Contents

Revised October 2005

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 E.Z.N.A.™ Fastfilter Plasmid Midi Kit combines the power of HiBind® technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality plasmid DNA. Omega Bio-Tek's midi 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 the 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 50 ml of overnight culture in LB medium typically produces 100-250 µg of high-copy number plasmid or 10-100 µg of low-copy number plasmid. Up to 100 ml culture may be processed when working with low-copy number plasmids. 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/RNase A at 4°C, all other material at 22-25°C.

Kit Contents

Product Number	D6905-00	D6905-01	D6905-03	D6905-04
Purification times	2 Preps	5 Preps	25 Preps	100
HiBind™ DNA Midi Columns	2	5	25	100
15 ml collection tubes	2	5	25	100
Lysate Clearance Filter Syringe	2	5	25	100
Solution I	6 ml	15 ml	80 ml	270 ml
Solution II	6 ml	15 ml	80 ml	270 ml
Buffer N3	3 ml	10 ml	40 ml	140 ml
Buffer GBT	6 ml	15 ml	70 ml	230 ml
Buffer GPS	6 ml	15 ml	60 ml	250 ml
Buffer HB	8 ml	20 ml	90 ml	2 x170 ml
DNA Wash Buffer Concentrate	4 ml	10 ml	40 ml	4 x 40 ml
RNase A	pre-added	50 µl	300 µl	1.2 ml
Elution Buffer	8 ml	20 ml	90 ml	2 x 160
Instruction Booklet	1	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 x g. Nuclease-free 15 ml centrifuge tubes Absolute (95%-100%) ethanol
IMPORTANT	1. **Add vial of RNase A to Solution I provided and store at 4°C.** 2. **DNA Wash Buffer Concentrate** is to be diluted with absolute ethanol (~96-100%) as follows and store at room temperature. D6905-00 Add 16 ml absolute ethanol to bottle D6905-01 Add 40 ml absolute ethanol to bottle D6905-03 Add 160 ml absolute ethanol to bottle D6905-04 Add 160 ml absolute ethanol per bottle ***Store diluted DNA Wash Buffer at room temperature !***

Protocol 1: Fastfilter Plasmid Midi Kit Spin Protocol

This Protocol is designed to isolate 100-250 µg of high Copy-Number plasmids or 50-100 µg of low Copy-Number Plasmids from 30-50 ml overnight cultures. For increasing yield of low Copy-Number plasid, proceed as Low Copy-Number Plasmids protocol on page 8.

■ **Growth of bacterial culture**

1. **Culture volume: Inoculate 30-50 ml LB/ampicillin (50 µg/ml) medium placed in a 200-400 milliliter 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).Using a flask or vessel with a volume of a least 3-4 times the volume of the culture and 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}. For maximal yields, the OD600 of cultures should be under 3.0.

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

■ **Lyse bacterial cells with Alkaline-SDS Solution**

2. **Pellet up to 30-50 ml bacteria 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 2.5 ml Solution I/RNase A.** Resuspend cells completely by vortexing or pipetting up and down. *Complete resuspension of cell pellet is vital for obtaining good yield.*

4. **Add 2.5 ml Solution II, cover, and mix gently but throughly by inverting and rotating tube 7-10 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. Prolonged incubation may lead to nicking of plasmid DNA**.** (Store Solution II tightly capped when not in use.)

5. Add 1.25 ml ice-cold Buffer N3, cover, and gently mix by inverting tube several times until a flocculent white precipitate forms. 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.

6. **Add 2 ml Buffer GBT, cover and gently mix by inverting tube 3-4 times.**

7. **Prepare the HiBind Midi Column.** Place a HiBind Midi Column into a 15 ml collection tube, provided. Add **2 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 assembled the column again into the 15 ml collection tube.

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

8. **Immediately pour the lysate into the barrel of the Lysate Clearance Filter Syringe. Allow the cell lysate to sit for 2 minutes.** The white precipitate should float to the top. Use a new 15 ml tube to collect the cell lysate. Insert the plunger back into the barrel of the syringe.

9. **Hold the Lysate Clearence filter syringe barrel over the 15 ml tube and gently insert the plunger to expel the cleared lysate to the tube.**

Note: 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 ≥12,000 x g for 10 min at 4°C, instead of using Clearance Filter Syring in step 8-9. 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.

Note: Step 10 to 16 should be performed in swinging-bucket rotor for maximal plasmid DNA yields. All of centrifugation steps must be carried out at room temperature.

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

10. **Transfer 4 ml of the clear lysate to the HiBind® DNA Midi column assembled in the 15 ml collection tube**. The Midi column has a maximum capacity of 4.5 ml. Centrifuge at 3,000-5,000 x g for 3-5 min at room temperature to completely pass lysate through column. Discard the flow-through liquid and repeat this step until the entire sample has been passed through. Finally discard the flow-through and reuse the collection tube in Step 11.

11. **Add 3 ml Buffer HB to the Midi column and centrifuge as above**. This step ensures that residual protein contamination is removed and must be included for downstream applications requiring high quality DNA. Discard flow-through liquid and reuse the collection tube in the next step.

12. **Wash the column by adding 3.5 ml of DNA Wash Buffer diluted with absolute 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.

13. **Optional Step**: **Repeat wash step with another 3.5 ml of DNA Wash Buffer. Centrifuge as above and discard fluid.**

14. **Centrifuge the empty capped column for 10-15 min at maximum speed (no more than 8,000 x g) to dry the column matrix.**

DO NOT skip this step - it is critical for removing traces of ethanol that may otherwise interfere with downstream applications.

■ **Elution Plasmid DNA From HiBind™ DNA Midi column.**
Optional: *For maximal yield and high concentration of plasmid, see alternative protocol of elution on page 7. For fast elution, proceed step 15-16.*

15. Further Drying The Column (Optional). Choose either of the methods below to further dry the column before eluting DNA (only if necessary):

A. **Place the column into a vacuum container to dry the ethanol for 10 minutes.** Then, 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 16.

B. **Bake the column in a vacuum oven or incubator at 65°C for 10 minutes. Remove the column and proceed to Step 16.**

16. **Place column into a clean 15 ml centrifuge tube. Add 0.5-1.0 ml (depending on desired concentration of final product) 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 8,000 x g) for 3-5 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. Also, preheating the water to 70°C prior to 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 150-400ug/ml. Some residual ethanol is present, but does not interfere with these downstream applications. One may get high concentration and absolutely remove ethanol with optional elution step as following.

- **Alternative of Elution Step from Column**

1. **Place HiBind™ DNA Midi column into a clean 15 ml centrifuge tube. Add 3 ml Elution Buffer (Water) directly onto the column matrix.** Allow column to sit 2 min at room temperature. Centrifuge for 3-5 min at maxi speed to elute DNA.

2. Carefully transfer eluted plasmid from 15 or 30 ml centrifuge tube to a clean tube suitable for precipitation. **add 130 ul 5M NaCl and 2.2 ml room temperature isopropanol.** Vortex to mix and centrifuge at >15,000 x g for 30 min at 4°C. Carefully decant the supernatant.

3. **Wash DNA pellet once with 1 ml ice-cold 70% ethanol and centrifuge at > 15,000 x 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 200µl-500µl (depending on desired concentration of final product) Elution Buffer or water.

Protocol 2: Fastfilter Plasmid Midi Kit Vacuum/Spin Protocol

1. **Prepare the cleared lysate by following step 1-9 of Spin procedure on page 4-5.**

2. **Prepare the vacuum manifold according to manufacturer' s instructions and connect the midi column to the manifold.**

3. **Transfer the cleared lysate into the HiBind® DNA Midi column,** be careful not to overfill the column, apply the vacuum to allow all sample pass through the column. Repeat to transfer the lysate into the column until the entire sample has been passed through.

4. **Add 3.0 ml Buffer HB to the column** and apply the vacuum to draw the liquid through the column.

5. **Wash the column: add 3.5 ml of DNA Wash Buffer (pre-diluted with absolute ethanol) into the column** and allow it to pass through the column.

6. **Wash the column again with 3.5 ml DNA Wash Buffer by repeating step 5.**

7. **Keep the vacuum on for another 10 minutes after the liquid pass through the column.** (This step to ensure the removal of residue ethanol).

8. **Centrifuge the empty capped column for 10-15 min at maxi speed (no more than 8,000 x g) to dry the column matrix.** Remove any traces of ethanol from the column's inner surface or O-ring using a pipette.

9. **Proceed Elution step as Centrifugation protocol Fast Elution Step 15-16 on Page 6 or Alternative elution step on page 7.**

Protocol 3: Low Copy-Number Plasmids Protocol

Low copy number plasmids generally give 0.1-1 µg 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 (1-3 µg/ml culture) bacteria, the method can be modified to essentially increase the yield if necessary.

Start with 50-100 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 Buffer N3 and Buffer GBT. Continue as above using only one HiBind® DNA Midi column. There is no need to increase the volumes of Buffer HB and DNA Wash Buffer used. The Buffer of Solution I, II, Buffer N3 and Buffer GBT can be purchase separately.

Note: This method is not recommended for high copy number plasmids because above 50 ml culture, the HiBind® DNA Midi column 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 (10- to 20-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

DNA concentration = Absorbance$_{260}$ x 50 x (Dilution Factor) µ***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 binding capacity of kit. In these factors, The copy number of vector, culture volume and binding capacity of kit are most important. Copy number of plasmid 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.

Plasmid	Replicon	Copy Number	expected Yield of 50ml culture
pUC vector	pMB1	500-700	180-220 µ*g*
pBR322 and its derivatives	pMB1	15-20	10-20µ*g*
PACYC and its derivatives	p15A	10-12	5-30 µ*g*
pSC101 and its derivatives	pSC101	~5	5-20 µ*g*
pBluescript	ColE14	300-500	100-150 µ*g*
ColE14	ColE14	15-20	5-20 µ*g*
pGEM	pMB1	300-700	150-200 µ*g*

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 50 ml with high copy number plasmids. 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 0.1µg DNA from a 1 ml overnight culture. Increase culture volume to 100 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 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 13 to dry .
Plasmid DNA will not perform in downstream application	Traces of ethanol remain on column prior to elution.	The column must be dried before elution. Ethanol precipitation may be required following elution

Ordering Information

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	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	up to 30 µg Endotoxin free Plasmid DNA	D6948
Endo-Free Plasmid Mini Kit	up to 70 µg Endotoxin free Plasmid DNA	D6950
Endo-Free Plasmid Mid Kit	up to 250 µg Endotoxin free Plasmid DNA featuring filter syringes for lysate clearance	D6915
Endo-Free Plasmid Maxi Kit	Isolation of 1.0 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	D7004
HP Plasmid Maxi Kit	Isolation of up to 1.5 mg of High Purity Plasmid	D7022
E.Z.N.A.™Single Strand Phage DNA Isolation Kits		
M13 Isolation Kit	Isolation of 15µg of single stranded phage DNA	D6900
E-Z 96 M13 Isolation Kit	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	Effective purification of BAC or PAC DNA	D2156
BAC/PAC DNA Isolation Kit	purification of BAC or PAC using a 96-well format	D1056