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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-Z 96® 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. The E-Z 96® Fastfilter Plasmid Kit is a high throughput system to purify plasmid DNA from 1 to 1.5 ml *E. Coli* bacterial cultures that are grown and processed in 96 well plates. This kit is designed and optimized for the use in vacuum manifolds, and it is compatible with liquid handling and pipetting work stations. This kit can also be used with centrifugation protocol. The new E-Z 96® Lysate Clearance Plate obviates time-consuming centrifugation for clearing of the bacterial alkaline lysates. 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 7-15 µg high-copy plasmid DNA.

The plasmid DNA purified with E-Z 96® Fastfilter Plasmid Kit is free of the proteins, chromosome DNA and RNA contamination and can be directly used in most of downstream applications.

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 No.	D1097-00	D1097-01	D1097-02
Purification times	1x 96 Preps	4 x 96 Preps	20 x 96 Preps
E-Z 96® DNA Plate	1	4	20
96-Well Collection Plate (2.0 mL)*	1	2	4
Elution Plate (300 µL)	1	4	20
SealPlate Sealing film	3	12	60
E-Z 96® Lysate Clearance Plate	1	4	20
Solution I	50 mL	200 mL	1000 mL
Solution II	50 mL	200 mL	1000 mL
Solution III	50 mL	200 mL	1000 mL
Buffer HB	100 mL	500 mL	2500 mL
DNA Wash Buffer Concentrate	50 mL	200 mL	4 x 200 mL
Elution Buffer	30 mL	100 mL	500 mL
RNase A Concentrate	200 µL	800 µL	4.0 mL
Instruction Booklet	1	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.

IMPORTANT

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

D1097-00 Add 200 mL ~96%-100% ethanol

D1097-01 Add 800 mL ~96%-100% ethanol

D1097-02 Add 800 mL ~96%-100% ethanol to each bottle

Store the diluted DNA Wash Buffer at room temperature !

E-Z 96® Fastfilter® Plasmid Vacuum Manifold Protocol

Materials Supplied By User

- Vacuum pump or vacuum aspirator capable of achieving a vacuum of 300-400mbar
- Standard vacuum manifold (i.e: Omega Product #VAC-03)
- Sealing film for 96-well plate
- Optional: Vacuum oven or incubator preset to 70°C
- Optional: Racked Microtubes (for elution)

1. Culture Volume: **Innoculate 1.0-1.5 mL LB or 2 x YT medium placed in a 96-well 2 mL 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 1,000-1500 x g for 10 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 3-4 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 shaking or vortexing the plate until a flocculent white precipitate forms.

   Note: Incubate on ice for 5-10 minutes for optimal forming of precipitate.

6. **Assemble the vacuum manifold**: 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.

7. **Transfer the crude lysate from step 5 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.

8. **Apply the vacuum until all the lysate passes through**. Adjust the vacuum as necessary to control the flow rate as 1-2 drop per second.
   Note: Apply vacuum too quicky can cause the clogging of the plate.

9. **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.

10. **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.**

11. **Apply the vacuum until all the lysate passes through**. Adjust the vacuum as necessary to control the flow rate as 1-2 drop per second. When the cleared lysate has drained off, turn off the vacuum and ventilate the manifold.

12. **Add 500 µl of HB Buffer to each well**, then apply the vacuum until all the liquid passes through. When the cleared lysate has drained off, turn off the vacuum and ventilate the manifold.

13. **Add 900 µl DNA Wash Buffer (diluted with ethanol) to each well of the E-Z 96® DNA Plate**. Apply the vacuum until all buffer passes through. When the cleared lysate has drained off, turn off the vacuum and ventilate the manifold.

    Note: DNA wash Buffer need to be diluted with ethanol before use. See the dilution instruction on page 3.

14. **Add another 900 µl 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 (300-400 mbar) for another 15-20 minutes to dry the membrane completely.**
    Note: It is critical to completely dry the membrane before next elution step, the residue of ethanol and salt from DNA wash Buffer might interfere some downstream enzymatic reactions.

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 65°C for 10 minutes to further dry the plate.

18. **Elution with Racked Microtubes (not supplied):** 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.

19. **Elution with a 96-well microplate (provided):** Assemble the vacuum manifold by place a new 500 µl 96-well collection plat 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.

20. **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 5 minutes. Apply maximum vacuum for 5-10 minutes to elute DNA from the plate. Turn off the vacuum and ventilate the manifold slowly.

21. Store eluted DNA 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 4,000 x g
- Adapter for 96-well collection plate
- DNase/RNase-free 96-well 2.0 mL plates
- Absolute (96%-100%) ethanol

1. **Culture Volume:** Innoculate 1.0-1.5 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 1,000-1,500 x g for 10 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 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 by gently shaking and rotating the plate for 1 minute to obtain a cleared lysate. A 3-4 min incubation at room temperature may be necessary. The solution should become viscous and slightly clear.
   Note: 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 shaking or vortexing the plate until a flocculent white precipitate forms.
   Note: Incubate on ice for 5-10 minutes for optimal forming of precipitate.

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

7. Transfer the crude lysate from step 5 into E-Z 96® Lysate Clearance Plate.

8. Place the Clearance/Collection plates complex into centrifuge's swing-bucket rotor with adapter for deep well plate. Centrifuge at 2,000 x g for 5 minutes.

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

10. 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.

11. **Centrifuge at 2,000-5,000 x g for 3 minutes at room temperature**.

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

13. **Add 500 µl HB Buffer to each well of E-Z 96® DNA Plate**. **Centrifuge at 2,000-5,000 x g for 3 minutes. Discard the flow-through and reuse the collection plate for next step.**

14. **Add 900 µl DNA Wash Buffer to each well.** Centrifuge at 2,000-5,000 x g for 3 minutes. Discard the flow-through and re-use the collection plate for next step.

15. **Add another 900 µl DNA Wash Buffer to each well.** Centrifuge at maximum speed **(>3,000 x g) for 10 minutes.** Discard the flow-through and re-use the collection plate for next step.
    Note: The longer centrifugation time at this step ensure the membrane is completely dried before the elution step.

16. Place the E-Z 96® DNA Plate on top of a new 96-well Elute Plate.

17. **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**. Incubate at room temperature for 5 minutes.

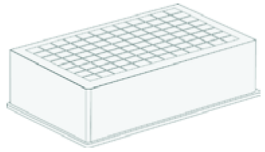
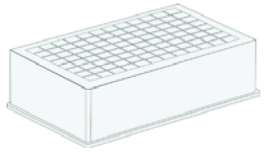
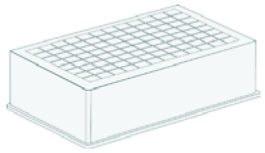
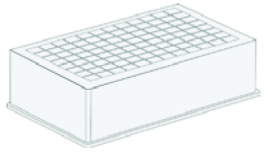
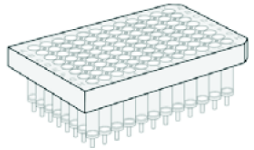
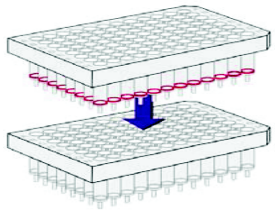
18. Centrifuge at 2,000 x g for 5 minutes to elute DNA. This represents approximately 75-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

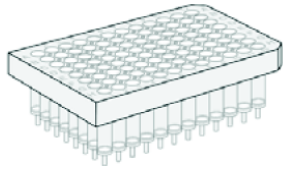
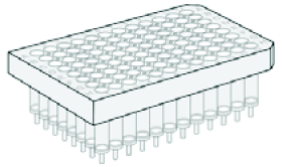
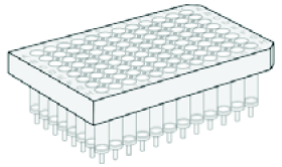
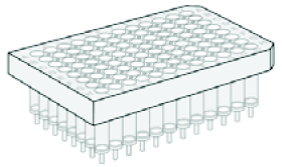
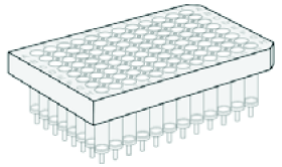
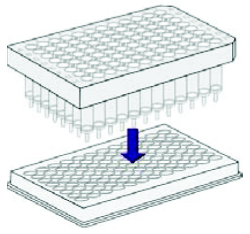
19. Store eluted DNA sample at -20°C.

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 5minutes, then rinse throughly with distilled water. 2mL 96-well collection plates can also be autoclaved after washing.

E-Z 96® Fastfilter Plasmid Kit Overview Protocol

1. Harvest bacterial cells	
2. Resuspend Bacterial cells with 250 µl Solution I	
3. Lyse the bacterial cells with 250 µl Solution II	
4. Neutralize with 350 µl Solution III by shaking or vortexing.	
6. Transfer crude lysate into the E-Z 96® Lysate clearance plate	
7. Clear the crude lysate with E-Z 96® Lysate Clearance Plate by vacuum or centrifugation	

8. Bind DNA to the HiBind membrane of the E-Z 96® DNA plate.	
9. Wash the E-Z 96® DNA plate with 500 µl HB Buffer	
10. Wash the E-Z 96® DNA plate with 900 µl DNA Wash Buffer	
11. Wash the E-Z 96® DNA plate with 900 µl DNA Wash Buffer	
12. Dry the E-Z 96® DNA plate by vacuum or centrifugation	
13. Place the E-Z 96® DNA plate on top of the Elution Plate. Elute Plasmid DNA with 75-150µl Elution Buffer.	

Troubleshooting Guide

Please use this guide to solve any problems that may arise. We hope that it will aid in clearing up any questions for you, if for any reason you need further assistance the scientists at Omega Bio-Tek, Inc. are always happy to answer any questions you may have about either the information and procedures of this manual or molecular biology applications (see page 16 for contact information).

Possible Problems and Suggestions

Low DNA yields	
a) Poor Cell Lysis	Only use LB or YT medium containing antibiotic. Do not use more than 5 ml (LB Culture) or 3 ml YT Culture.
	Cells may not have been dispersed adequately prior to the addition of Solution II. Make sure to 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.
b) Bacterial Clone is overgrown or not fresh.	Do not incubate cultures for more than 24 hr at 37°C. Storage of cultures for extended periods prior to plasmid isolation is detrimental.
c) Low elution efficiency	The pH of Elution Buffer or water must be ≥8.0

d) Low copy-number plasmid used	Such plasmids may yield as little as 0.5µg of DNA from a 5 ml overnight culture.
No DNA Eluted	
e) DNA Wash Buffer Concentrate not diluted with 96-100% ETOH.	Prepare DNA Wash Buffer Concentrate according to instructions on page 3.
High molecular weight DNA contamination of product.	
f) Over mixing of cell lysate upon addition of Solution II.	Do not vortex or mix aggressively after adding Solution II.
g) Culture overgrown	Overgrown culture contains lysed cells and degraded DNA. Do not grow cell for longer than 16 hours.
RNA visible on agarose gel	
h) RNase A not added to Solution I.	Check that RNase A provided with the kit has been used. If Solution I is more than 6 months old, add more RNase A.
Plasmid DNA floats out of well while loading agarose gel	
j) Ethanol has not completely been removed from E-Z 96 DNA Plate following wash steps.	Ensure applying the maximum vacuum. Place the DNA Plate into a vacuum oven to further dry the plate.
Plasmid DNA will not perform in downstream application	
Traces of ethanol remain on plate prior to elution.	Bake the plate as instructed in step 17 to further dry. 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.