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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® technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality plasmid DNA. Omega Bio-Tek'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, and other manipulations.

New in this edition

The following changes have been made to the E.Z.N.A.® 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.

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/RNase A at 4°C, all other material at 22-25°C.

Kit Contents

E.Z.N.A.® Plasmid Midiprep Kit

Product Number	D6904-00	D6904-03	D6904-04
Purifications	2	25	100
HiBind® DNA Midi Columns	2	25	100
15 ml collection tubes	2	25	100
Solution I	5 ml	35 ml	150 ml
Solution II	5 ml	35 ml	150 ml
Solution III	5 ml	50 ml	200 ml
Buffer HB	8 ml	80 ml	350 ml
Wash Buffer Concentrate	2 ml	25 ml	2 x 100 ml
RNase A, Concentrate	25 µl	150 µl	600 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
Swing-bucket centrifuge capable of 5000 x g with adaptor for 15 and 50ml tube.
Sterile 15 & 50ml 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. DNA Wash Buffer Concentrate is to be diluted with absolute ethanol as follows: D6904-00 Add 8 ml 100% ethanol D6904-03 Add 100 ml 100% ethanol D6904-04 Add 400 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 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.

   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 bacteria by centrifugation at 4,000 -5000 x g for 10 min at room temperature.

3. Decant or aspirate medium and discard. To the bacterial pellet add 1.25 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 1.25 ml Solution II, cover, and gently mix by inverting and rotating tube 7-10 times to obtain a cleared lysate. A 5 min incubation at room temperature may be necessary. *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 1.75 ml Solution III, cover, and gently mix by inverting several times until a flocculent white precipitate forms. Centrifuge at 12,000 x g for 15 minutes at room temperature to pellet the cellular debris and genomic DNA.

6. CAREFULLY aspirate and apply the clear supernatant to a HiBind® DNA Midi column assembled in an 15 ml collecting tube making sure that no cellular debris is carried over. The Midi column has a maximum capacity of 4.5 ml. Centrifuge 5 min at 5,000-8,000 x g at room temperature to completely pass lysate through column. 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 4,000 x g. Ensure that an appropriate rotor adaptor is in place to prevent damage to the collecting tube.

7. Add 3 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 4 ml of DNA Wash Buffer diluted with ethanol. Centrifuge 5 min at 5,000-8,000 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 4 ml DNA Wash Buffer. Centrifuge as above and discard fluid.

10 Centrifuge the empty capped column for 10 min at 5,000 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.

11. Place column into a clean 15 ml centrifuge tube. Add 1.0-1.5 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 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.

    If the column was **NOT** completely dried (step 10.) prior to elution, some ethanol from DNA Wash Buffer might be carried over to the eluted DNA and it is necessary to precipitate the plasmid DNA for final clean-up. To do so add sodium acetate to the eluate to a final concentration of 0.3 M followed by 2X volumes of absolute ethanol. Vortex to mix and centrifuge at 10,000 x g for 10 min. Wash the DNA pellet once with 5-10 ml 70% ethanol and centrifuge again at 10,000 x g for 10 min. Decant and discard supernatant and air-dry pellet. Finally resuspend DNA pellet in 100 µl-200 µl sterile deionized water or TE buffer.

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$_{260}$ × 50 × (Dilution Factor) µ*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.

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 37ºC. 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 quantization.
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.	Centrifuge column as instructed in step 10 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.

Ordering Information

PRODUCT NO.	PRODUCT NAME	DESCRIPTION
E.Z.N.A.® PLASMID MINIPREP SYSTEM		
D6942-01/02 D6943-01/02 D6944-01/02	PLASMID MINIPREP KIT I	ISOLATION OF UP TO 30MG PLASMID IN 15 MINUTES
D6945-01/02 D6946-01/02 D6947-01/02	PLASMID MINIPREP KIT II	ISOLATION OF UP TO 70MG PLASMID IN 15 MINUTES
D7042-01/02 D7043-01/02	HIGH PERFORMACE PLASMID MINIPREP KIT I	ISOLATION OF UP TO 30MG PLASMID FROM END A+ BACTERIAL IN 25 MINUTES
D7045-01/02 D7046-01/02	HIGH PERFORMACE PLASMID MINIPREP KIT II	ISOLATION OF UP TO 70MG 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 200MG PLASMID
D6905-01/02	FASTFILTER PLASMID MAXIPREP KIT	30 MINUTES PLASMID MAXI PREPARATION WITH FASTFILTER UNITE. YIELD UP TO 0.2 MG PLASMID
D6922-01/02	PLASMID MAXIPREP KIT	MAXIPREPS IN SPIN COLUMN FORMAT. YIELD UP TO 1MG PLASMID
D6924-01/02	FASTFILTER PLASMID MAXIPREP KIT	30 MINUTES PLASMID MAXI PREPARATION WITH FASTFILTER UNITE. YIELD UP TO 1MG PLASMID
D7004-01/02	HIGH PERFORMACE PLASMID MINIPREP KIT	ISOLATION OF UP TO 200MG PLASMID FROM END A+ BACTERIAL STRAINS.
E-Z 96® PLASMID ISOLATION SYSTEM		
D1097-01/02	FASTFILTER® 96 WELL PLASMID KIT	ISOLATION OF PLASMID IN 96 WELL FORMAT WITH LYSATE CLEARANCE PLATE