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 .

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6. **Add 20 mL Neutralization Buffer, cover, and gently mix by inverting tube several times until a flocculent white precipitate forms.** Incubate on ice for 10 minutes.

7. **Centrifuge at 15,000 × g for 20 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).

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

9. **Measure the volume of the supernatant and add 1/3 volume of the PFC Binding Buffer. Mix by inverting the bottle 10-15 times.**

10. **Insert a HiBind® DNA Mega column to the vacuum manifold.**

11. **Pour the cell lysate from step 9 into the HiBind® DNA Mega 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.

12. **To wash the DNA, add 50 ml Buffer GC into the column.** Turn on the vacuum source to draw all the liquid through the column. Turn off the vacuum source.

13. **Add 20 ml DNA Wash Buffer to the HiBind® DNA Mega column and apply the vacuum to draw all the liquid through the column.** Keep adding additional 20 ml DNA Wash Buffer until all the liquid pass through the column.

14. Apply the maximum vacuum on for another 10 minutes to further dry the column.

15. Transfer the **HiBind® DNA Mega column** into a 50 ml centrifuge tube (supplied). Centrifuge at 5,000 x g for 10 minutes at room temperature to dry the membrane.

Kit Contents

Product Number	D6228-00	D6228-01	D6228-02
Purification	2 Preps	5 Preps	20 Preps
HiBind® DNA Mega Columns	2	5	20
50 mL Collection tubes	2	5	20
Solution I	50 mL	120 mL	2 x 220 mL
Solution II	50 mL	120 mL	2 x 220 mL
Neutralization Buffer	50 mL	120 mL	2 x 220 mL
PFC Binding Buffer	50 mL	120 mL	2 x 220 mL
Buffer GC	120 mL	270 mL	5 x 220 mL
ETR Solution	5 mL	10 mL	30 mL
DNA Wash Buffer	25 mL	50 mL	5 x 50 mL
Endotoxin Free Elution Buffer	50 mL	100 mL	2 x 200 mL
RNase A	0.3 mL	0.6 mL	2 x 1 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 has to be diluted with absolute ethanol as follows:
	D6228-00 Add 100 mL ~96-100% absolute ethanol
	D6228-01 Add 200 mL ~96-100% absolute ethanol
	D6228-02 Add 200 mL ~96-100% absolute ethanol

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 Mega 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 500 mL of overnight culture in LB medium typically produces 2-5 mg high-copy plasmid DNA. Up to 0.5 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.™ Mega 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 4ºC, all other material at 22-25ºC. Solution II and PFC Binding Buffer may form precipitate under lower temperature during shipping or storage, incubate at 50°C to dissolve the precipitate.

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

17. **Centrifuge at 5,000 x g for 5 minutes at room temperature to elute the DNA.** The purified Plasmid is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, transfection of mammalian cells, and other manipulations. Optional step 18-24 can remove any residual endotoxin.

Optional steps: Removal residual Endotoxin in purified Plasmid.

18. Dilute Plasmid concentration to <300ng/ul with Elution Buffer or Water.

19. **Add 1 ml ETR Solution (blue) to the plasmid. Mix by inverting the tube 7-10 times and incubate on ice for 20 minutes.** Invert the tube several times during the incubation.

20. **Incubate the lysate at 45°C for 5 minutes. The lysate should appear turbid again.** Centrifuge at 5,000 x g for 5 minutes at 25°C (let centrifugation slow down). The ETR Solution will form a blue layer at the bottom of tube.

21. Carefully transfer the top aqueous phase into a 50 ml centrifuge tube capable of withstanding 15,000 × g (screw-cap polycarbonate or Corex® glass tubes will suffice). **Add 0.1 Volume of 3 M NaAc, pH5.2 and 0.7 volume of isopropanol. Vortex to mix.**

22. **Centrifuge at 15,000 x g for 10 minutes at 4°C.** Carefully decant the supernatant.

23. **Wash DNA pellet with 10 ml 70% ethanol** and centrifuge at 15,000 x *g* for 10 min at 4°C. Carefully decant the supernatant without disturbing the pellet.

24. **Air-dry the pellet for 10 min, and re-dissolve the DNA in a suitable volume of endotoxin-free Elution Buffer.**

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).

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.™ Endo-Free Plasmid Mega Prep Protocol

This Protocol is designed to isolate 2-5 mg of high Copy-Number plasmids or 400µg - 1mg of low Copy-Number Plasmids from0.5L overnight cultures using E.Z.N.A.™ Endo-Free Plasmid Mega Kit.

Materials provided by user

- Absolute ethanol (96-100%)
- Centrifuge capable of 15,000 x g with adapter for 250 ml or 500ml bottle
- Centrifuge with swinging bucket rotor capable of 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 Nalgen 3120)

Growth of bacterial culture

1. **Inoculate 0.5 L LB/ampicillin (50 µg/mL) medium placed in a 2-5 liter culture flask** with *E.coli* carrying desired plasmid and grow at 37°C with agitation for 12-16 hr.

2. **Pellet up to 0.5 L bacteria in appropriate vessels by centrifugation at 5,000 × g for 10 min at 4°C.**

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 20 mL Solution I/RNase A. Resuspend cells completely by vortexing or pipetting up and down.** Complete resuspension of cell pellet is vital for obtaining good yield.

5. **Add 20 mL Solution II, gently mix by inverting and rotating tube 10-15 times to obtain a cleared lysate.** Incubate 3-4 minutes at room temperature. Invert the tube several times during the incubation.