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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 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.™ 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 methods facilitate the binding, washing, and elution steps thus enabling multiple samples to be simultaneously processed. Yields vary according to plasmid copy number, *E.coli* strain, and conditions of growth, but 1000-2000 mL of overnight culture in LB medium typically produces 5-10 mg high-copy plasmid DNA. Up to 4 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.

Storage and Stability

All E.Z.N.A.™ Giga Plasmid Isolation Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: Solution I/RNase A at 4°C and OB protease mixtures at -20°C after received, 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.

Kit Contents

Product Number	D6920-00	D6920-01	D6920-02
Purification	2 Preps	5 Preps	20 Preps
HiBind® DNA Giga Columns	2	5	20
50 mL Collection tubes	2	5	20
Solution I	300 mL	700 mL	2800 mL
Solution II	300 mL	700 mL	2800 mL
Neutralization Buffer	300 mL	700 mL	2800 mL
PFC Binding Buffer	100 mL	235 mL	950 mL
DNA Wash Buffer	25 mL	100 mL	2 x 200 mL
Elution Buffer	15 mL	40 mL	160 mL
RNase A, Concentrate	1.12 mL	2.8 mL	11.2 mL
Instruction Booklet	1	1	1

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 as follows: D6920-00 Add 100 mL ~96-100% absolute ethanol D6920-01 Add 400 mL ~96-100% absolute ethanol D6920-02 Add 800 mL ~96-100% absolute ethanol

Growth and Culture of Bacteria

Bacterial Strain Selection

It is strongly recommended that an endA negative strain of E.coli be used for routine plasmid isolation. Examples of such strains include DH5α® , DH 1, and C600. These host strains yield high quality DNA with 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.g. JM101, JM110, HB101).One may reduce the amount of culture volume or double the volumes of Solution I, II, and III, 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, and then incubated for 12-16 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® spin column.
NOTE: As culture ages DNA yield may begin to decrease due to cell death and lysis within the culture.

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 OD600 is recommended.* When using nutrient-rich media, care should be taken to ensure that the cell density does not exceed an OD600 of 3.0. Using a high-density culture outside of the recommended OD range may overload the purification system.

E.Z.N.A.™ Plasmid Giga Prep Protocol

This Protocol is designed to isolate 10 mg of high Copy-Number plasmids or 400µg -1mg of low Copy-Number Plasmids from 2000ml overnight cultures using E.Z.N.A.™ Plasmid Giga Kit.

Materials provided by user

- Absolute ethanol (96-100%)
- Centrifuge capable of 13,000 x g with adapter for 250 ml or 500ml bottle
- Centrifuge with swinging bucket rotor capable of 3,000-5,000 x g with adapter for 50 ml centrifuge tube
- Vacuum pump capable of generate -200 to -600 mbar
- Vacuum manifold with standard leur connector
- 50 ml Centrifuge tube
- Centrifugation tube (i.e Nelgene 3120)
- Vacuum Manifold (**Cat No. Vac-08**)

Growth of bacterial culture

1. **Culture volume: Inoculate 2000 mL LB/ampicillin (50 µg/mL) medium placed in a 1-5 liter culture flask with *E.coli* carrying desired plasmid and grow at 37°C with agitation for 12-16 h.** For best results use overnight culture as the inoculum. 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®.

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 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 dilute the starter culture 1/500 to 1/1000 into growth medium.*

Following overnight bacterial growth, an OD_{600} of 1.5~2.0 indicates a well-grown culture. For the best result determination of OD_{600} 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 OD_{600}. We recommend a bacterial density of between 2.0 and 3.0 at OD_{600}. When using nutrient-rich media, care should be taken ensure that the cell density does not exceed an OD_{600} 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 picking a single colony and inoculate the 2-5mL starter culture as described above.

- **Lyse bacterial cells with alkaline-SDS Solution**

2. **Pellet up to bacteria in appropriate vessels by centrifugation at 13,000 × 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 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 yield.

5. **Add 125 mL Solution II, gently mix by inverting and rotating tube 7-10 times to obtain a cleared lysate. Incubate 3-5 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 Neutralization Buffer, cover, and gently mix by inverting tube several times until a flocculent white precipitate forms**.

   Note: The mix must be mixed throughly. If the mixture appears still viscous, brownish and conglobated, more mixing is required to completely neutralize the solution. Complete neutralization of the solution is vital of obtaining good yields. Increasing centrifugation speed is helpful to completely remove the precipitated bacterial cell material. After centrifugation, a tightly packed cell debris pellet indicates efficient lysis.

7. **Centrifuge at ≥13,000 × g for 10 minutes at 4°C to pellet the cellular debris and genomic DNA.** Centrifugation perform the best in 250 ml centrifugation tubes (i.e 250 ml PPCO tube from Nelgene, Type 3120). After the centrifugation, the supernatant should appear clear. When the lysaye is still cloudy after centrifugation, it should be filtered through a filter papersuch as Miracloth™ or a coffee filter paper before continuing.

■ Purify the plasmid with HiBind® Giga Column

8. **Measure the volume of the supernatant, add 1/3 volume of the PFC Binding Buffer. Mix throughly by vortexing.**

9. **Insert a HiBind® DNA Giga column to the vacuum manifold.**

10. **Pour the cell lysate from step 8 into the HiBind® DNA Giga column and turn on the vacuum source to draw all the liquid through the column.** Keep pouring the lysate until all the cell lysate pass through the column. Turn off the vacuum source.

11. **To wash the DNA, add 20 ml DNA Wash Buffer to the HiBind® DNA Giga column and apply the vacuum to draw all the liquid through the column. Keep adding additional 60 ml DNA Wash Buffer until all the liquid pass through the column.**

12. Transfer the **HiBind® DNA Giga column** into a 50 ml centrifuge tube (supplied). Centrifuge at 5000 x g for 10 minutes to dry the membrane.

13. Place the **HiBind® DNA Giga column** into a new 50 ml centrifuge tube (not supplied). Add 5 ml Elution Buffer (10mM Tris-HCl, pH 8.5) or water to the column. Incubate at room temperature for 2 minutes.

14. Centrifuge at 5000 x g for 5 minutes to elute the DNA.

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:

$$DNA\ concentration = Absorbance_{260} \times 50 \times (Dilution\ Factor)\ \mu g/mL$$

The ratio of ($Absorbance_{260}$)/($Absorbance_{280}$) 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 present.

Trouble Shooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	Use LB medium containing ampicillin. Do not excel the maximum recommended culture volume Cells may not be dispersed adequately prior to addition of Solution II. Vortex/ pipet cell suspension to completely disperse bacterial clumps. 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 to yield and quality.
	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 4000 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 column as instructed in the manual. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantitation.
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, does not freeze, or smells of ethanol.	Ethanol traces not completely removed from column following wash steps.	Centrifuge column as instructed in step 12 to dry the membrane completely. A swinging-bucket rotor is recommended for centrifugation. Alternatively, precipitate the eluted DNA with isopropanol as indicated in step .