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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 Biotek'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.

Applications such as transfection normally requires large quantity of plasmid that meed a number of stringent criteria, include high degree of purity and high percentage of supercoiling. The most common difficulty for large scale plasmid isolation is the contaminant of endotoxin which can have a negative effect on downstream applications such as transfection. The E.Z.N.A.® Endo-Free Plasmid Minipre Kit combines the power of HiBind™ technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality DNA with lower level of endotoxin. Omega Bio-Tek' s HiBind™ Maxi-columns facilitate the binding, washing, and elution steps thus enabling multiple samples to be simultaneously processed. Following the lysis, the cleared lysate are extracted with ETR Buffer which will almost completely remove the endotoxin, Then the lysate is applied to the HiBind™ Mini columns , plasmid DNA tis 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 30µg of high quality plasmid can be purified from 5 ml overnight culture. The product is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, transfection of mammalian cells, and other manipulations. .

Benefits

The E.Z.N.A.® Plasmid Miniprep Kit means:

- Speed - Plasmid DNA isolation in <15 min
- Reliability - optimized buffers guarantee pure DNA everytime
- Safety - No organic extractions
- Quality - purified DNA suitable for any application

Storage and Stability: All E.Z.N.A.™Plasmid Miniprep components are guaranteed for at least 12 months from the date of purchase when stored as follows: The mixture of Solution I/RNase A at 4°C, all other material at 22-25°C.

Kit Contents

E.Z.N.A.™ Plasmid Miniprep Kit

Product Number	D6948-00	D6948-01	D6948-02
Purifications	5	50	200
HiBind™ Miniprep Columns (I)	5	50	200
2 ml Collection Tubes	5	50	200
Solution I	5 ml	20 ml	60 ml
Solution II	5 ml	20 ml	60 ml
Neutralization Buffer	5 ml	20 ml	80 ml
ETR Reagent	5 ml	20 ml	80 ml
GBT Buffer	0.6 ml	6 ml	25 ml
Buffer HB	5 ml	25 ml	100 ml
Wash Buffer Concentrate	12 ml	40 ml	3 x 40 ml
RNase A, Concentrate	50 µl	100 µl	400 µl
Instruction Booklet	1	1	1

E.Z.N.A.™ Plasmid Miniprep Kit II

Product Number	D6950-00	D6950-01	D6950-02
Purifications	5	50	200
HiBind™ Miniprep Columns(II)	5	50	200
2 ml Collection Tubes	5	50	200
Solution I	5 ml	30 ml	120 ml
Solution II	5 ml	30 ml	120 ml
Neutralization Buffer	5 ml	40 ml	2 x 80 ml
Buffer HB	5 ml	30 ml	120 ml
GBT Buffer	5 ml	30 ml	120 ml
ETR Reagent	1.2 ml	12 ml	45 ml
Wash Buffer Concentrate	12 ml	40 ml	3 x 40 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:

Microcentrifuge capable of at least 10,000 x g.

Sterile 1.5 ml centrifuge tubes.

Sterile deionized water (or TE buffer)

Absolute (96%-100%) ethanol

15 ml centrifuge tubes (for Product No. D6950 only)

Centrifuge with swinging bucket rotor (D6950 only)

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:	
	D6948-00 D6945-00	Add 18 ml 100% ethanol
	D6948-01 D6950-01	Add 60 ml 100% ethanol to each bottle
	D6948-02 D6950-02	Add 60 ml 100% ethanol
	Store diluted DNA Wash Buffer at room temperature	

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

E.Z.N.A.™ Endo-Free Plasmid Miniprep Protocol

Product Number **D6948-01**

1. **Inoculate 5 ml LB/ampicillin (50 µg/ml) medium placed in a 10-20 ml culture tube 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 1.5-5 ml bacteria by centrifugation at 10,000 x g for 1 min at room temperature.**

3. **Decant or aspirate medium and discard. To the bacterial pellet add 250 µl Solution I/RNase A. Resuspend cells completely** by vortexing. Complete resuspension of cell pellet is vital for obtaining good yields.

4. **Add 250 µl Solution II and gently mix by inverting and rotating tube 4-6 times to obtain a cleared lysate.** A 2 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 250 µl Neutralization Buffer and gently mix by inverting several times until a flocculent white precipitate forms.** Centrifuge at 10,000 xg for 10 minutes at room temperature.

6. **CAREFULLY aspirate and transfer the cleared supernatant to a clean** 1.5 ml centrifuge tube and add 0.1 volume of ETR reagent to the cleared lysate. **Mix by inverting the tube 7-10 times and incubate in ice for 20 minutes. Note: After addition of ETR Reagent, the lysate should appear turbid, but it should became clear after incubation on ice.**

7. Incubate the lysate at 37° C for 5 minutes. The lysate should appear turbid again. Centrifuge at 10,000 x g for 5 minutes at 25° C. The ETR Reagent will form a blue layer at bottom of tube.

8. Transfer the top aqueous phase (cleared lysate) into a new 1.5 ml tube and add **250 µl of GBT Buffer** and gently mix by invert 1-2 times. **Note: avoid transferring any the blue ETR reagent since it contains high concentrated LPS.**

9. **CAREFULLY aspirate and add the clear supernatant to a clean Type I HiBind™ miniprep column (blue) assembled in a 2 ml collection tube (provided)**. Centrifuge 1 min at 10,000 x g at room temperature to completely pass lysate through column.

10. Discard the flow-through and load remaining of sample into the column and centrifuge at 10,000 x g. Discard the flow-through and re-use the collection tube.

11. Wash column with 500 µl Buffer HB and Centrifuge 1 min at 10,000 x g. This step ensures that residual protein contamination is removed and must be included for downstream applications requiring high quality DNA.

12 Discard flow-through liquid and wash the column by adding 750 µl of Wash Buffer diluted with ethanol. Centrifuge 1 min at 10,000 x g as above 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.

13 Optional step: repeat wash step with another 750 µl Wash Buffer.

14 Centrifuge the empty column for 1 min at 10,000 x g to dry the column matrix. **Do not skip this step - it is critical for removing ethanol from the column.**

15 **Place column into a clean 1.5 ml microcentrifuge tube. Add 50 µl to 100 µl (depending on desired concentration of final product) sterile deionized water (or TE buffer) directly onto the column matrix and centrifuge 1 min at 10,000 x g to elute DNA.** This represents approximately 75-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

16. **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:

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

High copy number plasmids generally yield up to 25 µg of DNA from 5 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.

E.Z.N.A.™ Endo-free Plasmid Miniprep Kit II

Product Number **D6950**

Note: Using the following protocol with product No. D6942 will not improve yields significantly with high-copy-plasmids due to the lower column binding capacity.

The E.Z.N.A.™ Plasmid Miniprep Kit II allows rapid and reliable isolation of greater than 50 µg plasmid DNA using the spin-column format. There is no need for organic extractions or alcohol precipitations, and the purified DNA is suitable for many downstream applications including double stranded DNA sequencing.

Procedure
Before starting, we recommend you refer to page 4 of this booklet for important information on preparation of components and required materials.

1. **Inoculate 10-15 ml LB/ampicillin (50 µg/ml) medium placed in a 50 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®. For low-copy plasmids use no more than 25 ml medium.

2. **Pellet bacteria by centrifugation at 5,000 x g for 10 min at room temperature preferably in a swinging bucket rotor.**

3. **Decant or aspirate medium and discard. To the bacterial pellet add 500 µl Solution I/RNase A. Resuspend cells completely** by vortexing or pipetting up and down. Complete resuspension of the cell pellet is vital for

obtaining good yields.

4. **Transfer cell suspension to a 2 ml microfuge tube and add 500 µl Solution II. Gently mix by inverting and rotating tube several 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. (Store Solution II tightly capped when not in use.)

5. **Add 700 µl Neutralization Buffer and gently mix by inverting several times until a flocculent white precipitate forms.** Centrifuge at 10,000 x g for 10 minutes at room temperature.

6. **CAREFULLY aspirate and transfer the cleared supernatant to a clean** 2.0 ml centrifuge tube and add 0.1 volume of ETR reagent to the cleared lysate. **Mix by inverting the tube 7-10 times and incubate in ice for 20 minutes. Note: After addition of ETR Reagent, the lysate should appear turbid, but it should became clear after incubation on ice**.

7. Incubate the lysate at 37° C for 5 minutes. The lysate should appear turbid again. Centrifuge at 10,000 x g for 5 minutes at 25° C. The ETR Reagent will form a blue layer at bottom of tube.

8. Transfer the top aqueous phase (cleared lysate) into a new 1.5 ml tube and add **500 µl of GBT Buffer** and gently mix by invert 1-2 times. **Note: avoid transferring any the blue ETR reagent since it contains high concentrated LPS.**

9. **CAREFULLY aspirate and add 750 µl of the clear supernatant to a clean Type II HiBind™ miniprep column (purple) assembled in a 2 ml collection tube (provided).** Ensure that the pellet is not disturbed and that no cellular debris is carried over into the column. Centrifuge 1 min at 10,000 x g at room temperature to completely pass lysate through column. Discard the flow-through liquid and add the remaining lysate to the column and centrifuge as above.

10. Discard liquid and wash column with 500 µl Buffer HB and centrifuge 1 min at 10,000 x g.This step ensures that residual protein contamination is removed and must be included for downstream applications requiring high quality DNA.

11. Discard flow-through liquid and wash the column by adding 750 µl of Wash Buffer diluted with ethanol. Centrifuge 1 min at 10,000 x g as above 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.

12. Optional step: repeat wash step with another 750 µl DNA Wash Buffer.

13. Centrifuge the empty column for 1 min at 10,000 x g to dry the column matrix. **Do not skip this step - it is critical for removing ethanol from the column.**

14. **Place column into a clean 1.5 ml microcentrifuge tube. Add 50 µl to 100 µl (depending on desired concentration of final product and plasmid copy-number) sterile deionized water (or TE buffer) directly onto the column matrix and centrifuge 1 min at 10,000 x g to elute DNA.** This represents approximately 75-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

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 5 ml (with high copy plasmids or 10 ml with low copy plasmids) culture with the basic protocol. 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 0.5µg DNA from a 5 ml overnight culture. Increase culture volume to 10 ml and follow suggested modifications with product No. D6942 or use the Plasmid Miniprep Kit II with 25 ml culture.
No DNA eluted.	Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare Wash Buffer Concentrate as instructed above.
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 and 8. 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.	Centrifuge column as instructed before elution