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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 buffers.

The Plasmid Midi Kit combines the power of HiBind® Midi-spin column technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality DNA. Omega Bio-tek's midi-columns 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 50 mL of overnight culture in LB medium typically produces 100-200 µg high-copy plasmid DNA. Up to 100 mL 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.

The E.Z.N.A.™ High Performance (HP) Plasmid Purification System is the modified version of E.Z.N.A.™ plasmid isolation system which is designed specially for those applications when high quality plasmid is required such as transfection, auto sequencing, etc. It also suitable for isolating plasmid from bacterial hosts (such as EndoA+ strains) with high level of endonuclease activity. The plasmid from this system has much better stability for long term storage.

Storage and Stability

All E.Z.N.A.™ High Performance (HP) Plasmid Midi 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.

Kit Contents

Product Number	D7004-00	D7004-01	D7004-02
Purification times	2 Preps	10 Preps	50 Preps
HiBind® DNA Midi Columns	2	10	50
15 mL collection tubes	2	10	50
Solution I	10 mL	30 mL	150 mL
Solution II	10 mL	30 mL	150 mL
Solution III	10 mL	40 mL	200 mL
Equilibration Buffer	3 mL	12 mL	60 mL
Buffer HB	10 mL	40 mL	200 mL
DNA Wash Buffer Concentrate	4 mL	15 mL	80 mL
Elution Buffer	5 mL	25 mL	100 mL
OB Protease Mixture	3 mg	16 mg	80 mg
RNase A, Concentrate	50 µL	100 µL	500 µL
Instruction Booklet	1	1	1

Before Starting

Supplied By User: High speed centrifuge capable of 12,000 x g
Sterile 15-30 mL centrifuge tubes. (Falcon® tubes recommended.)
High speed centrifuge tubes (polycarbonate or Corex®)
Absolute (96%-100%) ethanol

- IMPORTANT**
- Add vial of RNase A to bottle of Solution I and store at 4°C.
 - Dilute **OB Protease Mixture** with deionized water as follows. Aliquot and store reconstituted OB protease Mixture at -20°C.
 - D7004-00** Dissolve 110 µL deionized water
 - D7004-01** Dissolve 550 µL deionized water
 - D7004-02** Dissolve with 2.7 mL deionized water
 - DNA Wash Buffer Concentrate** is to be diluted with absolute ethanol as follows:
 - D7004-00** Add 16 mL ~96-100% ethanol
 - D7004-01** Add 60 mL ~96-100% ethanol
 - D7004-02** Add 320 mL ~96-100% ethanol

E.Z.N.A.™ HP Plasmid Midi Protocol

- Culture volume:** Inoculate 30-50 mL LB/ampicillin (50 µg/mL) medium placed in a 250 mL culture flask with *E.coli* carrying desired plasmid and grow at 37°C with agitation for 12-16 h.
- Pellet bacteria by centrifugation at 4,000 x g for 10 min at room temperature.
- Decant or aspirate medium and discard. **To the bacterial pellet add 2.25 mL Solution I/RNase A.** Resuspend cells **completely** by vortexing and/or pipetting.

Complete resuspension of cell pellet is vital for obtaining good plasmid yields.
- Transfer cell suspension to a 15-30 mL centrifuge tube capable of withstanding 12,000 x g (screw-cap polycarbonate or Corex® glass tubes will suffice). **Add 2.25 mL Solution II and 50 µL of OB Protease Mixture, cover, and gently mix by inverting and rotating tube 7-10 times to obtain a cleared lysate.** Incubated 3-5 min at room temperature.

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.)
- Add 3.2 mL Solution III, cover the tube, and gently mix by inverting several times until a flocculent white precipitate forms.** Centrifuge at ≥12,000 x g for 10 minutes at 4°C to pellet the cellular debris and genomic DNA.

Note: The Buffers must be mixed thoroughly. 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. A tightly packed cell debris pellet indicates efficient lysis.

- Take a HiBind® DNA Maxi column pre-inserted in a 50 ml collection tube. Add 1 ml Equilibration Buffer into the column. Let the column sit for 4 minutes at room temperature to equilibrate the membrane. Centrifuge at 3000 x g for 3 minutes..**
- CAREFULLY aspirate and add 3.75 mL of the clear supernatant to a clean HiBind® DNA Midi column** assembled in a 15 mL collecting tube making sure that no cellular debris is carried over. The Midi column has a maximum capacity of 4 ml. Centrifuge for 5 min at 3,000-8,000 x g at room temperature to completely pass lysate through column. Discard the flow-through liquid and **add an additional 3.75 mL of cleared lysate to the column.** Centrifuge as above and repeat until the entire sample has been passed through. Finally discard the flow-through and reuse the collection tube in step 7.

IMPORTANT: This and all subsequent steps must be performed using a

centrifuge capable of at least 3,000-8,000 x g. Ensure that an appropriate rotor adaptor is in place to prevent damage to the collection tube.

8. **Add 3.5 mL Buffer HB to the HiBind® DNA Midi column and centrifuge for 5 min at 3,000-8,000 x g 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.
9. **Wash the column by adding 3.5 mL of DNA Wash Buffer diluted with ethanol.** Centrifuge for 5 min at 3,000-8,000 x g at room temperature 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.
10. **Optional step:** repeat wash step with another 3.5 mL DNA Wash Buffer. Centrifuge as above and discard fluid.
11. **Wash the column with 3.5 mL absolute ethanol and centrifuge for 5 min at 3,000-8,000 x g at room temperature.** Discard flow-through liquid.
12. Centrifuge the empty capped column for 10 min at max speed (≤ 8000 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.** Remove any traces of ethanol from the column using a pipette.
13. Place column into a clean 15 mL centrifuge tube. **Add 0.5-1.0 mL (depending on desired concentration of final product) DNA Elution Buffer or sterile deionized water directly onto the column matrix.** Allow column to sit 2 min at room temperature. Centrifuge 5 min at maximal speed ($\leq 8,000$ x g) to elute DNA. This represents approximately 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. Also, preheating the water to 70°C prior to elution may significantly increase yields.
14. **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:

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

High copy number plasmids generally yield up to 200 μg of DNA from 50 mL culture. The ratio of $(\text{Absorbance}_{260})/(\text{Absorbance}_{280})$ 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.


Low Copy Number Plasmids

Low copy plasmids generally give 0.1-1 μg DNA per mL overnight culture. For isolation of plasmid from low copy-number plasmids (0.1-1 $\mu\text{g/mL}$ culture) or low-midi copy-number plasmids (1-2 $\mu\text{g/mL}$ culture) bacteria, the method can be modified to essentially increase the yield if necessary. Start with 50-100 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, III and OB Protease Mixture. Continue as above using only one HiBind® DNA Midi column. There is no need to increase the volumes of Buffer HB and DNA Wash Buffer used. The Buffer of Solution I, II, III and OB Protease Mixture can be purchased separately.

Note: This method is not recommended for high copy number plasmids because above 100 mL culture, the HiBind® DNA Maxi column quickly becomes saturated. In this situation we recommend processing of multiple samples from the same culture.

Short Protocol for Experienced User

Note: All steps are to be performed at room temperature. Refer to page 3-4 for important notes on preparation of components.



1. Pellet cells from 15-50 mL overnight culture. Resuspend cells in **2.25 mL Solution I/RNase A**.
2. **Add 2.25 mL Solution II with 50 µL protease.** Mix gently by inverting 4-6 times to obtain cleared lysate. A brief incubation at RT may be required.
3. **Add 3.2 mL Solution III** and mix to form white precipitate. Centrifuge at 12,000 x g at RT for 10 min.
4. Transfer cleared lysate to a HiBind® DNA Midi-column placed in a 15 mL collection tube. Centrifuge 5 min at 5000 x g. Discard liquid.
5. **Wash the column with 3.5 mL Buffer HB.**
6. **wash column with 3.5 mL DNA Wash Buffer diluted with ethanol.** Spin 5 min at 5000 x g for 5 minutes.
7. **Optional: Wash column again with 3.5 mL DNA Wash Buffer.**
8. Centrifuge 10 min at 8000 x g to completely dry the column .
9. Elute plasmid DNA with 0.5-1.0 mL sterile water or TE buffer.

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 50 mL with high copy plasmids. Cells may not be dispersed adequately prior to addition of Solution II. Vortex cell suspension to completely disperse. 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.
	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 100 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.
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	Ethanol not completely removed from column following wash steps.	Processing column as instructed in step 12 to dry .
Plasmid DNA will not perform in downstream application	Traces of ethanol remain on column prior to elution.	The column must be washed with absolute ethanol (step 10) and dried before elution. Ethanol precipitation may be required following elution.
Smear or degraded Plasmid observed from gel analysis	Protease Mixture not added or more protease is needed.	Add more protease Mixture and try to extend incubation time.