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**Revised March 2004**

## 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 96® Fastfilter Plasmid Purification 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. By using the E-Z 96® DNA Plate, up to 96 samples can be simultaneously processed in less than 90 minutes. The new E-Z 96® Lysate Clearance Plate obviates time-consuming centrifugation for clearing of the bacterial alkaline lysates. It also has an average DNA recovery rate 10 to 30% higher than the manual centrifuge method. Yields vary according to plasmid copy number, E.coli strain, and conditions of growth, but 1 ml of overnight culture in LB medium typically produces 10-15 µg high-copy plasmid DNA.

## Introduction

The following changes have been made to the E-Z 96® Fastfilter Plasmid Purification procedure for improving yield and purity:

- Newly designed E-Z 96® DNA Plate introduced
- New lysate clearance protocol introduced
- Only one wash step required with DNA Wash Buffer
- Buffer HB introduced
- A second wash step with absolute ethanol is introduced to reduce the drying time before elution

## Storage and Stability:

All E-Z 96® Fastfilter Plasmid Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: The mixture of Solution I/RNase A at 4°C; all other material at 22-25°C.

## E-Z 96™ Fastfilter Plasmid Purification Kit

Product Number	D1097-01	D1097-02
Purifications	4	20
E-Z 96® DNA Plate	4	20
96 Well Collection plate (1.0ml)	4	20
96 well Collection Plate (2.0 ml)*	2	4
96 well Collection Plate (300µl)	1	1
Lysate Clearance Plate	4	20
Solution I	100 ml	400 ml
Solution II	100 ml	400 ml
Neutralization Buffer	100 ml	400 ml
Buffer HB	200 ml	2 x 500 ml
Buffer GBT	100 ml	400 ml
Wash Buffer Concentrate	3 x 100 ml	6 x 200 ml
RNase A, Concentrate	400 µl	1500 µl
Instruction Booklet	1	1

\* The 2ml 96-well collection plates are reusable. See page 8 for detailed instructions.

### Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

<b>IMPORTANT</b>	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:	
	D1097-01	Add 150 ml ~96%-100% ethanol
	D1097-02	Add 300 ml ~96%-100% ethanol per bottle
	<b>Store diluted DNA Wash Buffer at room temperature</b>	

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

## E-Z 96® Fastfilter Plasmid Isolation With Vacuum Manifold:

### Supplied By User:

- Centrifuge with swinging-bucket rotor at room temperature capable of 4000 x g. (Such as Eppendorf 5810 with MTP rotor.)
- Adapter for 96 deep well plate
- DNase-/RNase-free 96-well 2.0 ml plates (Cat#9602)
- Vacuum pump or vacuum aspirator capable of achieving a vacuum of 20-24 inches Hg
- Standard Vacuum manifold ( i.e: Omega Product #VAC-03)
- Sterile deionized water (or TE buffer)
- Absolute (96%-100%) ethanol
- Vacuum oven or incubator preset to 70°C

1. Culture volume: inoculate 1.0 ml LB/antibiotic(s) medium placed in a 96-well 2ml culture plate/block 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. Seal the plate with tape or film and pellet bacteria by centrifugation at 4,000 x g in a swinging-bucket rotor at room temperature for 10-15 minutes at room temperature.
3. Remove the tape and discard supernatant by inverting the plate and dumping the supernatant into a waste container. Dry the plate by placing upside-down on a paper towel to remove excess media. Add 220 µl Solution I/RNase A to the bacterial pellet in each well of the plate. Resuspend cells completely by vortexing and/or pipetting. Complete resuspension of cell pellet is vital for obtaining good plasmid yields.
4. Add 220 µl Solution II and mix by gently shaking and rotating the plate for 1 minute 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. (Store Solution II tightly capped when not in use.)
5. Add 220 µl Neutralization Buffer and mix by gently shaking and rotating the plate for 1 minute until a flocculent white precipitate forms.
6. Add 220 µl of GBT Buffer and gently mix by shaking and rotating the plate for 1 minute.
7. Immediately transfer the lysate into the Lysate Clearance Plate. Allow the cell lysate to sit for 5 minutes. The white precipitate should float to the top.
8. Assemble the vacuum manifold by placing the E-Z 96® DNA plate with a collection container or a 300µl microplate into the base of the vacuum manifold and then place the E-Z 96® Lysate Clearance plate on the top plate of manifold. (Some manifolds might need adjustment for the inside by using a extra small plate.)
9. Apply the vacuum until all the lysate pass through the membrane.
10. Turn off the vacuum and discard the Lysate Clearance Plate. Carefully remove the E-Z 96® DNA Plate which contains cleared lysate from vacuum manifold. Place a 2 ml collection plate into the base of vacuum manifold.

Note: For manifolds that come with a waste collection container, the 2 ml collection plate can be replaced by the waste container.

11. Place E-Z 96<sup>®</sup> DNA plate on top plate of the manifold and apply vacuum until all lysate passes through the plate.
- 12.** Add 500 µl of HB Buffer to each well, apply the vacuum until all the liquid pass through.
13. Turn off the vacuum, remove the 2 ml collection plate from manifold and discard collected liquid. Place back the 2 ml collection plate and reassemble the manifold.
14. Wash the E-Z 96<sup>®</sup> DNA Plate by adding 750 µl DNA wash buffer. Apply the vacuum until all buffer pass through.
15. Wash the E-Z 96<sup>®</sup> DNA Plate again by repeating Step 14.
16. Add 500 µl absolute ethanol to each well, and apply vacuum to draw the liquid through the plate. Keep the vacuum on for 5 more minutes to dry the plate.
17. Remove the 2ml 96-well collection plate. Discard the liquid and clean the plate for future use.
18. Remove the E-Z 96<sup>®</sup> DNA plate from vacuum manifold, vigorously tap the plate on a stack of absorbent paper until no drops come out, clean the nozzles of the DNA Plate with clean absorbent paper.
19. (Optional) Place the E-Z 96<sup>®</sup> DNA plate into a vacuum oven or incubate preset at 70°C for 10 minutes to further dry the plate.
20. Place a collection plate in the manifold and place the Adjust position of collection plate in the vacuum manifold and place the E-Z 96<sup>®</sup> DNA plate on top of manifold. **Note:** Adjust the position of the collection is important for proper elution. For OBI's Vac-3 manifold, to give a proper elution position for the 300 µl plate, place the 300 µl plate on top of a 0.8ml collection plate (Supplied with manifold). For manifolds from other manufacturers, adjust the 300 µl plate to a proper collecting position with a suitable support plate according to the height of the manifold.
21. Add 50-200 µl water or TE buffer to each well and apply vacuum to elute DNA from the plate.
22. Remove the collection plate containing the eluted DNA and store the DNA sample at -20°C.

### **E-Z 96<sup>®</sup> Fastfilter Plasmid Isolation With Centrifugation:**

1. Centrifuge with swinging-bucket rotor at room temperature capable of 4000 x g. (Such as Eppendorf 5810 with MTP rotor.)
2. Adapter for 96 deep well plate
3. DNase-/RNase-free 96-well 2.0 ml plates (Cat#9602)
4. Vacuum pump or vacuum aspirator capable of achieving a vacuum of 20-24 inches Hg
5. Standard Vacuum manifold ( i.e: Omega Product #VAC-03)
6. Sterile deionized water (or TE buffer)
7. Absolute (96%-100%) ethanol
8. Vacuum oven or incubator preset to 70

1. Culture volume: inoculate 1.0 ml LB/antibiotic(s) medium placed in a 96-well 2ml culture plate/block 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<sup>®</sup> and JM109<sup>®</sup>.
2. Seal the plate with tape and pellet bacteria by centrifugation at 4,000 x g in a swinging-bucket rotor at room temperature for 10-15 minutes at room temperature.
3. Remove the tape and discard supernatant by invert the plate and dump the supernatant into a west container. Dry the plate by upside-down on a paper towel to remove excess media. Add 220 µl Solution I/RNase A mixture to the bacterial pellet in each well of the plate. Resuspend cells completely by pipetting. Complete resuspension of cell pellet is vital for obtaining good plasmid yields.
4. Add 220 µl Solution II and gently mix by gently shaking and rotating the plate for 1 minute 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. (Store Solution II tightly capped when not in use.)
5. Add 220 µl Neutralization Buffer and mix by gently shaking and rotating the plate for 1 minute until a flocculent white precipitate forms.
6. Add 220 µl of GBT Buffer and gently mix by rotating the plate for 1 minute.
7. Immediately transfer the lysate into the Lysate Clearance Plate. Allow the cell lysate to sit for 5 minutes. The white precipitate should float to the top.
8. Seal the Clearance plate with tape or film and place the plate on top of a 2ml collection plate. Place the Clearance/Collection plate Centrifuge the a plate adapter for centrifuge. Centrifuge at 4000 x g in a swinging-buck rotor for 15 minutes.
9. Transfer the cleared supernatant to a new E-Z 96 DNA plate.
10. Seal the E-Z 96<sup>®</sup> DNA plate and place it on top of a 2 ml 96-well collection plate. Centrifuge at 3000 x g for 5 minutes.
11. Remove the sealing film and add 500 µl of Buffer HB to each well and centrifuge at 3000 x g for 5 minutes. Discard the flow-through and reuse the collection plate for next step.
12. Add 700 µl DNA wash buffer to each well. Centrifuge at 3000 x g for 5 minutes. Discard the flow-through and reuse the collection plate for next step.
13. Add 700µl DNA Wash Buffer to each well. Centrifuge at 3000 x g for 5 minutes. Discard the flow-through and reuse the collection plate for next step.
14. Add 500 µl absolute ethanol to each well. Centrifuge at 3000 x g for 5 minutes.
15. Remove the E-Z 96<sup>®</sup> DNA plate, vigorously tap the plate on a stack of absorbent paper until no drops come out, clean the nozzles of the DNA Plate with clean absorbent paper.
16. (Optional): Place E-Z 96<sup>®</sup> DNA plate into a vacuum oven or incubator which was preset to 70°C for 10 minute. This step will ensure that the DNA plate is completely dried before DNA elution.
17. Add 50-100 µl Water or TE buffer to each well of the DNA plate.
18. Carefully place the E-Z 96<sup>®</sup> DNA plate on top of the 96-well 300µl collection plate(supplied). Centrifuge at 3000 x g for 5 minutes 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.

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

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

High copy number plasmids generally yield up to 200 µg of DNA from 50 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 presented

### Cleaning of 2ml 96-well plates:

The 2ml 96-well collection plates are reusable. To avoid cross-contamination, rinse the plates thoroughly with tap water after each user. Rinse with 0.5M Hcl for 5minutes, then rinse thoroughly with distilled water. 2ml 96-well collection plates can also be autoclaved after wash.

## Trouble Shooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	<p><b>Only use LB or YT medium</b> containing Antibiotec(s). Do not use more than 2 ml with <b>high copy plasmids</b>.</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 0.1µg DNA from a 1 ml overnight culture. Increase culture volume to 3 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 plate as instructed in steps 9-12. 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	Ethanol not completely removed from column following wash steps.	bake the plate 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 DNA plate must be washed with absolute ethanol and dried before elution. Ethanol precipitation may be required following elution.

If the above suggestions fail to resolve any problems you are having with E-Z®

96 plasmid Purification kit, please feel free to contact our technical specialist at:  
US customers:US customers: Tel: 800 832 8896  
All other customers: (770) 931 8400  
Fax: (770) 931 0230