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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-teks 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 Mini Kit combines the power of HiBind™ technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality DNA in under 20 minutes. Omega Bioteks mini-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 5 ml of overnight culture in LB medium typically yields 15-25 µg plasmid DNA with the Mini Kit I (D7042/3). The High performance Plasmid Mini Kit II (D7045) uses the same mini spin module format but is upscaled for isolation of low copy-number plasmids and yields 40-75 µg DNA from 10-15 ml culture when using high copy number plasmids. Mini Kit I is simple, efficient, and fast to facilitate screening of recombinant clones. Meanwhile Mini Kit II employs an additional wash step that produces high quality DNA 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 end A+ strains) with high level of endonuclease activity. The plasmid from this system has much better stability for long term storage.

Storage and Stability

All E.Z.N.A.™ High performance Plasmid Mini Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: **Solution I/RNase A mixture at 4°C, and protease mixture at -20°C**. all other material at 22-25°C.

Kit Contents

E.Z.N.A.™ HP (High Performance) Plasmid Mini Kit I

Product Number	D7042-00 D7043-00	D7042-01 D7043-01	D7042-02 D7043-02
Purification times	5 preps	50 preps	200 preps
HiBind™ DNA Minicolumn	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
Solution III	5 ml	25 ml	80 ml
Buffer HB	5 ml	30 ml	120 ml
DNA Wash Buffer Concentrate	2 ml	20 ml	3 × 20 ml
RNase A, Concentrate	50 µl	100 µl	400 µl
OB Protease	1.5 mg	15 mg	75 mg
Instruction Booklet	1	1	1

E.Z.N.A.™ HP (High Performance) Plasmid Mini Kit II

Product Number	D7045-00 D7046-00	D7045-01 D7046-01	D7045-02 D7046-02
Purifications	5 preps	50 preps	200 preps
HiBind™ DNA Minicolumn 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
Solution III	5 ml	40 ml	2 x 80 ml
Buffer HB	5 ml	30 ml	120 ml
DNA Wash Buffer Concentrate	2 ml	20 ml	3 × 20 ml
RNase A, Concentrate	50 µl	100 µl	400 µl
OB Protease	1.5 mg	15 mg	75 mg
Instruction Booklet	1	1	1

Column Specifications

Plasmid Kits	Kit I	Kit II
Product Number	**D7042** **D7043**	**D7045** **D7046**
Maximum Volume	750 µl	750 µl
Plasmid Binding Capacity	30 µg	75 µg
Yield:		
5 ml cultures (high copy number plasmids)	20-25 µg	20-25 µg
15 ml cultures (high copy number plasmids)	20-30 µg	60-70 µg
Applications:		
Screening Minipreps	✓✓✓	✓✓
DNA Sequencing	✓✓✓	✓✓✓
Subcloning - enzymatic reactions	✓✓	✓✓✓
Transfections	✓✓	✓✓✓

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 × g.
Sterile 1.5 ml & 2 ml centrifuge tubes.
Absolute (96%-100%) ethanol

IMPORTANT

1. **Dilute OB Protease with sterile deionized water** as follows: 70 µl (5 preps), 550 µl (50 preps) and 2.1ml (200 preps). Vortex gently to dissolve and store at -20°C.
2. **Add vial of RNase A to bottle of Solution I provided and store at 4°C.**
3. **DNA Wash Buffer Concentrate** is to be diluted with absolute ethanol (96-100%)as follows:

D7042-00, D7043-00 D7045-00, D7046-00	Add 8 ml absolute ethanol
D7042-01, D7043-01 D7045-01, D7046-01	Add 80 ml absolute ethanol
D7042-02, D7043-02 D7045-02, D7046-02	Add 80 ml absolute ethanol to each bottle

E.Z.N.A.™ HP Plasmid Mini Kit I Protocol

Product Number D7042/D7043

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** and resuspend cells by vortexing or pipetting. Complete resuspension of cell pellet is vital for obtaining good yields.

4. **Add 250 µl Solution II and 10 µl of OB protease, gently mix by inverting and rotating tube 4-6 times to obtain a cleared lysate. A 10 min incubation at room temperature is required**. Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. (Store Solution II tightly capped when not in use.)

5. **Add 350 µl Solution III 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 add the clear supernatant to a clean HiBind™ DNA Minicolumn I 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 for 1 min at 10,000 x g at room temperature to completely pass lysate through column.

7 **Discard liquid and wash column by adding 500 µl Buffer HB to the column.** Centrifuge for 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.

8. **Discard liquid and wash column with 700 µl of DNA Wash Buffer diluted with ethanol.** Centrifuge for 1 min at 10,000 x g and discard flow-through.

   Note: DNA 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 700 µl DNA Wash Buffer**.

10. **Centrifuge the empty column for 2 min at maximum speed (≥13,000 x g) to dry the column matrix.** Do not skip this step - it is critical for removing ethanol from the column.

11. **Place column into a clean 1.5 ml microcentrifuge tube. Add 30-50 µl (depending on desired concentration of final product) sterile deionized water (or TE buffer) directly onto the column matrix and let it sit at room temperature for 2 minutes.** Centrifuge for 1 min at 10,000 x g to elute DNA. This represents approximately 70-85% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

12. **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:

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

Low Copy-Number Plasmids

Low copy number plasmids generally give 0.1-0.5 µg DNA per ml overnight culture. For routine screening of recombinant clones, 5 ml culture should provide ample material for agarose gel visualization or restriction digest analysis. However, the method can be modified to essentially double the yield if necessary. Start with 10-15 ml bacterial culture, and pellet cells either successively 1.5 ml of culture at a time, or centrifuge for 10 min at 5,000 x g in a 15 ml centrifuge tube. Proceed to step 3 (page 5) and double the volumes of Solutions I, II, and III. Continue as above using only one HiBind™ DNA column per 10 ml culture. There is no need to increase the volume of Wash Buffer used.

Note: This method is not recommended for high copy number plasmids because above 5 ml culture, the HiBind™ mini-column quickly becomes saturated. In this situation we recommend processing of multiple samples from the same culture. Alternatively, use the E.Z.N.A.™ High performance Plasmid Mini Kit II (product No. D7045), a new member of the EaZy Nucleic Acid family that allows processing of 10-15 ml cultures using the mini-column format and generally yields up to 70 µg plasmid DNA with high-copy number plasmids.

Vacuum/Spin Protocol for Plasmid Extraction (V-Spin column only)

Carry out cell culture, lysis and neutralization as indicated previous protocols (step 1-5). Instead of continuing with centrifugation, follow steps blow.

Note: Please read through previous section of this book before using this protocol.

1. **Prepare the vacuum manifold according to manufacturer' s instructions and connect the V-Spin column to the manifold.**

2. **Load the clear supernatant from step 5 (page 5) to the V-Spin column.**

3. **Switch on vacuum source to draw the sample through the column** and add the remaining lysate to the column. Turn off the vacuum when all of the sample have passed through the column.

4. **Wash the column by adding 500 µl HB Buffer;** draw the buffer through the column by turning on the vacuum source.

5. **Wash the column by adding 700 µl DNA wash buffer diluted with absolute ethanol;** draw the wash buffer through the column by turning on the vacuum source. Repeat this step with another 700 µl DNA wash buffer.

6. **Assemble the column into a 2 ml collection tube and transfer the column to a micro centrifuge. Spin for 2 minute at maximum speed (≥13,000xg) to dry the column.**

7. **Place the column in a clean 1.5 ml microcentrifuge tube and add 30-50µl TE or water. Stand for 1-2 minute and centrifuge 1 minute to elute DNA.**

E.Z.N.A.™ HP Plasmid Mini Kit II Protocol

Product Number D7045,D7046

Note: Using the following protocol with product No. D7042, D7043 will not improve yields significantly with high-copy-number plasmid due to the lower column binding capacity (see column specifications on page 4).

The E.Z.N.A.™ High performance Plasmid Mini 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 number plasmids use no more than 25 ml medium.

2. **Pellet 10-15 bacteria by centrifugation at 5,000 × 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 and 10 µl OB protease.** Gently mix by inverting and rotating the tube several times to obtain a cleared lysate. A 10-15 min incubation at room temperature is required. 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 Solution III and gently mix by inverting several times until a flocculent white precipitate forms.** Centrifuge at ≥10,000 × g for 10 minutes at room temperature.

6. **CAREFULLY aspirate and add 700 µl of the clear supernatant to a clean HiBind™ DNA Minicolumn II 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 for 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.

7. **Discard liquid and wash column by adding 500 µl Buffer HB to the column.** Centrifuge for 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.

8. **Discard flow-through liquid and wash the column by adding 700 µl of DNA Wash Buffer diluted with ethanol.** Centrifuge for 1 min at 10,000 × g as above and discard flow-through.

   Note: DNA 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 700 µl DNA Wash Buffer**.

10. **Centrifuge the empty column for 2 min at maxi speed (≥13,000×g) to dry the column matrix.** Do not skip this step - it is critical for removing ethanol from the column.

11. **Place column into a clean 1.5 ml microcentrifuge tube. Add 80-100 µl (depending on desired concentration of final product and plasmid copy-number) sterile deionized water (or TE buffer) directly onto the column matrix and let it sit at room temperature for 2 minutes. Centrifuge for 1 min at ≥13,000 x g to elute DNA.** This represents approximately 70-85% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. Alternatively, apply the first eluate to the column to elute a second time.

12. **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 = Absorbance$_{260}$ × 50 × (Dilution Factor) µg/ml

Short Protocol For Experienced Users

Note: All steps are to be performed at room temperature. Refer to page 4 for important notes on preparation of components.

1. Pellet cells from 1.5-5 ml (Kit I) or 10-15 ml (Kit II) overnight culture.
2. Resuspend cells in 250 µl (Kit I) or 500 µl (Kit II) Solution I/RNase A.
3. Add 250 µl (Kit I) or 500 µl (Kit II) Solution II with 10 µl protease. Mix gently by inverting 4-6 times to obtain cleared lysate. A brief incubation at RT may be required.
4. Add 350 µl (Kit I) or 700 µl (Kit II) Solution III and mix well to form white precipitate.
5. Centrifuge at maximum (at least 10,000 x g) speed 10 min.
6. Transfer cleared lysate to a HiBind™ DNA Minicolumn I or II placed in a 2 ml collection tube. Centrifuge for 1 min at maxi speed. Discard liquid.
7. Wash column with 500 µl Buffer HB. Centrifuge 1 min at maxi speed. Discard liquid.
8. Using same collecting tube, wash column with 700 µl DNA Wash Buffer diluted with ethanol. Centrifuge 1 min at maxi speed.
9. Optional: Wash column a second time with 700 µl DNA Wash Buffer.
10. Centrifuge empty column 1 min at maxi speed to dry.
11. Elute plasmid DNA with 50-100 µl sterile water or TE buffer.

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. I ncrease incubate time of protease in step two. 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. D7042 or use the High performance 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 in step 9 to dry .

Ordering Information

Product	Applications	Cat. No.
Standard E.Z.N.A.™ Plasmid Isolation System		
Plasmid Mini Kit I	Isolation of up to 30 µg Plasmid DNA	D6942/3
Plasmid Mini Kit II	Isolation of up to 70 µg Plasmid DNA	D6945
Plasmid Midi Kit	Isolation of up to 250 µg Plasmid DNA	D6904
Plasmid Maxip Kit	Isolation of up to 1.5 mg Plasmid DNA	D6922
Fastfilter Plasmid Midi Kit	Isolation of up to 250 µg Plasmid DNA, featuring filter syringes for lysate clearance	D6905
Fastfilter Plasmid Maxi Kit	Isolation of up to 1.5 mg Plasmid DNA, featuring filter syringes for lysate clearance	D6924
E-Z 96 Fastfilter Plasmid Kit	Isolation of Plasmid DNA using a 96-well format	D1097
E-Z 96 SE Plasmid Kit	Isolation of plasmid DNA using a single plate	D1095
Yeast Plasmid Isolation Kit	Isolation fo Yeast Plasmid DNA	D3476
E.Z.N.A.™ Endotoxin Free Plasmid Isolation System		
Endo-Free Plasmid Mini Kit I	up to 30 µg Endotoxin free Plasmid DNA	D6948
Endo-Free Plasmid Mini Kit II	up to 70 µg Endotoxin free Plasmid DNA	D6950
Endo-Free Plasmid Mid Kit	up to 250 µg Endotoxin free Plasmid DNA featuring filter syringes for lysate clearance	D6915
Endo-Free Plasmid Maxi Kit	Isolation of 1.0 mg Endotoxin free Plasmid DNA featuring filter syringes for lysate clearance	D6926
E.Z.N.A.™ H P Plasmid Isolation System		
HP Plasmid Mini Kit I	Isolation of up to 30 µg of High Purity Plasmid DNA	D7042
HP Plasmid MidiKit	Isolation of up to 200 µg of High Purity Plasmid DNA	D7004
HP Plasmid Maxi Kit	Isolation of up to 1.5 mg of High Purity Plasmid DNA	D7022
E.Z.N.A.™ Single Strand Phage DNA Isolation Kits		
M13 Isolation Kit	Isolation of 15µg of single stranded phage DNA	D6900
E-Z 96 M13 Isolation Kit	up to 15µg of M-13 DNA using a 96-well format	D1900
E.Z.N.A.™ Large Construct DNA Isolation Kits		
BAC/PAC DNA Isolation Kit	Effective purification of BAC or PAC DNA	D2156
BAC/PAC DNA Isolation Kit	purification of BAC or PAC using a 96-well format	D1056