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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.

The Plasmid Miniprep 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 1 hour. Omega Biotek's 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 produces 15-25 µg plasmid DNA. The Plasmid Miniprep Kit II, is upscaled for isolation of low copy-number plasmids and yields 40-75 µg DNA from 10-15 ml culture when using high copy plasmids. The product is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, 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

New in this edition

- **!** New V-Spin column (Cat.# D6943 & D6946) ensures the elimination of potential contamination and provides the flexibility for vacuum & spin protocol

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 4ºC, all other material at 22-25ºC.

Kit Contents

E.Z.N.A.™ Plasmid Miniprep Kit

Product Number	D6942-00 D6943-00	D6942-01 D6943-01	D6942-02 D6943-02
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
Solution III	5 ml	20 ml	80 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	D6945-00 D6946-00	D6945-01 D6946-01	D6945-02 D6946-02
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
Solution III	5 ml	40 ml	2 x 80 ml
Buffer HB	5 ml	30 ml	120 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 (Product No. D6945 & D6946 only)

Centrifuge with swinging bucket rotor (D6945 & D6946 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: D6942-00 and D6943-00 — Add 18 ml 100% ethanol D6945-00 and D6946-00 D6942-01 and D6943-01 — Add 60 ml 100% ethanol to each bottle D6945-01 and D6946-01 D6942-02 and D6945-02 — Add 60 ml 100% ethanol D6945-02 and D6946-02 ***Store diluted DNA Wash Buffer at room temperature***

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

E.Z.N.A.™ Plasmid Miniprep Protocol

Product Number **D6942 & D6943**

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 DH5 ® 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 350 µl Solution III 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 add the clear supernatant to a clean Type I HiBind™ miniprep column (blue) 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.

7. 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. **This step can be skipped if the downstream applications don't require high quality plasmid, such as enzyme digestion or other screening methods**.

8 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.

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

10 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.**

11. **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.

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:

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

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.

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

Carry out cell culture, lysis, neutralization, and loading onto HiBind® RNA column 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 to the V-Spin column I (blue).

3. Switch on vacuum source to draw the sample through the column and turn off the vacuum.

4. Wash the column by adding 500 µl HB Buffer, draw the wash buffer through the column by turn on the vacuum source.

5. Wash the column by adding 750 µl DNA wash buffer, draw the wash buffer through the column by turn on the vacuum source. Repeat this step with another 750 µl DNA wash buffer.

6. **Assemble the column into a 2 ml collection tube and transfer the column to a micro centrifuge. Spin 1 minute 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.

Low Copy-Number Plasmids

Low copy 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 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 volumes of Buffer HB and DNA 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.™ Plasmid Miniprep Kit II (product No. D6945), 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 40-70 µg plasmid DNA with high-copy plasmids.

E.Z.N.A.™ Plasmid Miniprep Kit II

Product Number **D6945**

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 DH5 ® 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 Solution III 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 add 800 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.

7. 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.

8. 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.
9. Optional step: repeat wash step with another 750 µl Wash Buffer.

10. 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.**

11. **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.

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

    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.

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

Carry out cell culture, lysis, neutralization, and loading onto HiBind® RNA column 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 to the V-Spin column I (purple).

3. Switch on vacuum source to draw the sample through the column and add the remaining lysate to the column . Turn off the vacuum.

4. Follow the same steps in Vacuum/Spin protocol in page 6 to wash and elute DNA from column.

Short Miniprep 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 (D6942) or 10-15 ml (D6945) overnight culture.
2. Resuspend cells in 250 µl (D6942) or 500 µl (D6945) Solution I/RNase A.
3. Add 250 µl (D6942) or 500 µl (D6945) Solution II. Mix gently by inverting 4-6 times to obtain cleared lysate. A brief incubation at RT may be required.
4. Add 350 µl (D6942) or 700 µl (D6945) Solution II 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 white (D6942) or purple (D6945) HiBind™ DNA column placed in a 2 ml collection tube. Centrifuge 1 min at max speed. Discard liquid.
7. Wash column with 500 µl Buffer HB. Centrifuge 1 min at max speed. Discard liquid.
8. Using same collecting tube, wash column with 750 µl DNA Wash Buffer diluted with ethanol. Centrifuge 1 min at max speed.
9. Optional: Wash column a second time with 750 µl DNA Wash Buffer.
10. Centrifuge empty column 1 min at max 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. 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 in step 9 to dry .

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

NOW YOU CAN FIND US IN CYBERSPACE!

Visit our web site (http://www.omegabiotek.com) and learn more about Omega Bio-tek. You can browse our product list, place orders, or even ask technical questions.

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.