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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 E-Z96® Fastfilter Plasmid 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-Z96® 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.

New In this Edition

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

- New lysate clearance plate introduced

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. Store Solution II tightly capped.

Kit Contents

Product Number	D1097-00	D1097-01	D1097-02
E-Z 96® DNA Plate	1	4	20
96-Well Collection Plate (2.0 mL)*	1	2	4
Racked Microtubes (1.2 mL)	1	4	20
8-Strip Microtube Caps	12	50	250
Sealing film	3	12	60
E-Z 96® Lysate Clearance Plate	1	4	20
Solution I	30 mL	110 mL	550 mL
Solution II	30 mL	110 mL	550 mL
Solution III	40 mL	150 mL	750 mL
Equilibration Buffer	18 mL	70 mL	300 mL
HB Buffer	60 mL	220 mL	1050 mL
DNA Wash Buffer Concentrate	30 mL	120 mL	3 x 200 mL
Elution Buffer	20 mL	80 mL	300 mL
RNase A Concentrate	100 µL	400 µL	2.0 mL
Instruction Booklet	1	1	1

* The 2mL 96-well collection plates are reusable. See page 7 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.

IMPORTANT	Add vial of RNase A to bottle of Solution I and Store at 4°C.
	Dilute **DNA Wash Buffer** with **absolute ethanol** as follows **D1097-00** Add 120 mL ~96%-100% ethanol per bottle **D1097-01** Add 480 mL ~96%-100% ethanol per bottle **D1097-02** Add 800 mL ~96%-100% ethanol per bottle

E-Z 96® Fastfilter® Plasmid Vacuum Manifold Protocol

Materials Supplied By User

- Centrifuge with swinging-bucket rotor at room temperature capable of 4000 x g
- Adapter for 96-well collection plate
- DNase-/RNase-free 96-well 2.0 mL deep well 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)
- Vacuum oven or incubator preset to 70°C

1. Culture Volume: **Innoculate 1.0-1.2 mL LB/antibiotic(s) medium placed in a 96-well 2mL culture block with *E.coli* carrying desired plasmid and grow at 37°C with agitation (180-300 rpm) for 20-24 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 sealing film and pellet bacteria by **centrifugation at 1,500-2,000 x g for 5 minutes** in a swinging-bucket rotor at room temperature.

3. Remove the sealing film and discard supernatant into a waste container. Dry the plate by tapping the inverted block firmly a paper towel to remove excess media. **Add 250 µl Solution I/RNase A to the bacterial pellet in each well of the plate**. Resuspend cells completely by vortexing and/or pipetting. No cell clumps should be visible after resuspension of the pellet. Complete resuspension of cell pellet is vital for obtaining good plasmid yields.

4. **Add 250 µl Solution II into each well and mix throughly by gently shaking and rotating the plate for 1 minute to obtain a cleared lysate.** A 2-3 min incubation at room temperature may be necessary. The solution should become viscous and slightly clear. Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity. (Store Solution II tightly capped when not in use.)

5. **Add 350 µl Solution III to each well.** Dry the top of the plate with a paper tower. Seal the plate with sealing film and mix by gently inverting the plate for 5-6 times until a flocculent white precipitate forms.

6. Assemble the vacuum manifold: Place the HiBind® DNA Plate on top of the 2ml collection plate (supplied). **Add 150µl Equilibration Buffer into each well and let the plate sit for 4 minutes at room temperature. Apply the Vacuum til all liquid passes through. Discard the liquid flowthrough**

7. Assemble the vacuum manifold: 1). Place E-Z 96® Lysate Clearance Plate in the top plate of manifold; 2). Place the plate holder inside the manifold base; 3). Place the E-Z 96® DNA Plate into the plate holder. 4). Place the top plate of manifold over the base, the E-Z 96® DNA Plate now should be positioned under the E-Z 96® Lysate Clearance Plate. (Some manifolds might require internal height adjustment by using an extra small plate.) Seal the unused wells of E-Z 96® Lysate Clearance Plate with sealing film.

8. Immediately transfer the lysate into the wells of E-Z 96® Lysate Clearance Plate. Allow the lysate to stand for 5 minutes. The white precipitate should float to the top.

9. Apply the vacuum until all the lysate passes through.

10. Turn off the vacuum and discard the E-Z 96® Lysate Clearance Plate. Carefully transfer the E-Z 96® DNA Plate which contains cleared lysate to the top plate of vacuum manifold. Seal the unused wells of E-Z 96® DNA Plate with sealing film.

11. Place a waste collection tray inside the vacuum manifold and place top plate over the base. Making sure that the E-Z 96® DNA Plate is seated securely. Apply vacuum until all lysate passes through the plate

12. **Add 500µl of HB Buffer to each well**, then apply the vacuum until all the liquid passes through.

13. Turn off the vacuum, wash the samples by **adding 750µl DNA Wash Buffer diluted with absolute ethanol** to each well of the E-Z 96 DNA® Plate. Apply the vacuum until all buffer passes through.

14. Turn off the vacuum, wash the samples by **adding another 750µl DNA Wash Buffer diluted with absolute ethanol** to each well of the E-Z 96 DNA® Plate. Apply the vacuum until all buffer passes through.

15. After the all liquid pass through the membrane, dry the membrane by applying maximum vacuum for another 15-20 minutes.

16. Remove the E-Z 96® DNA Plate from the vacuum manifold, then vigorously tap the plate on a stack of absorbent paper towels until no drops come out. Remove any residual moisture from the tip ends of the DNA plate with clean absorbent paper towels.

17. (Optional) Place the E-Z 96® DNA Plate into a vacuum oven or incubate preset at 70°C for 10 minutes to further dry the plate.

18. Place the E-Z 96® DNA Plate back to the vacuum manifold and apply the maximum vacuum for another 5 minutes. This step will evaporate any remaining ethanol from membrane.

19. **Elution with provided Racked Microtubes:** Place a Racked Microtubes in the manifold, making height adjustments as necessary by using another plate, then place the E-Z 96® DNA Plate in top plate of the manifold.

20. **Elution with a 96-well microplate (not provided):** Assemble the vacuum manifold by place a new 300 µl 96-well collection plate (not provided) inside the base of manifold. If Omega manifold (Vac-03) is used in this procedure, a used E-Z 96® DNA Plate or a 800 µl plate should be placed under the 300 µl collection plate as a support to give the collection plate a proper position.

21. **Add 100-150 µl Elution Buffer (10mM Tris-HCl, pH 8.5) or sterile water** to each well of the E-Z 96® DNA Plate, let stand for 2 minutes. Apply maximum vacuum for 5-10 minutes to elute DNA from the plate. Turn off the vacuum and ventilate the manifold slowly.

22. Remove the Racked Microtubes or microplate containing eluted DNA and seal with caps or film. Store sample at -20°C.

E-Z 96® Fastfilter Plasmid Spin Protocol

Materials Supplied By User

- Centrifuge with swinging-bucket rotor at room temperature capable of 3000 xg (such as Eppendorf 5810 with MTP rotor.)
- Adapter for 96-well collection plate
- DNase-/RNase-free 96-well 2.0 mL plates
- Absolute (96%-100%) ethanol
- Vacuum oven or incubator preset to 70°C

1. **Culture Volume:** Innoculate 1.0-1.2 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 (180-300 rpm) for 20-24 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 sealing film and pellet bacteria by **centrifugation at 1,500-2,000 x g for 5 minutes** in a swinging-bucket rotor at room temperature

3. Discard supernatant into a waste container. Dry the plate by tapping the inverted block firmly a paper towel to remove excess media. **Add 250µl Solution I/RNase A to the bacterial pellet in each well of the plate**. Resuspend cells completely by vortexing and/or pipetting. No cell clumps should be visible after resuspension of the pellet. Complete resuspension of cell pellet is vital for obtaining good plasmid yields.

4. **Add 250 µl Solution II into each well** 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. The solution should become viscous and slightly clear.

   Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity. (Store Solution II tightly capped when not in use.)

5. **Add 350 µl Solution III.** Dry the top of the plate with a paper tower. Seal the plate with sealing film and mix by gently inverting the plate for 5-6 times until a flocculent white precipitate forms.

6. Place the HiBind® DNA Plate on top of the 2ml collection plate (supplied). Add 150µl Equilibration Buffer into each well and let the plate sit for 4 minutes at room temperature. Centrifuge at 3,000 to 5,000 g for 3 minutes. Discard the liquid flowthrough

7. Place a new E-Z 96® Lysate Clearance Plate on top of a new deep well plate.

8. Transfer the lysate into E-Z 96® Lysate Clearance Plate. Allow the cell lysate to sit

for 2-3 minutes. A white precipitate should float to the top.

9. Place the Clearance/Collection plates in centrifuge's swing-bucket rotor with adapter for deep well plate. Centrifuge at 3000 x g for 5 minutes.

10. Discard the E-Z 96® Lysate Clearance Plate.

11. Place a new E-Z 96® DNA Plate on top of a 96-well deep well plate (e.g., 2 mL deep well plate supplied with kit) and transfer the cleared cell lysate into E-Z 96® DNA Plate.

12. Centrifuge at 3000 x g for 5 minutes.

13. Discard the flow-through liquid and re-use the deep well collection plate for next step.

14. **Add 500µl HB Buffer to each well of E-Z 96® DNA Plate**. Centrifuge at 3000 x g for 5 minutes. Discard the flow-through and reuse the collection plate for next step.

15. **Add 900µl DNA Wash Buffer to each well.** Centrifuge at 3000 x g for 10 minutes. Discard the flow-through and re-use the collection plate for next step.

16. Remove the E-Z 96® DNA Plate, 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.

17. **(Optional)** Place E-Z 96® DNA Plate into a vacuum oven or incubator which was preset to 70°C for 10 minutes. This step will ensure that the DNA plate is completely dried before DNA elution.

18. **Elution with provided Racked Microtubes:** Place the E-Z 96® DNA Plate in top plate of the manifold.

19. **Add 100-150 µl Elution Buffer (10mM Tris-HCl, pH 8.5) or sterile water to each well of the E-Z 96® DNA Plate**. Let stand for 2 minutes.

20. Centrifuge at 3000 x g for 5 minutes to elute DNA and seal the tube with caps. 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

DNA concentration = Absorbance$_{260}$ × 50 × (Dilution Factor) µg/mL

Cleaning of 2mL 96-Well Collection Plates

The 2mL 96-well collection plates are reusable. To avoid cross-contamination, rinse the plates throughly with tap water after each use. Rinse with 0.5M HCl for 5 minutes, then rinse throughly with distilled water. 2mL 96-well collection plates can also be autoclaved after washing.

Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	Only use LB or YT medium containing Antibiotec(s). Do not use more than 2 mL with high copy 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 3 mL.
No DNA eluted.	DNA Wash Buffer is not pre-diluted with 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 plate 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	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.
	DNA is permanently denatured	Cell lysis process should not be over 5 minutes. It may cause DNA permanently denatured.