omega BIO-TEK

innovations in nucleic acid isolation

Product Manual

E-Z 96 Endo-Free Plasmid Kit

D1099-01	1 x 96 preps
D1099-02	4 x 96 preps
D1099-03	20 x 96 preps

For Research Use Only

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E-Z 96 Endo-free Plasmid Kit

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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® Endo-free 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. It 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 E-Z 96® Endo-free Plasmid Kit combines the power of HiBind technology with the time tested consistency of alkaline-SDS lysis of E-Z 96®DNA Plant facilitate the binding,washing, and elution steps thus enabling multiple samples to be simultaneously processed. The product is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, transfection of mammalian cells, and other manipulations.

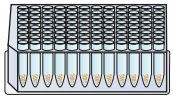
Yield and Quality of DNA

Determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

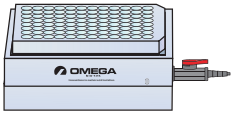
DNA concentration = Absorbance 260 × 50 × (Dilution Factor) µg/mL

A ratio greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) sometimes can be determined best 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 concatemers may also be present.

Illustrated Vacuum Protocol

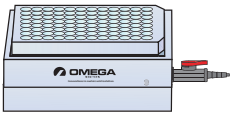


Resuspend
Lyse
Neutralize



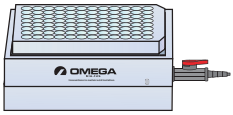
Clear Lysate with
Lysate Clearance
Plate

Vacuum



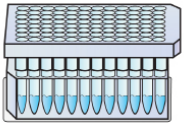
Bind and Wash 3x

Vacuum



Dry Membrane

Vacuum



Elute

Kit Contents

Product No.	D1099-01	D1099-02	D1099-03
Preps	1 x 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	25 mL	100 mL	2 x 250 mL
Solution II	25 mL	100 mL	2 x 250 mL
Neutralization Buffer	25 mL	100 mL	2 x 250 mL
PFC Binding Buffer	20 mL	80 mL	2 x 200 mL
Buffer GW1	100 mL	2 x 200 mL	8 x 250 mL
DNA Wash Buffer Concentrate	50 mL	4 x 50 mL	16 x 50 mL
Elution Buffer	20 mL	80 mL	2 x 200 mL
RNase A Concentrate	120 µL	480 µL	2 x 1.2 mL
User Manual	✓	✓	✓

* The 2mL 96-well collection plates are reusable. See page 5 for detailed instructions.

Storage and Stability

All E-Z 96® Endo-free Plasmid Kit components are guaranteed for at least 24 months from the date of purchase when stored as follows: Solution I/RNase A at 4℃, all other material at 22-25℃. Solution II and PFC Binding Buffer may form precipitate under lower temperature during shipping or storage, incubate at 50℃ to dissolve the precipitate.

Preparing Reagents

1. Add the vial of RNase A to the bottle of Solution I and store at 2-8˚C.

2. Dilute **DNA Wash Buffer Concentrate** with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D1099-01	200 mL
D1099-02	200 mL per bottle
D1099-03	200 mL per bottle

3. Check Solution II and Solution III for precipitation before use. Redissolve any precipitation by warming to 37˚C.

Cleaning of 96-well Collection Plates(2.0mL)

The 96-well Collection Plates(2.0mL) supplied with this kit are reusable. To avoid cross-contamination, rinse the plates thoroughly with tap water after each use. Soak the plates in 0.5M HCl for 5 minutes then wash thoroughly with distilled water. The 96-well Collection Plates(2.0mL) also can be autoclaved following washing.

Guidelines for Vacuum Manifold

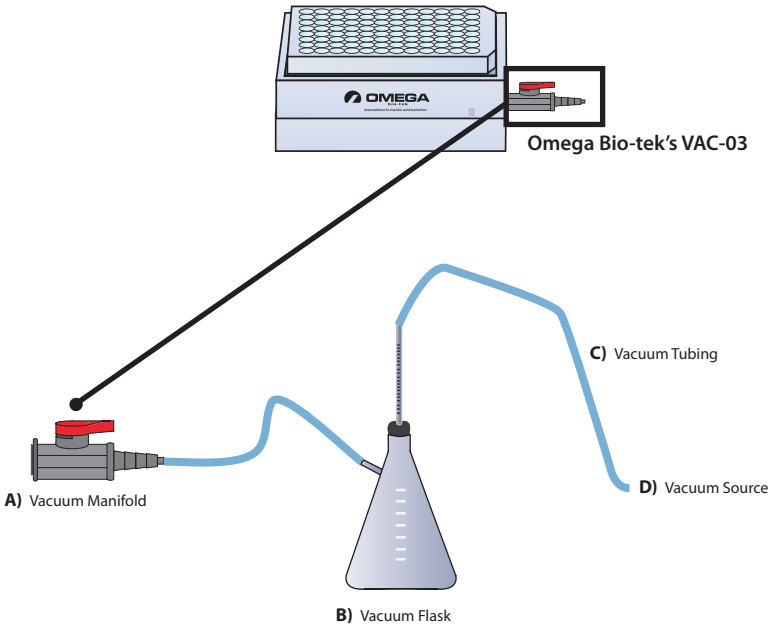
The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-03)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Manifold	Recommended Pressure (mbar)
VAC-03	-200 to -400

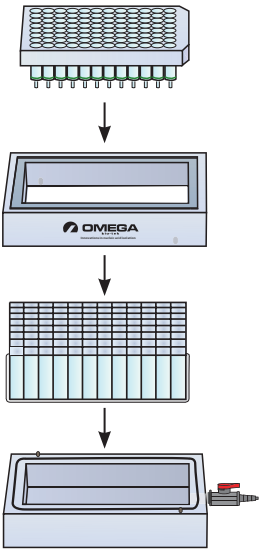
Conversion from millibars:	Multiply by:
Millimeters of Mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of Mercury (inchHg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Illustrated Vacuum Setup

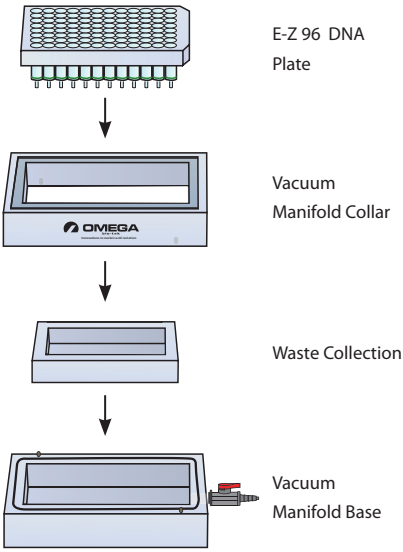


Guidelines for Vacuum Manifold

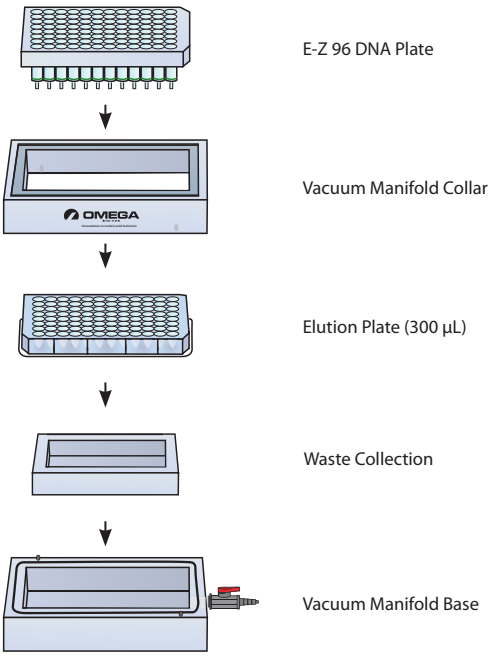
Lysate Clearance Setup



DNA Bind & Wash Setup



Standard Elution Setup



E-Z 96 Endo-free Plasmid Kit - Vacuum Protocol

Materials and Equipment to be 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℃
- 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 200 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 200 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 200 µl Neutralization Buffer 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.

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 2ml collection Plate inside the manifold base; 3). Place the top plate of manifold over the base. (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 acuum and discard the E-Z 96® Lysate Clearance Plate.**

10. **Add 150 µl PFC Binding Buffer to the cleared lysate in 2ml collection Plate.** Shaking for 30 seconds to mix well.

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.**

12. **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.

13. **Add 500 µl of Buffer GW1 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.

14. **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 5.

15. **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.

16. **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.

17. **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.

18. (Optional) Place the E-Z 96® DNA Plate into a vacuum oven or incubate preset at 65℃ for 10 minutes to further dry the plate.

19. **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.

20. **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.

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

22. Store eluted DNA sample at -20℃.

E-Z 96 Endo-free Plasmid DNA Kit - Spin Protocol

All centrifugation steps used are performed at room temperature.

Materials and Equipment to be 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 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 200 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 200 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 200 µl Neutralization Buffer 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. Add 150ul PFC Binding Buffer to the Collection Tube. Shaking to mix well.

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 Mixture into E-Z 96® DNA Plate.

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

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

13. **Add 500 µl Buffer GW1 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℃.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff , toll free, **at (800-832-8896).**

Possible Problems and Suggestions

Problem	Cause	Solution
Low DNA yields	Poor cell lysis	Only use LB or YT medium containing antibiotic(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.
	Bacterial culture overgrown or not fresh	Reduce quantity of starting material.
	Low copy number plasmid used	Such plasmids may yield as little as 0.1 µg DNA from a 1 mL overnight culture. Increase culture volume to 3 mL.
Problem	**Cause**	**Solution**
No DNA eluted	DNA Wash Buffer is not diluted with ethanol	Prepare DNA Wash Buffer as instructed on Page 5.
Problem	**Cause**	**Solution**
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.
Problem	Cause	Solution
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.

Troubleshooting Guide

Problem	Cause	Solution
RNA visible on agarose gel	RNase A not added to Solution I	Add vial of RNase A to bottle of Solution I.
Problem	**Cause**	**Solution**
Plasmid DNA floats out of well while loading agarose gel	Ethanol not completely removed from column following wash steps	Dry the plate in a vacuum oven or incubator set at 70°C for 10 minutes.
Problem	**Cause**	**Solution**
Plasmid DNA will not perform in downstream application	Traces of ethanol remain on column prior to elution	Extend Drying Time by 5 minutes during drying step. Dry the plate at 70°C for 5 minutes after drying step and before elution.
	DNA is permanently denatured	Cell lysis process should not exceed 5 minutes. It may cause DNA to permanently denature.

Ordering Information

The following components are available for purchase separately.
(Call Toll-Free Number (1-800-832-8896)

Product	Part Number
Vacuum Manifold	VAC-03
Solution I (250 mL)	PS001
Solution II (250 mL)	PS002
Elution Buffer (100 mL)	PDR048
DNA Wash Buffer (30 mL)	PDR044
RNase A (400 µL)	AC117

For more purification solutions, visit **www.omegabiotek.com**

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96-Well Silica Plates


Mag Beads

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Plasmid


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FFPE


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