µg of high quality plasmid can be purified from 50 overnight culture. The product is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, transfection of mammalian cells, and other manipulations.

Storage and Stability: All E.Z.N.A.[®] Plasmid isolation components are guaranteed for at least 12 months from the date of purchase when stored as follows: solution I (once RNase A is added) at 4°C, all other material at 22-25oC.

Kit Contents

Product Number	D6905-00	D6905-03	D6905-04
Purifications	2	25	100
HiBind™ DNA Midi Columns	2	25	100
Lysate Clearance filter syringe	2	25	100
Solution I	5 ml	35 ml	140 ml
Solution II	5 ml	35 ml	140 ml
Solution III	5 ml	50 ml	200 ml
Buffer HB	8 ml	90 ml	350 ml
Midi DNA Wash Buffer	6 ml	75 ml	2 x 150 ml
RNase A, Concentrate	30 µl	200 µl	800 µl
Instruction Booklet	1	1	1

Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

Supplied Laboratory centrifuge equipped with **swinging-bucket** rotor
By User: capable of 5000 x g.
 centrifuge capable of 5,000 x g
 Sterile 15 ml centrifuge tubes. (Falcon® tubes recommended.)
 Sterile 50 ml centrifuge tubes. (Falcon® tubes recommended.)
 Sterile deionized water (or TE buffer)
 Absolute (96%-100%) ethanol

IMPORTANT	1. Add vial of RNase A to bottle of Solution I provided. Store at 4°C.
	2. DNA Wash Buffer Concentrate is to be diluted with absolute ethanol as follows:
	D6905-00 Add 14 ml of 100% ethanol D6905-03 Add 175 ml of 100% ethanol
	D6905-04 Add 350 ml of 100% ethanol per bottle
Store diluted DNA Wash Buffer at room temperature	

E.Z.N.A.® Fastfilter Plasmid Midiprep Protocol (Centrifugation Protocol)

- Inoculate 75-100 ml LB/ampicillin (50 µg/ml) medium placed in a 1-4 liter culture flasks with *E.coli* carrying desired plasmid and grow at 37°C with agitation for 12-16 h. For best results use an overnight culture (~1 ml) as the inoculum. It is strongly recommended that an *endA* negative strain of *E.coli* be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®.
- Pellet up to 50 ml bacteria in appropriate vessels by centrifugation at 5,000 x g for 10 min at room temperature.
- Decant or aspirate medium and discard. To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the walls of the vessel. To the bacterial pellet add 1.25ml Solution I/RNase A. Resuspend cells completely by vortexing and/or pipetting. *Complete resuspension of cell pellet is vital for obtaining good yields.*
- Add 1.25 ml Solution II and gently mix by inverting and rotating tube 7-10 times to obtain a cleared lysate. A 3-5 min incubation at room temperature may be necessary. *Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity.* (Store Solution II tightly capped when not in use.)
- Prepare a Lysate Clearance Filter Syringe by removing the plunger and place the barrel in a tube rack to keep the syringe upright.
- Add 1.75 ml Solution III and gently mix by inverting several times until a flocculent white precipitate forms. This may require a 2-3 min incubation at room temperature with occasional mixing.
- Immediately pour the lysate into the barrel of the Lysate Clearance Filter Syringe. Allow the cell lysate to sit for 5 minutes. The white precipitate should float to the top. The cell lysate may start to pass through the filter, **use a new 50 ml tube (not supplied) to collect the cell lysate.** Insert the plunger back into the barrel of the syringe.
- Hold the filter syringe barrel over the 50 ml tube and gently insert the plunger to expel the cleared lysate to the tube. **Note: Some of the lysate may remain in the flocculent precipitate, do not force this residual lysate through the filter.**
- Add cleared lysate into the DNA midi column, centrifuge at 2000 x g for 3 minutes. Discard the flow-through and re-use the collection tube.

20°C.

10. Add 3.5ml Buffer HB to the DNA midi column. Centrifuge at 2000 x g for 2 minutes. Discard the flow-through and re-use the collection tube.
11. Add 4.0 ml DNA wash Buffer to the DNA Midi column and centrifuge at 2000 x g for 2 minutes. Discard the flow-through and re-use the collection tube.
12. Add 4.0 ml DNA wash Buffer to the DNA midi column and centrifuge at 3000 x g for 7 minutes. Discard the flow-through and collection tube
13. Transfer the DNA midi column to a new 15 ml collection tube (Not provided). Add 1 ml DNA Elution Buffer or water to the column. Centrifuge at 3000 x g for 3 minutes to elute the DNA.

E.Z.N.A.[®] Fastfilter Plasmid Midiprep Protocol (Vacuum Protocol)

1. Prepare cleared cell lysate by following step 1-8 of centrifugation procedure on page 4.
2. Transfer cleared cell lysate into the HiBind[®] DNA Midi column, be careful not to overfill the column, apply the vacuum to allow all sample pass through the column.
3. Add 3.5 ml HB buffer to the column and apply the vacuum to draw the liquid through the column.
4. Wash the column: add 4 ml of DNA wash buffer (pre-diluted with absolute ethanol) into the column and allow it pass through the column.
5. Wash the column again with 4 ml DNA wash buffer by repeating step 5. Keep the vacuum on for another 10 minutes after the liquid pass through the column. (This step to ensure the removal of residue ethanol).
6. Optional: Insert the HiBind[®] DNA Midi column to a 15 ml centrifuge tube and centrifuge at 3000 x g for 5 minutes to dry the column.
7. Transfer the HiBind[®] DNA Midi column to a new 15 ml centrifuge tube (not provided). Add 1 ml DNA elution Buffer or water to the column. Centrifuge at 3000 x g for 5 minutes to elute DNA. Second elution is optional.
8. Remove the column from collection tube and store the eluted DNA at -

Yield and quality of DNA: determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

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

High copy number plasmids generally yield up to 1 mg of DNA from 500 ml culture. The ratio of (Abs₂₆₀)/(Abs₂₈₀) gives an indication of nucleic acid purity. A value greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations. Typically, the majority of the DNA eluted is in monomeric supercoil form, though concatamers may also be present.

Trouble Shooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	<p>Only use LB or YT medium containing ampicillin. Do not use more than 100 ml.</p> <p>Cells may not be dispersed adequately prior to addition of Solution II. Vortex/ pipet cell suspension to completely disperse bacterial clumps.</p> <p>Increase incubation time with Solution II to obtain a clear lysate.</p> <p>Solution II if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS.</p>
	Bacterial culture overgrown or not fresh.	Do not incubate cultures for more than 16 hr at 37°C. Storage of cultures for extended periods prior to plasmid isolation is detrimental to yield and quality.
	Low copy-number plasmid used	Such plasmids may yield as little as 0.1µg DNA from a 1 ml overnight culture. Increase culture volume to 400 ml.
No DNA eluted.	DNA Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare Wash Buffer Concentrate as instructed on the label.
High molecular weight DNA contamination of product.	Over mixing of cell lysate upon addition of Solution II.	Do not vortex or mix aggressively after adding Solution II. Adequate mixing is obtained by simply inverting and rotating tube to cover walls with viscous lysate.
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 10-12. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantitation.
RNA visible on agarose gel.	RNase A not added to Solution I.	Add 1 vial of RNase to each bottle of Solution I.
Plasmid DNA floats out of well while loading agarose gel, does not freeze, or smells of ethanol.	Ethanol traces not completely removed from column following wash steps.	Centrifuge column at 3000 x g for 10 minutes to dry the column. A swinging-bucket rotor is recommended for centrifugation. Alternatively, precipitate the eluted DNA with isopropanol as indicated in step .

Ordering Information

Product No.	Product Name	Description
E.Z.N.A.® Plasmid Miniprep System		
D6942-01/02 D6943-01/02	Plasmid Miniprep Kit I	Isolation of up to 30µg plasmid in 15 minutes
D6945-01/02 D6946-01/02	Plasmid Miniprep Kit II	Isolation of up to 70µg plasmid in 15 minutes
D7042-01/02 D7043-01/02	High Performance Plasmid Miniprep Kit I	Isolation of up to 30µg plasmid from end A+ bacterial in 25 minutes
D7045-01/02 D7046-01/02	High Performance Plasmid Miniprep Kit II	Isolation of up to 70µg plasmid from end A+ bacterial in 25 minutes
E.Z.N.A.® Plasmid Midi/Maxi Isolation System		
D6904-03/04	Plasmid Midiprep Kit	Midipreps in spin column format. Yield up to 200µg plasmid
D6905-03/04	Fastfilter Plasmid Midiprep kit	Isolation of up to 200µg plasmid in less than 30 minutes
D6922-01/02	Plasmid Maxiprep Kit	Maxipreps in spin column format. Yield up to 1mg plasmid
D6924-01/02	Fastfilter Plasmid Maxiprep kit	Isolation of up to 1 mg plasmid in less than 30 minutes
E-Z 96® Plasmid Isolation System		
D1096-01/02	E-Z 96® Plasmid Isolation Kit	Isolation of plasmid in 96 well format
D1097-01/02	E-Z 96® Fastfilter Plasmid Isolation Kit	Isolation of plasmid in 96 well format with lysate clearance plate