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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 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 Plasmid Maxiprep 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 DNA Maxi-columns facilitate the binding, washing, and elution steps thus enabling multiple samples to be simultaneously processed. Yields vary according to plasmid copy number, *E.coli* strain, and conditions of growth, but 250-500 ml of overnight culture in LB medium typically produces 500-1000 µg high-copy plasmid DNA. Up to 500 ml culture may be processed when working with low-copy number plasmids. 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

E.Z.N.A.™ Plasmid Maxiprep Kit

Product Number	D6922-00	D6922-01	D6922-02
Purifications	2	5	20
HiBind™ DNA Maxi Columns	2	5	20
Solution I	20	40 ml	160 ml
Solution II	20	40 ml	160 ml
Solution III	30	55 ml	220 ml
Buffer HB	15	30 ml	110 ml
Wash Buffer Concentrate	12ml	36 ml	2 x 60 ml
RNase A, Concentrate	50 µl	100 µl	400 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 By User:
Laboratory centrifuge equipped with **swinging-bucket** rotor.
High speed centrifuge capable of 12,000 x g
Sterile 50 ml centrifuge tubes. (Falcon® tubes recommended.)
High speed centrifuge tubes (polycarbonate or Corex®)
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:

D6922-01	Add 84 ml 100% ethanol
D6922-02	Add 140 ml 100% ethanol per bottle

Store diluted DNA Wash Buffer at room temperature

Note: All steps must be carried out at room temperature.

E.Z.N.A.® Plasmid Maxiprep Protocol

1. Inoculate 200-500 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 a 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®.

2. Pellet up to 500 ml bacteria in appropriate vessels by centrifugation at 3,500 - 5,000 x g for 10 min at room temperature. A 250 ml centrifuge bottle is recommended.

3. 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 7.0 ml Solution I/RNase A. Resuspend cells completely by vortexing and/or pipetting. *Complete resuspension of cell pellet is vital for obtaining good yields.*

4. Transfer cell suspension to a 30 ml centrifuge tube capable of withstanding at least 12,000 x g (screw-cap polycarbonate or Corex® glass tubes will suffice). Add 7.0 ml Solution II and gently mix by inverting and rotating tube 7-10 times to obtain a cleared lysate. A 5-10 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.)

5. Add 10 ml Solution III and gently mix by inverting several times until a flocculent white precipitate forms. This may require a 5 min incubation at room temperature with occasional mixing. Centrifuge at 12,000 x g for 10 minutes at room temperature to pellet the cellular debris and genomic DNA. *A compact pellet should be obtained.*

6. CAREFULLY aspirate and add the clear supernatant to a clean HiBind® DNA Maxi column assembled in a 50 ml centrifuge tube (provided). Make sure that the pellet is not disturbed and no cellular debris is transferred as this may clog the column and reduce plasmid yield. It may be necessary to sacrifice 2-4 ml of the lysate when pipetting. The lysate may be passed through 4 layers of cheese cloth placed over the column. The Maxi column has a maximum capacity of 25 ml; for larger volumes add the lysate in two successive steps. Cap the tube and centrifuge 5 min at 3,000-4,000 x g in a swinging-bucket rotor at room temperature to completely pass lysate through column. Discard the flow-through liquid and reuse the 50 ml tube in step 6.

   IMPORTANT: THIS AND ALL SUBSEQUENT STEP MUST BE PERFORMED USING A CENTRIFUGE EQUIPPED WITH A SWINGING-BUCKET ROTOR. USING A FIXED ANGLE ROTOR WILL RESULT IN

BUCKLING OR CRACKING OF THE 50 ML CENTRIFUGE TUBE.

7. Add 5 ml Buffer HB to the Maxi column, cap the tube, and centrifuge 5 min at 3,000-4,000 x g as above. This step ensures that residual protein contamination is removed and must be included for downstream applications requiring high quality DNA. Discard flow-through liquid and reuse the 50 ml tube in the next step.

8. Wash the column by adding 10 ml of DNA Wash Buffer diluted with ethanol. Cap the tube and centrifuge 5 min at 3,000-4,000 x g at room temperature and discard flow-through.

   Note: Wash Buffer Concentrate must be diluted with absolute ethanol before use. See label for directions. *If refrigerated, DNA Wash Buffer must be brought to room temperature before use.*

9. *Optional step:* repeat wash step with another 10 ml DNA Wash Buffer and discard the flow-through liquid.

10. Wash the column with 10 ml absolute ethanol and centrifuge 5 min at 3,000-4,000 x g. Discard flow-through liquid.

11. Centrifuge the empty capped column for 10 min at 3,000-4,000 x g to dry the column matrix. **Do not skip this step - it is critical for removing traces of ethanol that may otherwise interfere with downstream applications.** Remove any traces of ethanol from the column using a pipette.

12. **Drying the column**: chose either of the methods below to further dry the column before eluting DNA.

12.a. Remove the column and place into a vacuum chamber at room temperature. Any device connected to a vacuum source may be used. Seal the chamber and apply vacuum for 15 min. Remove the column and proceed to step 13.

12.b. Bake the maxi column at 65°C in a vacuum oven or incubator for 10-15 minutes. If no vacuum chamber is available perform elution (step 13) now.

13. Place column into a clean 50 ml centrifuge tube. Add 1-2 ml (depending on desired final concentration) sterile deionized water (or TE buffer) onto the column matrix and centrifuge 5 min at 3,500 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. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration. Preheating the water to 70°C and allowing the column to soak 2 min at room temperature before elution may significantly increase yields.

    Note: remove any residue of the DNA wash buffer on the rim of the O-ring inside the maxi column is very important to get a good elution.

If the column was **NOT** vacuum-dried (step 12.a.) prior to elution, precipitate the plasmid DNA for final clean-up. To do so add sodium chloride to the eluate to a final concentration of 200 mM followed by 0.8 volumes of isopropanol. Vortex to mix and centrifuge at 10,000 x g for 10 min. Wash the DNA pellet once with 70% ethanol and centrifuge again at 10,000 x g for 10 min. Decant and discard supernatant and briefly air-dry pellet. Finally resuspend DNA pellet in 200 µl-500 µl sterile deionized water or TE buffer.

14. **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:

    DNA concentration = Absorbance260 × 50 × (Dilution Factor) µg/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	Only use LB or YT medium containing ampicillin. Do not use more than 500 ml. Cells may not be dispersed adequately prior to addition of Solution II. Vortex/ pipet cell suspension to completely disperse bacterial clumps. Increase incubation time with Solution II to obtain a clear lysate. Solution II if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS.
	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 500 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 7-9. 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 as instructed in step 10 or vacuum as indicated to dry. 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 Performace Plasmid Miniprep Kit I	Isolation of up to 30µg plasmid from end A+ bacterial in 25 minutes
D7045-01/02 D7046-01/02	High Performace 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		
D6904-01/02	Plasmid Midiprep Kit	Midipreps in spin column format. Yield up to 200µg plasmid
D6922-01/02	Plasmid Maxiprep Kit	Maxipreps in spin column format. Yield up to 1mg plasmid
D7004-01/02	High Performace Plasmid Miniprep Kit	Isolation of up to 200µg plasmid from end A+ bacterial strains.
E-Z 96® Plasmid Isolation System		
D1096-01/02	96 well Plasmid Kit	Isolation of plasmid in 96 well format

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References

1. Ausubel, F.M. et al. (1989) Current Protocols in Molecular Biology, Vol. 2, John Wiley & Sons, New York.
2. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Laboratory, Cold Spring Harbor, New York.
3. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1982) Molecular Cloning: A Laboratory Manual, 1st edition, Cold Spring Laboratory, Cold Spring Harbor, New York.

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