

E.Z.N.A.® Endo-Free Plasmid Giga Kit

Table of Contents

| | |
|--|----|
| Introduction | 2 |
| Kit Contents and Storage | 3 |
| Preparing Reagents | 4 |
| Recommended Settings | 5 |
| Endo-Free Plasmid Giga Vacuum/Spin Protocol | 8 |
| Troubleshooting Guide | 11 |
| Ordering | 13 |

Manual Revision: July 2010



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 the 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.™ Endo Free Plasmid Giga Kit combines time-tested consistency of alkaline SDS lysis of bacterial cells with Omega Bio-tek's innovative high efficiency DNA binding technology to recovery large scale high quality plasmid DNA. This new method facilitates the binding, washing, and elution steps, enabling multiple samples to be simultaneously processed. This kit uses a Syringe-Format System is designed to allow for faster processing and eliminating the need to for time consuming binding and washing steps with a centrifuge.

Yields vary according to plasmid copy number, E.coli strain, and conditions of growth, but 1L of overnight culture in LB medium typically produces 5-10 mg high-copy plasmid DNA. Up to 1 liter overnight 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.

New In this Edition

- The latest edition of this manual has been redesigned to enhance readability and protocol quality.
- Redesigned HiBind DNA Giga Columns

Kit Contents

| Product Number | D6234-00 | D6234-01 | D6234-02 |
|------------------------|----------|------------|-------------|
| Purification | 2 Preps | 5 Preps | 20 Preps |
| HiBindDNA Giga Columns | 2 | 5 | 20 |
| Solution I | 270 mL | 3 x 230 mL | 3 x 900 mL |
| Solution II | 270 mL | 3 x 230 mL | 3 x 900 mL |
| Neutralization Buffer | 270 mL | 3 x 230mL | 3 x 900mL |
| PFC Binding Buffer | 270 mL | 3 x 230 mL | 3 x 900 mL |
| Buffer EWR | 90 mL | 250 mL | 2 x 1000 mL |
| ETR Binding Beads | 35 mL | 80 mL | 160 mL |
| DNA Wash Buffer | 50 mL | 2 x 50 mL | 2 x 200 mL |
| Endotoxin-Free Water | 50 mL | 100 mL | 400 mL |
| Rnase A | 1.0 mL | 3 x 0.7 mL | 11 mL |
| Instruction Booklet | 1 | 1 | 1 |

Storage and Stability

All E.Z.N.A.® Endo Free Giga Plasmid Isolation Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: Solution I/RNaseA at 2-8°C after being combined, see page 4. Store ETR binding Beads at 2-8°C. All other material at 22-25°C. Solution II and PFC Binding Buffer may form precipitate under lower temperature during shipping or storage, incubate at 50°C to dissolve the precipitate.

Preparing Reagents

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

Dilute DNA Wash Buffer with absolute ethanol (96-100%) as follows:

| Kit | Ethanol To Be Added |
|----------|---|
| D6234-00 | Add 100 mL ~96-100% absolute ethanol |
| D6234-01 | Add 500 mL ~96-100% absolute ethanol |
| D6234-02 | Add 800 mL ~96-100% absolute ethanol per Bottle |

Store diluted DNA Wash Buffer at room temperature.

Prepare ETR Binding Beads as Follows:

1. Aliquote the amount of ETR Binding Beads. Prepare ETR Binding beads fresh within 4 hours of processing. Each Prep will require 15 mL of Beads
2. Centrifuge at 3,000 x g for 3 minutes and remove the supernatant
3. Add 2 Volumes of Water(not provided) to the ETR Binding Beads
4. Mix by Vortexing until the beads are resuspended
5. Centrifuge at 3,000 x g for 3 minutes and remove the supernatant
6. Add 2 Volumes of Water (not provided) to the ETR Binding Beads
7. Mix by Vortexing until the beads are resuspended
8. Centrifuge at 3,000 x g for 3 minutes and remove the supernatant
9. Add 1 Volume of Water (not provided) and vortex to fully resuspend the beads.

Recommended Settings

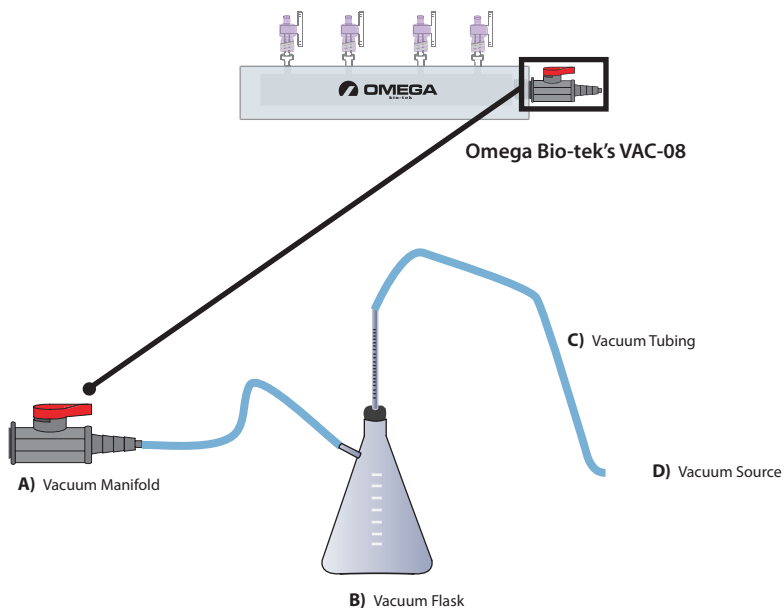
The Vacuum/Spin Protocol is the recommended protocol as it decreases hands on and procedure time. The following is required for use with the Vacuum /Spin Protocol:

- A) Vacuum Manifold (We recommend Omega Bio-tek VAC-08)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, Promega Vacman®, or any manifold with standard Luer connectors
- B) Vacuum Flask
- C) Vacuum Tubing
- D) Vacuum Source (review tables below for pressure settings)

| Manifold | Recommended Pressure (mbar) |
|----------|-----------------------------|
| VAC-08 | -200 to -600 |

| Conversion from millibars: | Multiply by: |
|--------------------------------|--------------|
| Millimeters of mercury (mm Hg) | 0.75 |
| Kilopascals (kPa) | 0.1 |
| Inches of mercury (inch Hg) | 0.0295 |
| Torrs (Torr) | 0.75 |
| Atmospheres (atmos) | 0.000987 |
| Pounds per Square Inch (psi) | 0.0145 |

Illustrated Vacuum Setup:



Recommended Settings Continued

Growth and Culture of Bacteria

Bacterial Strain Selection

It is strongly recommended that an end A negative strain of E.coli be used for routine plasmid isolation. Examples of such strains include DH5 α [™], DH1, and C600. These host strains yield high quality DNA with the E.Z.N.A.[®] Plasmid Isolation protocols. XL1-Blue, although a slower growing strain, is also recommended due to its yield of high quality DNA.

Host strains derivatives from HB101 such as TG1 and the JM100 series release large amounts of carbohydrates during lysis which may inhibit enzyme activities when not completely removed. Some strains may also lower DNA quality due to having high levels of endonuclease activity and therefore are not recommended (i.e. JM101, JM110, HB101). One may reduce the amount of culture volume or double the volumes of Solution I, II, and N3, if problems are encountered with strains such as TG1 and Top10F.

Inoculation

Bacterial cultures for plasmid preparations should always be grown from a single colony picked from a freshly streaked plate. Subculturing directly from glycerol stock or liquid cultures may lead to uneven yields or plasmid loss. Optimal results are obtained by using one single isolated colony from a freshly transformed or freshly streaked plate to inoculate an appropriate volume of starter culture containing the appropriate antibiotic. It should then be incubated for 12-16 hours at 37°C with vigorous shaking (~300rpm; shaking incubator).

Note: Aeration is very important. The culture volume should not exceed 1/4 the volume of the container.

Culture Media

The E.Z.N.A.[®] Plasmid Kits are specially designed for use with cultures grown in Luria Bertani (LB) medium. Richer broths such as TB(Terrific Broth) or 2 x YT lead to high cell densities that can overload the purification system, and therefore are not recommended. If rich media has to be used, growth times have to be optimized, and the recommended culture volumes must be reduced to match the capacity of the HiBind DNA column.

Note: As culture ages, DNA yield may begin to decrease due to cell death and lysis within the culture.

Recommended Settings Continued

Culture Volume and Cell Density

Do Not Exceed Maximum Recommended Culture Volumes

For optimal plasmid yields, the starting culture volume should be based on culture cell density. A bacterial density between 2.0 and 3.0 at OD_{600} is recommended. When using nutrient-rich media, care should be taken to ensure that the cell density does not exceed an OD_{600} of 3.0. Using a high-density culture outside of the recommended OD range may overload the purification system.

Endo-Free Plasmid Giga - Vacuum/Spin Protocol

E.Z.N.A. Endo-Free Plasmid Giga Vacuum Protocol

This Protocol is designed to isolate 10 mg of high Copy-Number plasmids or 1 mg of low copy-Number Plasmids from 1 L overnight cultures using the E.Z.N.A.® Endo-Free Plasmid Giga Kit. Binding more than 10 mg of Plasmid DNA to the HiBind DNA Giga Column can increase processing time and cause the column to clog.

User Supplied Equipment:

- Absolute ethanol (96-100%)
- Centrifuge capable of 20,000 x g with adapter for 500 mL Centrifuge bottles
- Vacuum pump capable of generating -200 to -600 mbar
- Vacuum manifold with standard leur connector
- 500 mL Centrifuge Bottles
- Vacuum Manifold (Cat No. Vac-08)
- Ice
- 50 mL Collection Tube

Things to do before starting:

- Pre chill Neutralization Buffer on Ice
 - Prepare ETR Binding Beads and DNA Wash Buffer and Solution 1 according to directions on page 4
1. Culture volume: Inoculate 2 L LB/ampicillin (50 µg/mL) medium placed in a 5 liter culture flask with E. Coli carrying desired plasmid and grow at 37°C with agitation for 12-16 hours. For best results, use overnight culture as the inoculum. It is strongly recommended that an end A negative strain of E.coli be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®.

Note: 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 grown plate to inoculate a 2-5mL starter culture containing the appropriate antibiotic. Incubate for ~8 hours 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 diluting the starter culture 1/500 to 1/1000 into the growth medium.

Following overnight bacterial growth, an OD₆₀₀ of 1.5~2.0 indicates a well-grown culture. For the best result determination of OD₆₀₀ 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 OD₆₀₀. We recommend a bacterial density of between 2.0 and 3.0 at OD₆₀₀. When using nutrient-rich media,

Care should be taken ensure that the cell density does not exceed an OD₆₀₀ of 3.0. If using a frozen glycerol stock as inoculum, streak it onto an agar plate containing the appropriate antibiotic for single colony isolation. Then pick a single colony and inoculate the 2-5mL starter culture as described above.

Endo-Free Plasmid Giga - Vacuum/Spin Protocol

2. Pellet up to bacteria in appropriate vessels by centrifugation at $5,000 \times g$ 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.
4. Add 125 mL of Solution I/RNase A. Resuspend cells completely by vortexing or pipetting up and down.

Note: Complete resuspension of cell pellet is vital for obtaining good yields.

5. Add 125 mL of Solution II, gently mix by inverting and rotating the tube 7 times to obtain a cleared lysate. Incubate 3 minutes at room temperature.

Note: 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.)

6. Add 125 mL of Ice-cold Neutralization Buffer, cover, and gently mix by inverting tube several times until a flocculent white precipitate forms.

Note: The mix must be mixed thoroughly. If the mixture appears viscous, brownish or conglobated, more mixing is required to completely neutralize the solution. Complete neutralization of the solution is vital of obtaining good yields.

7. Centrifuge at $15,000 \times g$ for 20 minutes at 4°C to pellet the cellular debris and genomic DNA. Centrifugation performs best in 500 or 1000 ml centrifugation tubes.

8. Transfer the supernatant into a new 500 mL Centrifugation bottle. After the centrifugation, the supernatant should appear clear. If some precipitation presents in the supernatant, it should be filtered through a filter paper such as Miracloth™ or a coffee filter paper before continuing.

9. Add 15 ml of ETR Binding Beads. Mix throughly by vortexing for 10-20 seconds, and incubate for 5 minutes at room temperature.

Important: ETR Binding Beads must be prepared according to preparing reagents section on page 4 before use.

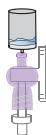
10. Centrifuge at $3,000 \times g$ for 10 minutes.

11. Carefully remove the cleared supertenant to a new 500 mL vessel avoid transferring the ETR Binding Bead Pellet

Note: Pelleted ETR Binding Beads contains high levels of Endo-toxins and should not be transferred to the new vessel.

Endo-Free Plasmid Giga - Vacuum/Spin Protocol

12. Measure the volume of the supernatant, add 1/3 volume of the PFC Binding Buffer. Mix thoroughly by vortexing.
13. Insert a HiBind DNA Giga column to the vacuum manifold.
14. Pour the lysate from step 13 in to the HiBind DNA Giga column. Switch on the vacuum source to draw the solution through the HiBind DNA Giga column. Continue applying lysate from step 10 to the HiBind DNA Giga column. Do not allow the HiBind DNA Giga column to become empty until you have applied all of the lysate from step 10.



By not allowing the HiBind DNA Giga column to empty until the end of step 14, clogging or foaming of the HiBind DNA Giga column will be reduced.

15. Add 40 ml of Buffer EWR to the HiBind DNA Giga column and apply the vacuum to draw all the liquid through the column.
16. To wash the DNA, add 50 ml of DNA Wash Buffer to the HiBind DNA Giga column and apply the vacuum to draw all the liquid through the column.
17. Add an additional 45 ml of DNA Wash Buffer to the HiBind DNA Giga column and apply the vacuum to draw all the liquid through the column.
18. With the HiBind DNA Giga Column empty continue to apply vacuum for 15 minutes to dry the HiBind DNA Matrix
19. Place the HiBind DNA Giga column into a new 50 ml centrifuge tube (not supplied). Add 10-15 ml of Endotoxin Free Water. Incubate at room temperature for 5 minutes.
20. Hold the HiBind DNA Giga Column barrel over the a 50 mL vessel and gently insert the plunger to expel the EndoToxin Free Water containing the Plasmid DNA to the tube.
21. Repeat Step 20 with 10-20 mL of Endotoxin Free Water. To Maintain a higher Concentration of Plasmid DNA the eluate from step 20 can be reused.

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

| Low DNA Yields | |
|---|---|
| Poor cell lysis | Reduce the initial volume of culture or increase the lysis time while monitoring the lysis visually. Cells may not have been dispersed adequately prior to the addition of Solution II. Make sure to vortex cell suspension to completely disperse bacterial cells. Solution II if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS. |
| 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. |
| Low elution efficiency | If using Endotoxin-free water for elution, adjust the pH of the water to pH 8.0 |
| Low copy-number plasmid used | Such plasmids may yield as little as 0.1 µg of DNA from a 1 ml overnight culture. Double culture volume and follow suggested modifications with low copy number plasmid protocol. |
| Binding columns were spun in a fixed angle rotor or with insufficient g-force | For midi and Midi kit, binding columns must be spun in a swinging bucket rotor at 4,000 x g for liquids to pass through efficiently. |
| Alkaline lysis is prolonged | Reduce the lysis time (Solution II) to 3 minutes or until the suspended cells form a clear viscous solution |
| Too many or too few cells were used | Confirm the cell density by measuring OD . To calculate the volume of culture to use, take the desired cell mass and divide by the absorbance of the overnight culture at 600 nm. |
| DNA Wash Buffer not diluted with ethanol. | Prepare DNA Wash Buffer Concentrate according to instructions on page 4. |

Troubleshooting Guide

| 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. |
| Culture overgrown | Overgrown culture contain lysed cells and degraded DNA. Do not grow cell longer than 16 hours |
| Plasmid DNA floats out of well while loading agarose gel | |
| Ethanol has not completely been removed from column following wash steps. | Incubate columns for 10 minutes at 65°C to completely dry membrane after centrifugation step for drying |
| Absorbance of purified DNA does not accurately reflect quantity of the plasmid (A_{260} / A_{280} ratio is high or low). | |
| DNA Wash Buffer is diluted with ethanol containing impurities. | Check the absorbance of the ethanol between 250nm and 300nm. Do not use ethanol with high absorbance. Traces of impurities may remain on the binding column after washing and contribute to the absorbance in the final product. |
| Plasmid DNA is contaminated with RNA; RNase A treatment is insufficient. | Confirm that the RNase A Solution was added to Solution I prior to first use. The RNase A Solution may degrade due to high temperatures (>65°C) or prolonged storage (> 6 months at room temperature) |
| Background reading is high due to silica fines. | Spin the DNA sample at maximum speed for 1 minutes; use the supernatant to repeat the absorbance readings. |
| Purification is incomplete due to column overloading. | Reduce the initial volume of culture. |
| Plasmid DNA is contaminated with chromosomal DNA | Do not use cultures that have grown for more than 24 hours or are in the cell death phase. Do not vortex or vigorously shake the cells during the lysis reaction or Neutralization Buffer step. |

Ordering Information

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

| Buffer (Size) | Part Number |
|--------------------------------------|-------------|
| Solution I (250 mL) | PS001 |
| Solution II (250 mL) | PS002 |
| Neutralization Buffer (250mL) | PS004 |
| DNA Wash Buffer Concentrate (100 mL) | PS010 |
| RNase A (5mL) | RNA-03 |

E.Z.N.A and MicroElute are registered trademarks of Omega Bio-tek, Inc.

Qiagen®, QIAvac® and Vacman® are all trademarks of their respected companies.

PCR is a patented process of Hoffman-La Roche. Use of PCR process requires a license.

