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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 Maxi 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™ Maxi 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 4°C, all other material at 22-25^{0}C.

Kit Contents

Product Number	D6924-00	D6924-01	D6924-03	D6924-04
Purification times	2 Preps	5 Preps	25 Preps	100 Preps
HiBind™ DNA Maxi Column	2	5	25	100
50 ml collection tubes	2	5	25	100
Lysate Clearance filter syringe	2	5	25	100
Solution I	25 ml	60 ml	270 ml	5 x 220 ml
Solution II	25 ml	60 ml	270 ml	5 x 220 ml
Buffer N3	15 ml	30 ml	140 ml	2 x 270 ml
Buffer GBT	20 ml	60 ml	230 ml	4 x 220 ml
Buffer GPS	15 ml	30 ml	150 ml	2 x 270 ml
Buffer HB	25 ml	60 ml	270 ml	4 x 260 ml
DNA Wash Buffer	12 ml	40 ml	3 x50 ml	6 x 50 ml
RNase A	100 µl	300 µl	1 ml	5 x 800 µl
Elution Buffer	15 ml	40 ml	180 ml	3 x 220 ml
Instruction Booklet	1	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 4°C.
2. **DNA Wash Buffer** is to be diluted with absolute ethanol (96-100%) as follows:

D6924-00	Add 48 ml of absolute ethanol
D6924-01	Add 160 ml of absolute ethanol
D6924-03	Add 200 ml of absolute ethanol per bottle
D6924-04	Add 200 ml of absolute ethanol per bottle

Store diluted DNA Wash Buffer at room temperature !

Protocol 1: Fastfilter Plasmid Maxi Kit Spin Protocol

This Protocol is designed to isolate 600-1200 µg of high Copy-Number plasmids or 50-400 µg of low Copy-Number Plasmids from 200 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 200 ml LB/ampicillin (50 µg/ml) medium placed in a 1-4 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 maximal 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 100-200 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 10 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 10 ml Solution II, cover, and mix gently but throughly by inverting and rotating tube 10-15 times to obtain a cleared lysate.** A 2 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 5 ml ice-cold Buffer N3, cover, and gently mix by inverting tube several times until a flocculent white precipitate forms.** Prepare a Lysate Clearance Filter Syringe by placing the barrel in a tube rack to keep the syringe upright.

   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. **Add 8.3 ml Buffer GBT, cover, and gently mix by inverting tube 3-5 times.**

7. **Prepare the HiBind Maxi Column.** Place a HiBind Maxi Column into a 50 ml collection tube, provided. Add **5 ml** of Buffer GPS to the column and Lit it sit at room temperature for 3-10 min. Spin in a swinging bucket rotor at 3,000-5,000 x *g* for 5 minutes at room temperature. Discard the eluate and assemble the column again in the 50 ml Collection tube.

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

8. **Immediately pour the lysate into the barrel of the Lysate Clearance Filter Syringe. Allow the cell lysate to sit for 2-3 minutes.** The white precipitate should float to the top. Use a new 50 ml tube to collect the cell lysate. Insert the plunger back into the barrel of the syringe.

9. **Hold the Lysate Clearance filter syringe barrel over the 50 ml tube and gently insert the plunger to expel the cleared lysate to the tube.** *Some of the lysate may remain in the flocculent precipitate, do not force this residual lysate through the filter.*

   Step 10 to 16 should be performed in swinging-bucket rotor for maximal plasmid DNA yields. All of centrifugation steps must be carried out at room temperature.

■ **Purify Plasmid DNA with HiBind™ DNA Maxi Column**

10. **Transfer 16 ml of the cleared lysate to the HiBind® DNA maxi column assembled in the 50 ml collection tube**. The Maxi column has a maximum capacity of 20 ml. Centrifuge at 3,000-5,000 × g for 3-5 min at room temperature to completely pass lysate through column. Discard the flow-through liquid and repeat this step until the entire sample has been passed through. Finally discard the flow-through and reuse the collection tube in Step 11.

11. **Add 10 ml Buffer HB to the Maxi column and centrifuge as above**. This step ensures that residual protein contamination is removed and must be included for downstream applications requiring high quality DNA. Discard flow-through liquid and reuse the collection tube in the next step.

12. **Wash the column by adding 15 ml of DNA Wash Buffer diluted with ethanol.** Centrifuge as above and discard flow-through.

    Note: DNA Wash Buffer Concentrate must be diluted with absolute ethanol before use. See label for directions. If refrigerated, DNA Wash Buffer must be brought to room temperature before use.

13. **Optional Step: Repeat wash step with another 10ml DNA Wash Buffer.** Centrifuge as above and discard flow-through.

14. **Centrifuge the empty capped column for 10 min at maxi speed (no more than 6,000 x g) to dry the column matrix.**

    DO NOT skip this step - it is critical for removing traces of ethanol that may otherwise interfere with downstream applications.

■ **Elution Plasmid DNA From HiBind™ DNA Maxi column**

Optional: *For maximal yield and high concentration of plasmid, see alternative protocol of elution on page 7. For fast elution, proceed step 15-16.*

15. **Further Drying The Column (Optional)**. Choose either of the methods below to further dry the column before eluting DNA (only if necessary):

    A. **Place the column into a vacuum container to dry the ethanol for 10 minutes.** Then, remove the column and place into a vacuum chamber at room temperature. Any device connected to a vacuum source may be used. Seal the chamber and apply vacuum for 15 min. Remove the column and proceed to Step 16.

B. Bake the column in a vacuum oven or incubator at 65°C for 10 minutes. Remove the column and proceed to Step 16.

16 **Place column into a clean 50 ml centrifuge tube. Add 1.0-3.0 ml (depending on desired concentration of final product) Elution Buffer (or water) directly onto the column matrix**. Allow column to sit 2 min at room temperature. Centrifuge at maxi speed (no more than 6,000 x g) for 5 min to elute DNA. This represents approximately 60-80% 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. Also, preheating the water to 70°C prior to elution may significantly increase yields.

Note: The plasmid DNA obtained using this protocol performs well in PCR, restriction digests, lipid mediated transfection and transformation. The expected concentration of plasmid is vary between different copy number vector. However, the concentration of high copy-number plasmid is 150-400ug/ml. Some residual ethanol is present, but does not interfere with these downstream applications. One may get high concentration and absolutely remove ethanol with optional elution step as following.

■ **Alternative protocol of Elution Plasmid from Column**

1. **Place HiBind™ DNA Maxi column into a clean 50 ml centrifuge tube. Add 6 ml sterile deionized water (or TE buffer) directly onto the column matrix.** Allow column to sit 2 min at room temperature. Centrifuge at maxi speed(no more than 6,000 x g) for 5 min to elute DNA.

2. **Carefully transfer eluted plasmid from 50 ml centrifuge tube to a clean tube suitable for precipitation and add 260ul 5M NaCl and 4.4 ml room temperature isopropanol.** Vortex to mix and centrifuge at >15,000 × g for 30 min at 4°C. Carefully decant the supernatant.

3. **Wash DNA pellet with 2ml ice-cold 70% ethanol and centrifuge at > 15,000 × g for 10 min.** Carefully decant the supernatant without disturbing the pellet. Air-dry the pellet for 5-10 min.

4. **Finally resuspend DNA pellet in 200-500µl (depending on desired concentration of final product) Elution Buffer or water.**

Protocol 2. Fastfilter Plasmid Maxi Kit Vacuum Protocol

1. **Prepare the cleared lysate by following step 1-9 of centrifugation procedure on page 4-5.**

2. **Transfer the cleared lysate into the HiBind® DNA Maxi column,** be careful not to overfill the column, apply the vacuum to allow all sample pass through the column. Repeat transfer the lysate into the column until the entire sample has been passed through.

3. **Add 10.0 ml HB buffer to the column** and apply the vacuum to draw the liquid through the column.

4. **Wash the column: add 15 ml of DNA Wash Buffer** (pre-diluted with absolute ethanol) into the column and allow it pass through the column.

5. **Wash the column again with 10 ml DNA Wash Buffer by repeating step 4.**

6. **Keep the vacuum on for another 10 minutes after the liquid pass through the column.** (This step to ensure the removal of residue ethanol).

7. **Centrifuge the empty capped column for 10-15 min at maxi speed (no more than 6,000 x g) to dry the column matrix.**

8. Proceed Elution Step as Centrifugation protocol Fast Elution Step 15-16 on Page 6 or Alternative elution step on page 7.

Protocol 3: 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 200-400 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, and Buffer N3. Continue as above using only one HiBind® DNA Maxi 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 200 ml culture, the HiBind® DNA Maxi 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:

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

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.

Plasmid Copy-Number and Expected Yield

The Yield and quality of the plasmid DNA depend on a number of factors including plasmid copy number, size of insert, host strain, culture volume, culture medium and binding capacity of kit. In these factors, The copy number of vector, culture volume and binding capacity of kit are most important. Copy number of plasmid is vary from one copy to several hundred copies per cell as dicated by their origin of replication. But very large plasmids are often maintained at very low copy number per cell.

Plasmid	Replicon	Copy Number	expected Yield of 50ml culture
pUC vector	pMB1	500-700	180-220 µg
pBR322 and its derivatives	pMB1	15-20	10-20µg
PACYC and its derivatives	p15A	10-12	5-30 µg
pSC101 and its derivatives	pSC101	~5	5-20 µg
pBluescript	ColE14	300-500	100-150 µg
ColE14	ColE14	15-20	5-20 µg
pGEM	pMB1	300-700	150-200 µg

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 .

Ordering Information

Product	Applications	Cat. No.
Standard E.Z.N.A.™ Plasmid Isolation System		
Plasmid Mini Kit I	Isolation of up to 30 µg Plasmid DNA	D6942/3
Plasmid Mini Kit II	Isolation of up to 70 µg Plasmid DNA	D6945
Plasmid Midi Kit	Isolation of up to 250 µg Plasmid DNA	D6904
Plasmid Maxip Kit	Isolation of up to 1.5 mg Plasmid DNA	D6922
Fastfilter Plasmid Midi Kit	up to 250 µg Plasmid DNA, featuring filter syringes	D6905
Fastfilter Plasmid Maxi Kit	up to 1 mg Plasmid DNA, featuring filter syringes for lysate clearance	D6924
E-Z 96 Fastfilter Plasmid Kit	Isolation of Plasmid using a 96-well format	D1097
E-Z 96 SE Plasmid Kit	Isolation of plasmid using a single plate	D1095
Yeast Plasmid Isolation Kit	Isolation fo Yeast Plasmid DNA	D3476
E.Z.N.A.™ Endotoxin Free Plasmid Isolation System		
Endo-Free Plasmid Mini Kit I	Isolation of up to 30 µg Endotoxin free Plasmid	D6948
Endo-Free Plasmid Mini Kit	Isolation of up to 70 µg Endotoxin free Plasmid	D6950
Endo-Free Plasmid Mid Kit	Isolation of up to 250 µg Endotoxin free Plasmid	D6915
Endo-Free Plasmid Maxi Kit	Isolation of up to 1.5 mg Endotoxin free Plasmid	D6926
E.Z.N.A.™ H P Plasmid Isolation System		
HP Plasmid Mini Kit I	Isolation of up to 30 µg of High Purity Plasmid	D7042
HP Plasmid MidiKit	Isolation of up to 200 µg of High Purity Plasmid	D7004
HP Plasmid Maxi Kit	Isolation of up to 1.5 mg of High Purity Plasmid	D7022
E.Z.N.A.™ Single Strand Phage DNA Isolation Kits		
M13 Isolation Kit	Isolation of 15µg of single stranded phage DNA	D6900
E-Z 96 M13 Isolation Kit	15µg of M-13 DNA using a 96-well format	D1900
E.Z.N.A.™ Large Construct DNA Isolation Kits		
BAC/PAC DNA Isolation Kit	Effective purification of BAC or PAC DNA	D2156
BAC/PAC DNA Isolation Kit	purification of BAC/PAC using a 96-well format	D1056
Fastfilter BAC/PAC DNA Kit	purification of BAC/ PAC using a 96-well format	D1055