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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.

Application such as transfection normally requires large quantity of plasmid that need a number of stringent criteria, including 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 Mini Kit combines the power of HiBind technology with the time-tested consistency of alkaline-SDS lysis of HiBind Maxi-columns facilitate the binding, washing, and elution steps thus enabling multiple samples to be simultaneously processed. Following the lysis, the cleared lysate is extracted with ETR Solution which will almost completely remove the endotoxin. Then the lysate is applied to the HiBind Mini-column, plasmid DNA is bound to the silica membrane and contaminants are removed with a simple wash step.Yield vary according to plasmid copy number, *E.coli* strain and conditions of growth, but up to 30 µg of high quality plasmid can be purified from 5ml 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.® Endo-free Plasmid Mini Kit means:

- Speed–Plasmid DNA Isolation in<60 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.® Endo-Free Plasmid isolation components are guaranteed for at least 12 months from the date of purchase when stored as follows: Solution I (once RNase A is added) and ETR Solution at 4ºC (for long time storage), all other material at 22-25ºC.

Kit Contents

E.Z.N.A.™ Endo-free Plasmid Mini Kit I

Product No.	D6948-00B	D6948-01B	D6948-02B
Purification times	5 Preps	50 Preps	200 Preps
HiBind™ DNA Mini column 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
Buffer N3	5 ml	10 ml	30 ml
ETR Binding Buffer	5 ml	40 ml	160 ml
ETR Wash Buffer	5 ml	30 ml	120 ml
Buffer EHB	5 ml	30 ml	120 ml
DNA Wash Buffer	2 ml	20 ml	3 × 20 ml
RNase A, Concentrate	Pre-added	100 µl	300 µl
Endotoxin-Free Elution Buffer	5 ml	10 ml	30 ml
Instruction Booklet	1	1	1

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

Product Number	D6950-00B	D6950-01B	D6950-02B
Purification times	5 Preps	50 Preps	200 Preps
HiBind™ DNA Mini column 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
Buffer N3	5 ml	15 ml	60 ml
ETR Binding Buffer	10 ml	80 ml	2 × 160 ml
ETR Wash Buffer	5 ml	30 ml	120 ml
Buffer EHB	5 ml	30 ml	120 ml
DNA Wash Buffer	2 ml	20 ml	3 × 20 ml
RNase A, Concentrate	Pre-added	100 µl	400 µl
Endotoxin-Free Elution Buffer	5 ml	10 ml	30 ml
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 13,000×g.

Sterile 1.5ml & 2ml centrifuge tubes.

Absolute (96%-100%) ethanol (room temperature)

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

IMPORTANT

1. **Add 1 vial of RNase A to bottle of Solution I and store at 4°C.**
2. **DNA Wash Buffer Concentrate** is to be diluted with absolute ethanol (96-100%) as follows:

D6948-00B D6950-00B	Add 8 ml of absolute ethanol
D6948-01B D6950-01B	Add 80 ml absolute ethanol to each bottle
D6948-02B D6950-02B	Add 80 ml absolute ethanol to each bottle

Store diluted DNA Wash Buffer at room temperature

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

E.Z.N.A.® Endo-free Plasmid Mini Kit I Spin Protocol

Product Number D6948B

The E.Z.N.A.® Endo-free Plasmid Mini Kit I allows rapid and reliable isolation of 20- 40µg of high copy-Number plasmid and 1-10µg of low copy-Number plasmid using the spin-column format. If high yields of low copy-number plasmid are desired, Start with 10-15ml bacterial culture as "Low Copy-Number Plasmids" on page 7.

1. **Inoculate 5ml LB/ampicillin (50 µg/ml) medium placed in a 10-20ml 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 1.5-5ml bacteria in appropriate vessels by centrifugation at 10,000 × 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 or pipetting up and down. *Complete resuspension of cell pellet is vital for obtaining good yields.*

4. **Add 250 µl Solution II and mix gently but throughly 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 125 µl ice-cold Buffer N3 and mix gently but throughly by inverting tube several times until a flocculent white precipitate forms.** Centrifuge at ≥12,000 × g for 10 minutes at room temperature (preferably at 4°C).

   Note: The Buffers must be mixed throughly. If the mixture appears still viscous, brownish and conglobated, more mixing is required to completely neutralize the solution. Complete neutralization of the solution is vital of obtaining good yields.

6. **CAREFULLY aspirate and transfer the cleared supernatant to a clean 1.5ml centrifuge tube. Add Equal volume of ETR Binding Buffer to the cleared lysate.** Mix by inverting tube 7-10 times. Incubate at room temperature for 5 minutes.

7. **Transfer 700 µl of the mixture(from Step 6) into a clean HiBind™ DNA Mini column I assembled in a 2ml collection tube(provided).** Centrifuge at 8,000 × g for 1 min **at room temperature** to pass solution through column. Discard the flow-through and re-use the collection tube.

8. **Repeat step 7 until all of the cleared lysate pass through the Mini column.**

9. Add 500ul ETR Wash Buffer I to the mini column Centrifuge at 8,000 × g for 1 min. Discard the flow-through and re-use the collection tube.

10. Add 500ul Buffer EHB to the mini column Centrifuge at 8,000 × g for 1 min. Discard the flow-through and re-use the collection tube.

11. **Add 700 µl DNA Wash Buffer diluted with ethanol.** Centrifuge 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.

12. **Repeat wash step 11 with another 700 µl DNA Wash Buffer diluted with ethanol.**

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

14. **Place column into a new clean 1.5 ml micro-centrifuge tube. Add 30-50 µl (depending on desired concentration of final product) Endotoxin-Free Elution Buffer (or water) directly onto the column matrix and centrifuge at ≥13,000 × g for 1 min 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.

Low Copy-Number Plasmids

Low copy-number plasmids generally give 0.1-1µg 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, Buffer N3 and ETR Binding Buffer. Continue as above using only one HiBind DNA Mini 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® DNA 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.® Endo-free Plasmid Mini Kit II (Product No. D6950B), 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.® Endo-free Plasmid Mini Kit II Spin Protocol

Product Number D6950B

The E.Z.N.A.® Endo-free Plasmid Mini Kit II allows rapid and reliable isolation of 40- 75µg of high copy-Number plasmid and 5-30µg of low copy-Number plasmid using the spin-column format. **Note: Using the following protocol with product No.D6948 will not improve yields significantly with high-copy-number-plasmids due to the lower column binding capacity.**

Procedure

1. **Inoculate10-15ml LB/ampicillin(50 µg/ml) medium placed in a 50ml culture flask with E.*coli* carrying desired plasmid isolation.** 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 bacteria in appropriate vessels 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 cell pellet is vital for obtaining good yields.*

4. **Transfer the cell suspension to a new 2 ml micro-centrifuge tube. Add 500 µl Solution II and mix gently but throughly by inverting and rotating tube 7-10 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 ice-cold Buffer N3 and mix gently but throughly by inverting tube several times until a flocculent white precipitate forms.** Centrifuge at ≥12,000 × g for 10 minutes at room temperature (preferably at 4°C).
   Note: The Buffers must be mixed throughly. If the mixture appears viscous, brownish and conglobated, more mixing is required to completely neutralize the solution. Complete neutralization of the solution is vital of obtaining good yields.

6. **CAREFULLY aspirate and transfer the cleared supernatant to a clean 1.5ml centrifuge tube. Add Equal volume of ETR Binding Buffer to the cleared lysate.** Mix by inverting tube 7-10 times.

7. **Transfer 700 µl of the mixture(from Step 6) into a clean HiBind™ DNA Mini column assembled in a 2ml collection tube(provided).** Centrifuge at 10,000 × g for 1 min **at room temperature** to pass solution through column. Discard the flow-through and re-use the collection tube.

8. **Repeat step 7 until all of the cleared lysate pass through the Mini column.**

9. **Add 500ul ETR Wash Buffer I to the mini column.** Centrifuge at 10,000 × g for 1 min.

Discard the flow-through and re-use the collection tube.

10. **Add 500ul Buffer EHB to the mini column.** Centrifuge at 10,000 × g for 1 min. Discard the flow-through and re-use the collection tube.

11. **Add 700 µl DNA Wash Buffer diluted with ethanol.** Centrifuge 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.

12. **Repeat wash step 12 with another 700 µl DNA Wash Buffer.**

13. **Discard the flow-through liquid and centrifuge the empty column at maximum speed (≥13,000×g) for 3 min to dry the column matrix.** Do not skip this step-it is critical for removing ethanol from the column.

14. **Place column into a new clean 1.5ml micro-centrifuge tube. Add 60-100 µl (depending on desired concentration of final product) Endotoxin-Free Elution Buffer directly onto the column matrix and let it sit at room temperature for 2 minutes. Centrifuge at ≥13,000 × g for 1 min 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.

Low Copy-Number Plasmids

Low copy-number plasmids generally give 0.1-1µg DNA per ml overnight culture. For routine screening of recombinant clones, 10-15 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 15-25 ml bacterial culture, centrifuge for 10 min at 5,000 x g in a 50 ml centrifuge tube. Proceed to Step 3 (Page 8) and double the volumes of Solutions I, II, and Buffer N3. Continue as above using only one HiBind DNA Mini column II per 15-25 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 15 ml culture, the HiBind® DNA Mini column II quickly becomes saturated. In this situation we recommend processing of multiple samples from the same culture.

Yield and quality of DNA

Determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260nm and then at 280nm. The DNA concentration is calculated as follows:

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

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.

Short Protocol For Experienced Users

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

1. Pellet cells from 1.5-5 ml (D6948B) or 10-15 ml (D6950B) overnight culture.
2. Resuspend cells in 250 µl (D6948B) or 500 µl (D6950B) Solution I/RNase A.
3. Add 250 µl (D6948B) or 500 µl (D6950B) Solution II. Mix gently by inverting 4-6 times to obtain cleared lysate.
4. Add 125 µl (D6948B) or 250 µl (D6950B) Buffer N3 and mix well to form white precipitate. A brief incubation at RT may be required.
5. Centrifuge at maximum speed 10 min.
6. Add equal volume of ETR Binding Buffer.
7. Transfer the lysate to a HiBind™ DNA Minicolumn placed in a 2 ml collection tube. Centrifuge 1 min at max speed. Discard liquid.
8. Wash column with 500 µl ETR Wash Buffer. Centrifuge 1 min at max speed. Discard liquid.
9. Wash column with 500 µl Buffer EHB. Centrifuge 1 min at max speed. Discard liquid.
10. wash column with 700 µl DNA Wash Buffer diluted with ethanol. Centrifuge 1 min at max speed.
11. Optional: Wash column a second time with 700 µl DNA Wash Buffer.
12. Centrifuge empty column 2 min at max speed to dry.
13. 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(D6948) or 15ml(D6950). Cells may not be dispersed adequately prior to addition of Solution II. Vortex/ pipet cell suspension to completely disperse bacterial clumps. 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 to yield and quality.
	Low copy-number plasmid used	Such plasmids may yield as little as 0.1µg DNA from a 1 ml overnight culture. Increase culture volume to 400 ml.
No DNA eluted.	DNA Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare Wash Buffer Concentrate as instructed on the label.
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 Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantization.
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, does not freeze, or smells of ethanol.	Ethanol traces not completely removed from column following wash steps.	Centrifuge column as instructed to dry the column before elution .