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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 (OBI) 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.® Fastfilter Plasmid Mega Kit combines the power of HiBind™ technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality DNA. Omega Bio-Tek' s HiBind™ Mega columns facilitate the binding, washing, and elution steps thus enabling multiple samples to be simultaneously processed. This kit also include a special filter cartridge, which replaces the centrifugation step following alkaline lysis. Following lysis the DNA is bound to the silica membrane and contaminants are removed with a simple wash step.

Yields vary according to plasmid copy number, *E.coli* strain, and conditions of growth, but up to 600-1200 ug of high copy number plasmid or 50-400 ug of low copy number plasmid can be purified from 200 ml overnight culture. Up to 500ml bacterial cultures can be used when working with low copy number plasmid. 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.® Plasmid isolation components are guaranteed for at least 12 months from the date of purchase when stored as follows: Solution I (once RNase A is added) at 4ºC, all other material at 22-25^{0}C.

Kit Contents

Product Number	D6929-01	D6929-02	D6929-03
Purification times	2 Preps	5 Preps	20 Preps
HiBind™ DNA Mega Columns	2	5	20
50ml Collection Tubes	2	5	20
Lysate Clearance Filter syringe	2	5	20
Solution I	60 ml	150 ml	2 x 270 ml
Solution II	60 ml	150 ml	2 x 270 ml
Neutralization Buffer	60 ml	150 ml	2 x 270 ml
PFC Binding Buffer	60 ml	150 ml	2 x 270 ml
Buffer GC	60 ml	150 ml	2 x 270 ml
DNA Wash Buffer	20 ml	50 ml	4 x 50 ml
RNase A	300 µl	700 µl	2 x 1 ml
Elution Buffer	10 ml	50 ml	100 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.

Supplied By User
Laboratory centrifuge equipped with **swinging-bucket** rotor capable of 3000-5000 x g.
Sterile 50 ml centrifuge tubes.
Absolute (96%-100%) ethanol

IMPORTANT

1. Add RNase A to bottle of Solution I provided and store at 4ºC.
2. **DNA Wash Buffer** is to be diluted with absolute ethanol (96-100%) as follows:

   D6929-01 Add 80 ml of absolute ethanol

   D6929-02 Add 200 ml of absolute ethanol

   D6929-03 Add 200 ml of absolute ethanol per bottle

Store diluted DNA Wash Buffer at room temperature !

Protocol 1: Fastfilter Plasmid Mega Kit Spin Protocol

This Protocol is designed to isolate 1-5 mg of high Copy-Number plasmids or 500-2000 µg of low Copy-Number Plasmids from 500 ml overnight cultures. For increasing yield of low Copy-Number plasmid, proceed as "Low Copy-Number Plasmids protocol" on page 8.

- **Growth of bacterial culture**

1. **Culture volume: Inoculate 500 ml LB/ampicillin (50 µg/ml) medium placed in a 4 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®.

   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 ~8hr 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 at 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}. For Megamal yields, the OD600 of cultures should be under 3.0.

   If using a frozen glycerol stock as inoculun, 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.

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

2. **Pellet up to 500 ml bacteria in appropriate vessels by centrifugation at 3,500-5,000 × 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. **To the bacterial pellet add 25 ml Solution I/RNase A and resuspend cells completely by vortexing or pipetting.** *Complete resuspension of cell pellet is vital for obtaining good yield.*

4. **Add 25 ml Solution II, cover, and mix gently but throughly by inverting and rotating tube 15-20 times to obtain a cleared lysate.** A 5 min incubation at room temperature may be necessary.

   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.)

5. Add 25 ml Neutralization Buffer, cover, and gently mix by inverting tube 15-20 times until a flocculent white precipitate forms.

   Note: The Buffers 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.

6. **Centrifugation at 3,500-5,000 × g for 15 min at room temperature.**

- **Clear the lysate with Lysate Clearance Filter Syringe**

7. **Immediately pour the supernatant into the barrel of the Lysate Clearance Filter Syringe.** Use a new 150ml bottle to collect the cell lysate. Insert the plunger back into the barrel of the syringe.

8. Hold the Lysate Clearance filter syringe barrel over the bottle and gently insert the plunger to expel the cleared lysate to the tube.

9. Measure the volume of the flow-through and add 1/3 volume of the PFC Binding Buffer. Mix by inverting the bottle 10-15 times. Incubate at room temperature for 5 minutes.

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

11. Transfer the cleared lysate (<20ml) into the HiBind® DNA Mega column, be careful not to overfill the column, apply the vacuum to allow all sample pass through the column.

12. Repeat transfer the lysate into the column until the entire sample has been passed through.

13. **To wash the DNA, add 20 ml Buffer GC into the column.** Turn on the vacuum source to draw all the liquid through the column. Turn off the vacuum source.

14. **Add 20 ml DNA Wash Buffer into the column.** Turn on the vacuum source to draw all the liquid through the column. Keep adding additional 20 ml DNA Wash Buffer until all the liquid pass through the column.

15. **Add 5 ml absolute ethanol to the HiBind DNA Mega Column and apply vacuum to draw all the liquid through the column.**

16. Apply the maximum vacuum on for another 15 minutes to further dry the column.

17. Transfer the **HiBind® DNA Mega column** into a 50 ml centrifuge tube (supplied). Centrifuge at 3,000-5,000 x g for 10 minutes at room temperature to dry the membrane.

18. Place the **HiBind® DNA Mega column** into a new 50 ml centrifuge tube (not supplied). Add 1.5-3ml Elution Buffer or water to the column. Incubate at room temperature for 3 minutes.

19. **Centrifuge at 5,000 x g for 5 minutes at room temperature to elute the DNA.**

    This represents approximately 60-70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration. Preheating the water to 70°C and allowing the column to soak 2 min at room temperature before elution may significantly increase yields.

Protocol 2: Low Copy-Number Plasmids Protocol

Low copy number plasmids generally give 0.1-1 µg DNA per ml overnight culture. For isolation of plasmid from low copy-number plasmids (0.1-1 µg/ml culture) or low-midi copy-number plasmids (1-2 µg/ml culture) bacteria, the method can be modified to essentially increase the yield if necessary.

Start with 500-1000 ml bacterial culture, centrifuge for 10 min at 3,500-5,000 x g in a centrifuge tube. Proceed to Step 3 (Page 4) and double the volumes of Solution I, II, Neutralization Buffer and PFC Binding Buffer. Continue as above using only one HiBind® DNA Mega column. There is no need to increase the volumes of Buffer HB and DNA Wash Buffer used. The Buffer of Solution I, II, Buffer N3 can be purchase separately.

Note: This method is not recommended for high copy number plasmids because above 500 ml culture, the HiBind® DNA Mega column quickly becomes saturated. In this situation we recommend processing of multiple samples from the same culture.

Yield and Quality of Plasmid

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$_{260}$ × 50 × (Dilution Factor) µg/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	Only use LB or YT medium containing ampicillin. Do not use more than 500 ml. 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 400 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. 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 at 3000 x g for 10 minutes to dry the column. A swinging-bucket rotor is recommended for centrifugation. Alternatively, precipitate the eluted DNA with isopropanol as indicated in step .