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	Isolation of up to 30 µg Endotoxin free Plasmid DNA	D6948
Endo-Free Plasmid Mini Kit II	Isolation of up to 70 µg Endotoxin free Plasmid DNA	D6950
Endo-Free Plasmid Mid Kit	Isolation of up to 250 µg Endotoxin free Plasmid DNA, featuring filter syringes for lysate clearance	D6915
Endo-Free Plasmid Maxi Kit	Isolation of up to 1.5 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 up to 15µg of single stranded phage DNA	D6900
E-Z 96 M13 Isolation Kit	Isolation of 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	Parallel purification of BAC or PAC using a 96-well format	D1056

Contents

binding capacity of the kit. In these factors, The copy number of vector, culture volume and binding capacity of the kit are most important. Copy number of plasmid is vary from one copy to several hundred copies per cell as dicated by their origin of replication. But very large plasmids are often maintained at very low copy number per cell. The expected yield of 200 ml overnight cultures (LB medium) are indicated in the table.

Plasmid	Replicon	Copy Number	expected Yield of 200ml culture
pUC vector	pMB1	500-700	700-1200 µ*g*
pBR322 and its derivatives	pMB1	15-20	40-80µ*g*
PACYC and its derivatives	p15A	10-12	20-40 µ*g*
pSC101 and its derivatives	pSC101	~5	10-20 µ*g*
pBluescript	ColE14	300-500	600-900 µ*g*
ColE14	ColE14	15-20	10-40 µ*g*
pGEM	pMB1	300-700	800-1000 µ*g*

Trouble Shooting Guide

Kit Contents

Product Number	D6926-00B	D6926-01B	D6926-03B	D6926-04B
Purification times	2 Preps	6 Preps	25 Preps	100 Preps
HiBind™ DNA Maxi Column	2	6	25	100
Lysate Clearance filter syringe	2	6	25	100
Solution I	25 ml	70 ml	270 ml	5 x 220 ml
Solution II	25 ml	70 ml	270 ml	5 x 220 ml
Buffer N3	15 ml	35 ml	135 ml	2 x 270 ml
ETR Binding Buffer	55 ml	170 ml	2 x 220 ml	10x270 ml
Buffer GPS	15 ml	40 ml	150 ml	2 x 270 ml
ETR Wash Buffer	25 ml	70 ml	270 ml	5 x 220 ml
Buffer EHB	25 ml	70 ml	270 ml	5 x 220 ml
DNA Wash Buffer	15 ml	40 ml	4 x 50 ml	11 x 50 ml
RNase A	120 µl	350 µl	1.4 ml	5 x 1.1 ml
Endotoxin-Free Elution Buffer	10 ml	40 ml	90 ml	2 x 160 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

Laboratory centrifuge equipped with **swinging-bucket** rotor capable of 3,000-5,000 × g.
Water bath preset at 42°C
Sterile 50 ml centrifuge tubes.
Absolute (96%-100%) ethanol

IMPORTANT

1. **Add a vial RNase A to bottle of Solution I provided and store at 4°C.**
2. **DNA Wash Buffer** is to be diluted with absolute (96-100%) ethanol as follows and store at room temperature.
   D6926-00B: Add 60 ml of absolute ethanol
   D6926-01B: Add 160 ml of absolute ethanol
   D6926-03B: Add 200 ml of absolute ethanol per bottle
   D6926-04B: Add 200 ml of absolute ethanol per bottle

1. **Prepare the cleared lysate by following step 1-9 of centrifugation procedure on page 4-6.**

2. **Prepare the vacuum manifold according to manufacturer's instructions, and connect the Maxi Column to the manifold.**

3. **Transfer the lysate into the HiBind® DNA Maxi Column, be careful not to overfill the column, apply the vacuum to allow all sample pass through the column.**

4. Add 10 ml ETR Wash Buffer to the column and apply the vacuum to draw the liquid through the column.

5. Add 10 ml EHB buffer to the column and apply the vacuum to draw the liquid through the column.

6. Wash the column: add 15 ml DNA Wash Buffer (pre-diluted with absolute ethanol) into the column and allow it pass through the column.

7. Wash the column again with 10 ml DNA Wash Buffer by repeating step 5. Keep the vacuum on for another 10-15 minutes after the liquid pass through the column.

8. Proceed step 18 on page 7.

Protocol 3: Low Copy-Number Plasmids Protocol

Low copy plasmids generally give 0.1-1 µg plasmid DNA per ml overnight culture. For isolation of plasmid from low copy-number plasmids (0.1-1 µg/ml culture) or low-midi copy-number plasmids (1-2 µg/ml culture), the method can be modified to essentially increase the yield if necessary.

Start with 200-400 ml bacterial culture, centrifuge for 10 min at 3,500-5,000 x g in a centrifuge tube. Proceed to Step 3 (Page 4) and double the volumes of Solution I, II, and Neutralization Buffer. Continue as above using only one HiBind® DNA Maxi Column. There is no need to increase the volumes of Buffer EHB and DNA Wash Buffer used. The Buffer of Solution I, II, Buffer N3, ETR Binding Buffer can be purchase separately.

Note: This method is not recommended for high copy number plasmids because above 200 ml culture, the HiBind® DNA Maxi Column quickly becomes saturated. In this situation we recommend processing of multiple samples from the same culture.

Yield and quality of Plasmid

Determine the absorbance of an appropriate dilution (10- to 20-fold) of the sample at 260

completely by vortexing or pipetting up and down. Complete resuspension of cell pellet is vital for obtaining good yield.

4. **Add 10 ml Solution II and mix gently but throughly by inverting and rotating tube 10-15 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 5 ml Buffer N3 and mix gently but throughly by inverting the tube several times until a flocculent white precipitate forms.** This may require a 2-3 min incubation at room temperature with occasional mixing. **Prepare a Lysate Clearance Filter Syringe by placing the barrel in a tube rack to keep the syringe upright.**

   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. Using ice-cold Buffer N3 is helpful to precipitate more bacterial proteins.

6. **Prepare the HiBind Maxi Column.** Place a HiBind Maxi Column into a 50 ml collection tube, provided. Add **5 ml** of Buffer GPS to the column and lit it sit at room temperature for 3-10 min. Spin in a swinging bucket rotor at 3,000-5,000 x *g* for 5 minutes at room temperature. Discard the eluate and assemble the column again in the 50 ml collection tube.

- **Clear the lysate with Lysate Clearance Filter Syringe**

7. **Pour the lysate into the barrel of the Lysate Clearance Filter Syringe.** Use a new 50 ml tube to collect the cell lysate. Insert the plunger back into the barrel of the syringe.

8. **Hold the Lysate Clearence filter syringe barrel over the 50 ml tube and gently insert the plunger to expel the cleared lysate to the tube.** Some of the lysate may remain in the flocculent precipitate, do not force this residual lysate through the filter.

   Alternatively, the cell debris and KDS-precipitation can be removed by centrifugation at 15,000 x g for 10 min at 4°C, *instead of using Clearance Filter Syring in step 6-7. A tightly packed cell debris pellet indicates efficient lysis. Using this alternatively cleared step may improve the yield because all of the solution can be collected comparing with Lysate Clearance Filter Syringe.*

- **Remove endotoxins with ETR Binding Buffer**

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-Tek's (OBI) 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.

Endotoxins are lipopolysaccharides (LPS), found in the outer cell membrane of the gram negative bacteria such as *E.Coli*. In Mammalia system, the endotoxins are pyrogenic, it can cause fever and endotoxin shock syndrome. The endotoxin contamination is one of the major concern for gene therapy. The endotoxin contamination in plasmid can cause lower transfection efficiency for endotoxin sensitive cell lines. Since Endotoxin is negative charged molecular like DNA, both DNA and endotoxin moleculars behave similarly on the surface of silica and anion-exchange chromatography which are the most common used technologies for plasmid purification.

The E.Z.N.A.® Fastfilter Endo-free Plasmid Maxi Kit combines the power of HiBind™ technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality DNA. Omega Bio-Tek's HiBind™ Maxi columns facilitate the binding, washing, and elution steps thus enabling multiple samples to be simultaneously processed. This kit also include a special filter cartridge, which replaces the centrifugation step following alkaline lysis. Following lysis of the cell, endotoxins are removed from the cleared cell lysate with simple extraction-phase-separation steps. Then DNA is bound to the silica membrane and contaminants are removed with a simple wash step. Yields vary according to plasmid copy number, *E.coli* strain, and conditions of growth, but up to 600-1200 µg of high copy number plasmid or 50-400 µg of low copy number plasmid can be purified from 200 ml overnight culture. Up to 500 ml culture may be processed when working with low-copy number plasmid. The product is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, transfection of mammalian cells, and other manipulations.

Storage and Stability

All E.Z.N.A.® 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 4°C, all other material at 22-25°C.

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	Only use LB or YT medium containing ampicillin. Do not use more than 500 ml. 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 500 ml.
No DNA eluted.	DNA Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare DNA 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 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, does not freeze, or smells of ethanol.	Ethanol traces not completely removed from column following wash steps.	Centrifuge column at 3000 x g for 10 minutes to dry the column. A swinging-bucket rotor is recommended for centrifugation. Alternatively, precipitate the eluted DNA with isopropanol as indicated in Page 8.

Ordering Information

Protocol 1: Endo-Free Plasmid Maxi Kit Spin Protocol

This Protocol is designed to isolate 600-1200 µg of high Copy-Number plasmids or 50-400 µg of low Copy-Number Plasmids from 200 ml overnight cultures using E.Z.N.A.® Fastfilter Endo-Free Plasmid Maxi Kit. For increasing yield of low Copy-Number plasmid, proceed as "Low Copy-Number Plasmids protocol" on page 9.

- **Growth of bacterial cultures**

1. **Inoculate 200 ml LB/ampicillin (50 µg/ml) medium placed in a 1-4 liter culture flask with *E.coli* carrying desired plasmid and grow at 37˚C with agitation for 12-16 h.** For best results use overnight culture as the inoculum. 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®.

   Optimal growth conditions of bacteria is vital of obtaining maximal plasmid DNA yields. The best conditions are achieved by picking a single isolated colony from a freshly transformed or freshly plate to inoculate a 2-5ml starter culture containing the appropriate antibiotic. Incubate for ~8hr at 37˚C with vigorous shaking (~300rpm). Then used to inoculate appropriate volume of Pre-warmed liquid growth medium with antibiotic. Grow at 37˚C for 12-16 hr with vigorous shaking(~300rpm), Use a flask or vessel with a volume of at least 3-4 times the volume of the culture, then dilute the starter culture 1/500 to 1/1000 into growth medium.

   Following overnight bacterial growth, an OD_{600} of 1.5~2.0 indicates a well-grown culture. For the best result, determination of OD_{600} for each culture is recommended. It is important to dilute the bacterial culture (10 to 20 fold) to enable photometric measurement in the linear range between 0.1 and 0.5 OD_{600}. We recommend a bacterial density of between 2.0 and 3.0 at $OD_{600.}$ When using untrient-rich media, care should be taken to ensure that the cell density does not exceed an OD_{600} of 3.0.

   If using a frozen glycerol stock as inoculum, streak it onto an agar plate containing the appropriate antibiotic for single colony isolation. Then picking a single colony and inoculate the 2-5ml starter culture as described above.

   Growth of bacteria in rich media such as 2 x YT or TB is not recommended for use with this kit.

- **Lyse the bacterial cells with alkaline-SDS Solution**

2. **Pellet up to 100-200 ml bacterial culture in appropriate vessels by centrifugation at 3,500-5,000 x g for 10 min at room temperature.**

3. Decant or aspirate medium and discard. To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the vessel. **To the bacterial pellet add 10 ml Solution I/RNase A.** Resuspend cells

nm and then at 280 nm. The DNA concentration is calculated as follows:

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

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.

Plasmid Copy-Number and Expected Yield

The Yield and quality of the plasmid DNA depend on a number of factors including plasmid copy number, size of insert, host strain, culture volume, culture medium and

9. **Add Equal volume of ETR Binding Buffer to the filtered lysate, mix by inverting the tube 7-10 times**.

■ **Purify Plasmid DNA with HiBind™ DNA Maxi Column**

10. **Carefully transfer the lysate into the HiBind® DNA Maxi Column assembled into the 50 ml Collection Tube**. Centrifuge at 3,000-5,000 x g for 3-5 minutes. Discard the flow-through and re-use the collection tube.

11. **Repeat Step 10 until the remaining of the lysate has been passed through the column**. Discard the flow-through and re-use the collection tube.

12. **Add 10 ml ETR Wash Buffer to the DNA Midi column and centrifuge as above.** Discard the flow-through and re-use the collection tube.

13. **Add 10 ml Buffer EHB to the DNA Maxi Column.** Centrifuge at 3,000-5,000 × g for 3-5 minutes. Discard the flow-through and re-use the collection tube.

14. **Add 15 ml DNA Wash Buffer (diluted with absolute ethanol) to the DNA Maxi Column and centrifuge as above.** Discard the flow-through and re-use the collection tube.

    Note: DNA Wash Buffer 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.

15. **Add 10 ml DNA Wash Buffe diluted with absolute ethanol to the DNA Maxi Column and centrifuge as above.** Discard the flow-through and re-use collection tube.

16. **Centrifuge the empty HiBind® DNA Maxi Column at maxi speed (no more than 6,000 x g) for 10-15 min to dry the column matrix**. Do not skip this step - it is critical for removing ethanol from the column.

■ **Elution Plasmid DNA From HiBind™ DNA Maxi Column.**
Optional: *For maximal yield and high concentration of plasmid, see alternative protocol of elution on page 7. For fast elution, proceed step 17-18.*

17. **Drying the column**: choose either of the methods below to further dry the column before eluting DNA.
    A. Remove the column and place into a vacuum chamber at room temperature. Any device connected to a vacuum source may be used. **Seal the chamber and apply vacuum for 15 min.** Remove the column and proceed to step 18.

    B. **Bake the Maxi column at 65°C in a vacuum oven or incubator for 10-15 minutes.** Proceed to step 19.

18. **Place HiBind™ DNA Maxi Column into a clean 50 ml centrifuge tube. Add 1-3 ml (depending on desired final concentration) Endotoxin-Free Elution Buffer (or Water) onto the column matrix and let it sit at room temperature for 2 minutes. Centrifuge at maxi speed (no more than 6,000 x g) for 5 min to elute DNA.**

    This represents approximately 60-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration. Preheating the water to 70°C and allowing the column to soak 2 min at room temperature before elution may significantly increase yields.

    Note: The plasmid DNA obtained using this protocol performs well in PCR, restriction digests, lipid mediated transfection and transformation. The expected concentration of plasmid vary between different copy number vector. However, the concentration of high copy-number plasmid is 150-600ug/ml. Some residual ethanol may present, but does not interfere with these downstream applications. One may get high concentration and absolutely remove ethanol with alternative elution step as following.

■ **Alternative Protocol of Elution Plasmid from Column**

1. **Place HiBind™ DNA Maxi Column into a clean 50 ml centrifuge tube. Add 6 ml Endotoxin-Free Elution Buffer (or Water) directly onto the column matrix**. Allow column to sit 2 min at room temperature. Centrifuge at maxi speed (no more than 6000 xg) for 5 min to elute DNA. Preheating the water to 70°C and allowing the column to soak 2 min at room temperature before elution may significantly increase yields.

2. **Carefully transfer eluted, purified plasmid from 50 ml centrifuge tube to a clean tube suitable for precipitation. Add 260 µl 5M NaCl and 4.4 ml room temperature isopropanol.** Vortex to mix and centrifuge at >15,000 × g for 30 min at 4°C. Carefully decant the supernatant.

3. **Wash DNA pellet once with 2ml 70% ethanol and centrifuge at > 15,000 × g for 10 min.** Carefully decant the supernatant without disturbing the pellet. Air-dry the pellet for 5-10 min.

4. Finally resuspend DNA pellet in 200-500 µl (depending on desired concentration of final product) Endotoxin-Free Eluiton Buffer or Water.

Protocol 2: Endo-Free Plasmid Maxi Kit Vacuum Protocol