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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 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 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 less than 20 minutes. Omega Bio-Tek'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 yields 15-25 µg plasmid DNA with the HP Mini Kit I (D7042/D7043). 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 plasmids. HP Mini Kit I is simple, efficient, and fast to facilitate screening of recombinant clones. Meanwhile HP 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 the E.Z.N.A.™ plasmid isolation system; it is specially designed for those applications in which high quality plasmids are required, such as transfection, autosequencing, etc. It is also suitable for isolating plasmids from bacterial hosts with high levels of endonuclease activity (such as EndoA+ strains). The plasmids from this system have excellent stability for long term storage.

New in this edition

- Equilibration Buffer Introduced to increase yield and performance

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**, **OB Protease Mixture at -20°C**, and all other material at 22-25°C.

Kit Contents

E.Z.N.A.™ 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
DNA HiBind® Mini 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	30 ml	2 x 40 ml
Equilibration Buffer	1.5 mL	7 mL	25 mL
Buffer HB	5 ml	30 ml	110 ml
Elution Buffer	1.5 mL	15 mL	50 mL
DNA Wash Buffer Concentrate	1.5 ml	15 ml	3 x 25 ml
RNase A, Concentrate	50 µl	100 µl	400 µl
OB Protease Mixture	1.8 mg	16 mg	63 mg
Instruction Booklet	1	1	1

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

Product Number	D7045-00	D7045-01	D7045-02
Purification Times	5 Preps	50 Preps	200 Preps
DNA HiBind® Mini 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
Equilibration Buffer	1.5 mL	7 mL	25 mL
DNA Wash Buffer Concentrate	1.5 ml	15 ml	3 x 25 ml
Elution Buffer	1.5 mL	15 mL	50 mL
RNase A, Concentrate	50 µl	100 µl	400 µl
OB Protease Mixture	1.8 mg	16 mg	63 mg

Column Specifications

Plasmid Kits	D7042 (Q-Spin) D7043 (V-Spin)	D7045 (Q-Spin)
Maximum Volume	750 µl	750 µl
Plasmid Binding Capacity	30 µg	75 µg
Yield: from 5 ml cultures (high copy) from 15 ml cultures (high copy)	20-25 µg 20-30 µg	20-25 µg 60-70 µg
Applications: Screening Minipreps DNA Sequencing Subcloning - enzymatic reactions Transfections	 ✓ ✓ ✓ ✓	 ✓ ✓ ✓ ✓

Before Starting

IMPORTANT	Dilute OB Protease Mixture with deionized water as follows. Store aliquots at -20°C. D7042/7043/7045-00 Add 60ul deionized water D7042/7043/7045-01 Add 530 ul deionized water D7042/7043/7045-02 Add 2.1ml deionized water
	Add vial of RNase A to bottle of Solution I provided. Store at 4°C.
	Dilute DNA Wash Buffer with absolute ethanol as follows D7042/7043/7045-00 Add 6ml absolute ethanol D7042/7043/7045-01 Add 60ml absolute ethanol D7042/7043/7045-02 Add 100ml absolute ethanol per bottle

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

Materials Supplied By User

- Microcentrifuge capable of at least 13,000 x g.
- Nuclease-free 1.5 ml centrifuge tubes.
- Sterile deionized water (or TE buffer)
- Absolute (96%-100%) ethanol
- 15 ml centrifuge tubes (Product No. D7045 only)
- Centrifuge with swinging bucket rotor (D7045 only)

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 10 µl of OB Protease Mixture. Gently mix by inverting and rotating tube 4-6 times to obtain a cleared lysate. A 3-5 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.**
6. **Centrifuge at ≥10,000 x g for 10 minutes at room temperature.**
7. **Prepare the column by adding 100 µl of Equilibration Buffer placed in a 2 mL collection tube. Centrifuge at ≥ 13,000 x g for 30-60 seconds. Discard the flowthrough liquid.**
8. **CAREFULLY aspirate and add the clear supernatant to a clean Type I HiBind[®] mini column 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.**
9. **Discard liquid and wash column with 500 µl Buffer HB and 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.

10. **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 x 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, Wash Buffer must be brought to room temperature before use.

11. **Optional step: repeat wash step 8 with another 700 µL DNA Wash Buffer.**
12. **Using the same collection tube, centrifuge the empty column for 1 min at maximum speed (≥13,000 x g) to dry the column matrix. Do not omit this step; it is *critical* for removal of ethanol from the column.**
13. **Place column into a clean 1.5 ml microcentrifuge tube. Add 50 µl to 100 µl (depending on desired concentration of final product) Elution Buffer (10mM Tris-HCl, pH 8.5) or water directly onto the column matrix and centrifuge for 1 min at ≥13,000 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.**
14. **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 (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.

Low Copy-Number Plasmids

Low copy plasmids generally yield 0.1-0.5 µg DNA per ml overnight culture. For routine screening of recombinant clones, a 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 volume of 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.™ High performance Plasmid Miniprep 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 plasmids.

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

Carry out cell culture, lysis, neutralization, and loading onto HiBind® DNA 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 6 to the V-Spin column I.**
- 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. Wash the column by adding 500 µL HB Buffer, draw the wash buffer through the column by turning on the vacuum source.**
- 5. Wash the column by adding 700 µL DNA wash buffer, 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 microcentrifuge. Spin at maximum speed at maximum speed ($\geq 13,000$ x g) for 1 minute to dry the column.**
- 7. Place column into a clean 1.5 ml microcentrifuge tube. Add 50 µl to 100 µl (depending on desired concentration of final product) Elution Buffer (10mM Tris-HCl, pH 8.5) or water directly onto the column matrix and centrifuge for 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.

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

Note: Using the following protocol with product No. D7043/D7042 will not improve yields significantly with high-copy-plasmids due to the lower column binding capacity (see column specifications on page 5).

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.

Before starting, we recommend you refer to page 5 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 and 10 µl OB Protease Mixture. Gently mix by inverting and rotating tube several times to obtain a cleared lysate. A 3-5 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.**
- 6. Centrifuge at 10,000 x g for 10 minutes at room temperature.**
- 7. Prepare the column by adding 100 µl of Equilibration Buffer placed in a 2 mL collection tube. Centrifuge at $\geq 13,000$ x g for 30-60 seconds. Discard the flowthrough liquid.**

8. CAREFULLY aspirate and add 700 µl of the clear supernatant to a clean Type II HiBind® Mini 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 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.

9. Discard liquid and wash column with 500 µl Buffer HB and 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.

10. Discard flow-through liquid and wash the column by adding 700 µl of DNA Wash Buffer diluted with absolute ethanol. Centrifuge for 1 min at 10,000 x 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.

11. Optional step: repeat wash step with another 700 µl DNA Wash Buffer.

12. Centrifuge the empty column for 1 min at maximum speed ($\geq 13,000$ x g) to dry the column matrix. Do not skip this step - it is critical for removing ethanol from the column.


13. Place column into a clean 1.5 ml microcentrifuge tube. Add 100 µl to 200 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 for 1 min at $\geq 13,000$ 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. Alternatively, apply the first eluate to the column to elute a second time.

14. 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 50-70 µg of DNA from 10-15 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.

Note: All steps are to be performed at room temperature. Refer to page 5 for important notes on preparation of components.



1. Pellet cells from 1.5-5 ml (D7042& D7043) or 10-15 ml (D7045) overnight culture.
2. Resuspend cells in 250 µl (D7042& D7043) or 500 µl (D7045) Solution I/RNase A.
3. Add 250 µl (D7042&D7043) or 500 µl (D7045) Solution II with 10 µl protease. Mix gently by inverting 4-6 times to obtain cleared lysate. A 3-5 minutes incubation at RT is required.
4. Add 350 µl (D7042&D7043) or 700 µl (D7045) Solution III and mix well to form white precipitate.
5. Centrifuge at maximum speed ($\geq 13,000$ x g) for 10 min.
6. Transfer cleared lysate to a blue (D7042&D7043) or purple (D7045) HiBind® DNA column placed in a 2 ml collection tube. Centrifuge for 1 min at max speed. Discard liquid. Add the remaining lysate to the column (D7045) and Centrifuge as above. Discard the liquid.
7. Wash column with 500 µl Buffer HB. Centrifuge for 1 min at max speed. Discard liquid.
8. Using same collecting tube, wash column with 700 µl DNA Wash Buffer diluted with ethanol. Centrifuge for 1 min at max speed.
9. Optional: Wash column a second time with 700 µl DNA Wash Buffer.
10. Centrifuge empty column for 1 min at max speed to dry.
11. Elute plasmid DNA with 50-100 µl (D7042/7043) or 100-200 µl (D7045) sterile water or TE buffer.

Short Protocol for Experienced User

Troubleshooting 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. Increase incubate time of protease in step two.</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 0.5µg DNA from a 5 ml overnight culture. Increase culture volume to 10 ml and follow suggested modifications with product No. D7043 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 .