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E.Z.N.A. Plasmid Mini Prep Kit

Revised June 2005

Introduction

The E.Z.N.A.[®] family of products are an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. The key to the system is Omega Bio-Tek's proprietary HiBind[®] matrix that avidly, but reversibly, binds nucleic acids under specific conditions, the removal of interfering compounds. Nucleic acids are subsequently eluted with de-ionized water or low salt buffer.

The Plasmid Mini-prep 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 Bio-Tek's mini-columns facilitate binding, washing, and elution steps enabling multiple samples to be simultaneously processed. Yields vary according to plasmid strain, copy number and reproductive efficiency, but 5 ml of overnight culture in LB medium typically yields approximately 30 µg of plasmid DNA. The Plasmid Mini-prep Kit II, is optimized for isolation of low copy-number plasmids and yields up to 75 µg DNA from 5 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 Mini-prep Kit means:

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

New in this edition

- Newly introduced V-Spin column (#D6943/D6946) features an attached cap and a standard outlet luer at the bottom. The attached cap eliminates the possibility of contamination.
- Optional vacuum/spin protocol is available for the V-Spin column format.

Storage and Stability: All E.Z.N.A.[®] Plasmid Mini-prep components are guaranteed for 12 months from the date of purchase when all components are stored at room temperature (22-25°C). Note: RNase A should be stored at 4°C for optimal life.

Kit Contents

E.Z.N.A.[®] Plasmid Mini-prep Kit

Product Number	D6942-01	D6942-02
	D6943-01	D6943-02
	D6944-01	D6944-02
Purifications	50	200
HiBind [®] Miniprep Columns (I)	50	200
2 ml Collection Tubes	50	200
Solution I	20 ml	60 ml
Solution II	20 ml	60 ml
Solution III	20 ml	80 ml
Buffer HB	30 ml	110 ml
Wash Buffer Concentrate	40 ml	3 x 40 ml
RNase A, Concentrate	100 µl	400 µl
Instruction Booklet	1	1

E.Z.N.A.[®] Plasmid Miniprep Kit II

Product Number	D6945-01	D6945-02
	D6946-01	D6946-02
	D6947-01	D6947-02
Purifications	50	200
HiBind [®] Mini-prep Columns(II)	50	200
2 ml Collection Tubes	50	200
Solution I	30 ml	120 ml
Solution II	30 ml	120 ml
Solution III	40 ml	2 x 80 ml
Buffer HB	30 ml	120 ml
Wash Buffer Concentrate	40 ml	3 x 40 ml
RNase A, Concentrate	100 µl	400 µl
Instruction Booklet	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.
 - Nuclease-free 1.5 ml centrifuge tubes.
 - Sterile de-ionized 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, D6943 and D6944-01 D6945, D6946 and D6947-01	Add 60 ml 100% ethanol to each bottle
D6942, D6943 and D6944-02 D6945, D6946 and D6947-02	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.® Plasmid Mini-prep Protocol

Product Number D6942 & D6943, D6944

1. **Inoculate 5 ml desired growth 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 (based on expected yield) by centrifugation at 10,000 x g for 1 min at room temperature.**
3. **Remove growth medium from pellet, discard and add 250 µl Solution I/RNase A to tube containing pelleted bacterial cells. Resuspend cells completely in buffer by vortexing gently.** Complete resuspension of cell pellet is vital for obtaining good yields.
4. **Add 250 µl Solution II to re-suspended cell Solution I mixture and invert tube to mix. Lysate should clear at this step.** A 2 minute incubation at room temperature may be required if solution does not clear. Avoid vigorous mixing during this step as this may 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. A flocculent white precipitate should form at this step.** Centrifuge at 10,000 x g for 10 minutes at room temperature to pellet precipitate.
6. **CAREFULLY remove cleared supernatant, taking care not to disturb the pellet, and add to the supplied HiBind® Mini-prep column (blue) assembled in a 2 ml collection tube .** Centrifuge at 10,000 x g for 1 minute at room temperature to pass lysate through column.
7. **(Optional) Discard liquid from collection tube and add 500 µl Buffer HB to HiBind® column and centrifuge at 10,000 x g for 1 minute.** 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 from collection tube, add 750 µl of Wash Buffer to HiBind® column and centrifuge at 10,000 x g for 1 minute 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 : Repeat wash step as outlined in Step 8.**

10. **Centrifuge the empty column at 10,000 x g for 1 minute to dry the column matrix.** *Do not skip this step - it is critical for removing ethanol from the column. Residual ethanol might interfere with downstream applications.*
11. **Place HiBind® column into a clean 1.5 ml microcentrifuge tube and add 50 µl (depending on desired concentration of final product) of Elution Buffer directly onto the column matrix and centrifuge at 10,000 x g for 1 minute 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:

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

High copy number plasmids generally yield up to 35 µg of DNA from 5 ml culture. The ratio of $(\text{absorbance}_{260})/(\text{absorbance}_{280})$ 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 Steps 1-5 in Standard E.Z.N.A.® Plasmid Mini-prep Protocol, when reaching step 6 follow steps blow.

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

- 6a. **Prepare the vacuum manifold according to manufacturer's instructions and connect the V-Spin column to the manifold.**
- 7a. **Apply cleared supernatant from Step 5 to the V-Spin column (blue).**
- 8a. **Switch on vacuum source to draw the sample through the column, then turn off the vacuum.**
- 9a. **Wash the column by adding 500 µl HB Buffer; draw the wash buffer through the column by turning on the vacuum source.**
- 10a. **Wash the column by adding 750 µl DNA wash buffer; draw the wash buffer through the column by turning on the vacuum source. Repeat this step with another 750 µl DNA wash buffer.**

11a. Assemble the column into a 2 ml collection tube and transfer the column to a micro centrifuge. Spin 1 minute to dry the column.

12a. Place the column in a clean 1.5 ml microcentrifuge tube and add 50µl Elution Buffer and centrifuge at 10,000 x g for 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 digestion analysis. However, the method can be modified to double the yield if necessary. Start with 10 ml bacterial culture, and centrifuge for 10 min at 10,000 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 I quickly becomes saturated. In this situation we recommend processing of multiple samples from the same culture. Alternatively, use the E.Z.N.A.® Plasmid Mini-prep Kit II (Product No. D6945), a new member of the EaZy Nucleic Acid family that allows processing of up to 15 ml cultures using the mini-column format. E.Z.N.A.® Plasmid Mini-prep Kit II generally yields 40-70 µg plasmid DNA with high-copy plasmids.

E.Z.N.A.® Plasmid Mini-prep Kit II

Product Number D6945, D6946, D6947

Note: Using the following protocol with E.Z.N.A.® Plasmid Mini-prep Kit I will not improve yields due to the lower column binding capacity.

The E.Z.N.A.® Plasmid Mini-prep Kit II allows rapid and reliable isolation of up to 75 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 manual for important information on preparation of components and required materials.

1. **Inoculate 10-15 ml growth medium placed in a 50 ml culture flask with desired bacteria 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 10,000 x g for 10 min at room temperature.**
3. **Carefully remove growth media from tube, being careful not to disturb pellet. To resuspend bacterial pellet add 500 µl Solution I/RNase A, and resuspend cells completely by vortexing gently or pipetting up and down.** Complete resuspension of the cell pellet is vital for obtaining good yields.
4. **Transfer cell suspension to a new 2 ml microcentrifuge tube and add 500 µl Solution II. Gently mix by inverting and rotating tube several times to mix. Lysate will clear at this step.** A 5 minute incubation at room temperature may be necessary if lysate does not clear immediately. 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 x g for 10 minutes at room temperature to collect precipitate.

6. CAREFULLY removed cleared lysate from tube, taking care to not disturb pellet and add 800 µl to a clean Type II (supplied with Plasmid Kit II) HiBind® Mini-prep column (purple) assembled in a 2 ml collection tube (provided). Ensure that the pellet is not disturbed and that no cellular Centrifuge at 10,000 x g for 1 minute at room temperature to completely pass lysate through column. Discard flow-through liquid and add the remaining lysate to the column and repeat as above.

7. **(Optional) Add 500 µl Buffer HB and centrifuge at 10,000 x g for 1 minute at room temperature. Discard flow through and place HiBind® column back into collection tube.** This step ensures that residual protein contamination is removed and for downstream applications requiring high quality DNA.
8. **Add 750 µl of Wash Buffer to HiBind® column and centrifuge at 10,000 x g 1 min 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) Repeat wash step with another 750 µl Wash Buffer if high purity DNA is required.**
10. **Centrifuge the empty column at 10,000 x g for 1 minute to remove residual ethanol from the column matrix.** Residual ethanol can interfere with downstream applications.
11. **Place column into a clean 1.5 ml microcentrifuge tube, and add 50 µl of Elution Buffer directly onto the column matrix and centrifuge at 10,000 x g for 1 minute 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:

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

High copy number plasmids generally yield up to 25 µg of DNA from 5 ml culture. The ratio of (absorbance₂₆₀)/(absorbance₂₈₀) 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 Steps 1-5 in Standard E.Z.N.A.® Plasmid Mini-prep Protocol, when reaching step 6 follow steps blow.

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

- 6a. **Prepare the vacuum manifold according to manufacturer's instructions and connect the V-Spin column to the manifold.**
- 7a. **Apply cleared supernatant from Step 5 to the V-Spin column (purple).**
- 8a. **Switch on vacuum source to draw the sample through the column, then turn off the vacuum.**

- 9a. **Wash the column by adding 500 µl HB Buffer; draw the wash buffer through the column by turning on the vacuum source.**
- 10a. **Wash the column by adding 750 µl DNA wash buffer; draw the wash buffer through the column by turning on the vacuum source. Repeat this step with another 750 µl DNA wash buffer.**
- 11a. **Assemble the column into a 2 ml collection tube and transfer the column to a micro centrifuge. Spin 1 minute to dry the column.**
- 12a. **Place the column in a clean 1.5 ml microcentrifuge tube and add 50µl Elution Buffer and centrifuge at 10,000 x g for 1 minute to elute DNA.**

Short Mini-prep 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 μ l (D6942) or 500 μ l (D6945) Solution I/RNase A.
3. Add 250 μ l (D6942) or 500 μ l (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 μ l (D6942) or 700 μ l (D6945) 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 blue (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 μ l Buffer HB. Centrifuge 1 min at max speed. Discard liquid.
8. Using same collecting tube, wash column with 750 μ l DNA Wash Buffer diluted with ethanol. Centrifuge 1 min at max speed.
9. Optional: Wash column a second time with 750 μ l DNA Wash Buffer.
10. Centrifuge empty column 1 min at max speed to dry.
11. Elute plasmid DNA with 50-100 μ l sterile water or TE buffer.

Trouble Shooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	<p>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.</p> <p>Cells may not be dispersed adequately prior to addition of Solution II. Vortex cell suspension to completely disperse.</p> <p>Increase incubation time with Solution II to obtain a clear lysate.</p> <p>Solution II if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS.</p>
	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 0.5 μ g 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 Mini-prep 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 quantification.
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 Mini-prep System		
D6942-01/02 D6943-01/02 D6944-01/02	Plasmid Mini-prep Kit I	Isolation of up to 30µg plasmid in 15 minutes
D6945-01/02 D6946-01/02	Plasmid Mini-prep Kit II	Isolation of up to 70µg plasmid in 15 minutes
D7042-01/02 D7043-01/02	High Performance Plasmid Mini-prep Kit I	Isolation of up to 30µg plasmid from end A+ bacterial in 25 minutes
D7045-01/02 D7046-01/02	High Performance Plasmid Mini-prep 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 Midi-prep Kit	Midi-preps in spin column format. Yield up to 200µg plasmid
D6922-01/02	Plasmid Maxi-prep Kit	Maxi-preps in spin column format. Yield up to 1mg plasmid
D7004-01/02	High Performance Plasmid Mini-prep 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.

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