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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 Midiprep Kit combines the power of HiBind™ Midi-spin column technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality DNA. Omega Biotek's midi-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 50 ml of overnight culture in LB medium typically produces 100-200 µg high-copy plasmid DNA. Up to 100 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.

The E.Z.N.A.® High Performance Plasmid Purification System is the modified version of E.Z.N.A plasmid isolation system which is designed specially for those applications when high quality plasmid is required such as transfection, autosequencing, etc. It also suitable for isolating plasmid from bacterial hosts (such as EndoA+ strains) with high level of endonuclease activity. The plasmid from this system has much better stability for long term storage.

New in this edition

The following changes have been made to the E.Z.N.A.® High performance Plasmid Midiprep procedure for improving yield and purity.

- New midi spin column introduced.
- DNA Wash Buffer has been improved.
- Only one wash step required with DNA Wash Buffer.
- A second wash step with absolute ethanol is introduced.

Storage and Stability: All E.Z.N.A.® High performance Plasmid isolation components are guaranteed for at least 12 months from the date of purchase when stored as follows: Solution I/RNase A at 4°C and OB protease mixtures at -20°C after received, all other material at 22-25°C.

Kit Contents
E.Z.N.A.™ (HP) Plasmid Midiprep Kit

Product Number	D7004-00	D7004-01	D7004-02
Purifications	2	10	50
HiBind™ DNA Midi	2	10	50
8 ml collection tubes	2	10	50
Solution I	10 ml	35 ml	160 ml
Solution II	10 ml	35 ml	160 ml
Solution III	10 ml	50 ml	245 ml
Buffer HB	10 ml	40 ml	200 ml
Wash Buffer	12 ml	36 ml	2 x 75 ml
OB Proteases Mixture	3 ug	15 ug	75 ug
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:

High speed centrifuge capable of 12,000 x g
Sterile 15 ml centrifuge tubes. (Falcon® tubes recommended.)
High speed centrifuge tubes (polycarbonate or Corex®)
Sterile deionized water (or TE buffer)
Absolute (95%-100%) ethanol

IMPORTANT	1. Add vial of RNase A to bottle of Solution I provided. Store at 4°C. 2. Dilute supplied OB Protease Mixture with deionized water as follows: D7004-00: Add 100 ul water or TE D7004-01: Add 500 ul water or TE D7004-02 Dissolve with 2.5 ml water or TE
	3. DNA Wash Buffer Concentrate is to be diluted with absolute ethanol as follows: D7004-00 Add 18 ml 100% ethanol D7004-01 Add 84 ml 100% ethanol D7004-02 Add 175 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.® High Performance Midiprep Protocol

1. **Culture volume:** innoculate 50 ml LB/ampicillin (50 µg/ml) medium placed in a 250 ml culture flask with *E.coli* carrying desired plasmid and grow at 37°C with agitation for 12-16 h.

2. Pellet bacteria by centrifugation at 4,000 x g for 10 min at room temperature**.**

3. Decant or aspirate medium and discard. To the bacterial pellet add 2.25 ml Solution I/RNase A. Resuspend cells **completely** by vortexing and/or pipetting. Complete resuspension of cell pellet is vital for obtaining good plasmid yields.

4. Transfer cell suspension to a 15-30 ml centrifuge tube capable of withstanding 12,000 x g (screw-cap polycarbonate or Corex® glass tubes will suffice). Add 2.25 ml Solution II and 50 µl of OB Protease Mixtures, cover, and gently mix by inverting and rotating tube 7-10 times to obtain a cleared lysate. Incubated 10-20 min at room temperature. *Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity.* Prolonged incubation may lead to nicking of plasmid DNA. (Store Solution II tightly capped when not in use.)

5. Add 3.2 ml Solution III, cover, and gently mix by inverting several times until a flocculent white precipitate forms. Centrifuge at 12,000 x g for 10 minutes at room temperature to pellet the cellular debris and genomic DNA.

6. CAREFULLY aspirate and add 3.75 ml of the clear supernatant to a clean

HiBind® DNA Midi column assembled in an 8 ml collecting tube making sure that no cellular debris is carried over. The Midi column has a maximum capacity of 4 ml. Centrifuge 5 min at 5,000-8,000 x g at room temperature to completely pass lysate through column. Discard the flow-through liquid and add an additional 3.75 ml of cleared lysate to the column. Centrifuge as above and repeat until the entire sample has been passed through. Finally discard the flow-through and reuse the collecting tube in step 7.

IMPORTANT: This and all subsequent steps must be performed using a centrifuge capable of at least 5,000 x g. Ensure that an appropriate rotor adaptor is in place to prevent damage to the collecting tube.

7. Add 3.50 ml Buffer HB to the Midi column and centrifuge 5 min at 5,000-8,000 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 collecting tube in the next step.

8. Wash the column by adding **3.5** ml of DNA Wash Buffer diluted with ethanol. Centrifuge 5 min at 5,000-7,000 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, Wash Buffer must be brought to room temperature before use.

9. **Optional step**: repeat wash step with another 3.5 ml DNA Wash Buffer. Centrifuge as above and discard fluid.

10. Wash the column with 3.5 ml absolute ethanol and centrifuge 5 min at 5,000-7,000 x g at room temperature. Discard flow-through liquid.

11. Centrifuge the empty capped column for 10 min at 5,000-7,000 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.
A. Place the column into a vacuum container to dry the ethanol for 10 minutes.
B. Bake the column in a vacuum oven at 65°C for 10 minutes.

13. Place column into a clean 15 ml centrifuge tube. Add 0.5-1.0 ml (depending on desired concentration of final product) sterile deionized water (or TE buffer) directly onto the column matrix. Allow column to sit 2 min at room temperature. Centrifuge 5 min at 5,000-7,000 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. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration. Also, preheating the water to 70°C prior to elution may significantly increase yields.

14. **Yield and quality of DNA:** determine the absorbance of an appropriate dilution (10- to 20-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

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

High copy number plasmids generally yield up to 200 µg of DNA from 50 ml culture. 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, 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.

Low Copy Number Plasmids

Such plasmids can sometimes yield as little as 0.1 µg DNA per 1 ml culture. To increase yields, innoculate up to 100 ml LB medium and incubate at 37°C for 12-16 h in a 500 ml flask. Adequate aeration can be achieved with a shaker set to 220-250 rpm. Follow the protocol above (page 4), ensuring that in step 3, the cells are completely resuspended in Solution I/RNase A. Also make sure that the cells have been completely lysed in step 4.

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 50 ml with high copy plasmids. Cells may not be dispersed adequately prior to addition of Solution II. Vortex cell suspension to completely disperse. 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.
	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 100 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	Ethanol not completely removed from column following wash steps.	Processing column as instructed in step 12 to dry .
Plasmid DNA will not perform in downstream application	Traces of ethanol remain on column prior to elution.	The column must be washed with absolute ethanol (step 10) and dried before elution. Ethanol precipitation may be required following elution.
Smear or degraded Plasmid observed from gel analysis	Protease Mixture not added or more protease is needed.	Add more protease Mixture and try to extend incubation time.

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

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.