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Introduction

The E.Z.N.A.® Mag-Bind® 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 Omega Bio-Tek's proprietary Mag-Bind® Particle 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.® Mag-Bind® Plasmid Purification Kit combines the power of Mag-Bind® technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality plasmid DNA. Yields vary according to plasmid copy number, *E.coli* strain, and conditions of growth, but 1 ml of overnight culture in LB medium typically produces 5-10 µg high-copy number plasmid DNA. The purified plasmid can be used directly for automated fluorescent DNA sequencing, such as with BigDye sequencing chemistry, as well as for other standard molecular biology techniques including restriction enzyme digestion.

Storage and Stability

All E.Z.N.A.® Mag-Bind® Plasmid Purification Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: The mixture of Solution I/RNase A and Mag-Bind® particle solution should be stored at 4°C; all other material at 22-25°C.

Kit Contents

Product Number	M1260-01	M1260-02	M1260-03	M1260-04
Purification times	50 Preps	200 Preps	500 Preps	1000 Preps
Mag-Bind® Particle Solution E	550 ul	2x1.05ml	5.2 ml	10.4 ml
MGC Binding Buffer	10 ml	30 ml	2 x 35 ml	4 x 35 ml
Solution I	20 ml	40 ml	100 ml	2 x 100 ml
Solution II	20 ml	40 ml	100 ml	2 x 100 ml
Neutralization Buffer	20 ml	40 ml	100 ml	2 x 100 ml
SPM Wash Buffer	15 ml	50 ml	3 x 40 ml	6 x 40 ml
RNase A Concentrate	300 µl	500 µl	1200 µl	2.4 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.

IMPORTANT

1. **Add 1 vial of RNase A to bottle of Solution I provided. Store at 4°C.**
2. SPM Wash Buffer has to be diluted with ethanol as
   - M1260-01 Add 60 ml absolute ethanol (96-100%)
   - M1260-02 Add 200 ml absolute ethanol (96-100%)
   - M1260-03/04 Add 160 ml absolute ethanol per bottle
3. MGC Binding Buffer has to be diluted with ethanol as
   - M1260-01 Add 40 ml absolute ethanol (96-100%)
   - M1260-02 Add 120 ml absolute ethanol (96-100%)
   - M1260-03/04 Add 140 ml 96%-100% ethanol per bottle

Store diluted SPM Wash Buffer & MGC Binding Buffer at room temperature

Note: All steps must be carried out at room temperature.

E.Z.N.A.® Mag-Bind® Plasmid Kit Magnetic Protocol

Supplied By User

- Microcentrifuge capable of at least 12,000 x g.
- Nuclease-free 1.5 ml & 2 ml centrifuge tubes.
- Sterile deionized water (or TE buffer)
- Absolute (96%-100%) ethanol

1. Culture Volume: Innoculate 1.0-3.0 ml LB/antibiotic(s) medium placed in a 10-20 ml culture tube with *E.coli* carrying desired plasmid and grow at 37°C with agitation for 12-16 h.

   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®.

2. Pellet 1.0-3.0 ml bacteria by centrifugation at 10,000 x g for 1 min at room temperature.

3. **Decant or aspirate medium and discard. To the bacterial pellet add 150 µl Solution I/RNase A. Resuspend cells completely** by vortexing. Complete resuspension of cell pellet is vital for obtaining good yields.

4. **Add 150 µl Solution II and gently mix by inverting and rotating tube 4-6 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. (Store Solution II tightly capped when not in use.)

5. **Add 150 µl ice-cold Neutralization Buffer and gently mix by inverting several times until a flocculent white precipitate forms.** Centrifuge at ≥14,000 × g for 10 minutes at 4°C.

6. CAREFULLY aspirate and transfer the cleared supernatant to a clean 1.5ml centrifuge tube.

7. **Add 10 µl of Mag-Bind® Particles Solution E and follow by addition of 450µl of MGC Binding Buffer.** Mix well by inverting the tube several times. **NOTE: The Mag-Bind® Particles will settle and bead together at the bottom of their container after several hours. Please check container before use. If beading has occurred, gently shake or vortex container until particles have been redispersed in solution. (IMPORTANT)**

Tip: MGC Binding Buffer and Mag-Bind® Particles Solution E can be combined in appropriate proportions to make a master mix before starting the procedure.

8. **Incubate for 5 minutes at room temperature,** mixing once by inverting the centrifuge tube.

9. Place the tube into the magnetic separation stand and remove the supernatant after the magnetic particles have completely migrated to the walls of tube adjacent to the magnets. (Supernatant should be clear when migration is complete.)

10. **Remove tube from the Magnetic Separation stand, then wash the pelleted Mag-Bind® particles by adding 500µl SPM Wash Buffer.** Resuspend the particles in SPM Wash Buffer by shaking or briefly vortexing the tube. Again place the tube on the magnet separation stand and remove the supernatant after Mag-Bind® particles have completely migrated to the walls of the tube. **NOTE: For better washing efficiency, Mag-Bind® particles should be fully resuspended. Resuspension can be performed by pipetting or by vortexing.**

11. **Remove the tube from magnetic separation stand and wash the Mag-Bind® particles by adding 500µl SPM Wash Buffer.** Resuspend the Mag-Bind® particles by shaking or briefly vortexing the tube. Place the tube on the magnetic separation stand to pellet the Mag-Bind® particles. Aspirate the supernatant.

12. Optional: **Remove the tube from magnetic separation stand and wash the Mag-Bind® particles by adding 500 µl absolute ethanol to the tube.** Resuspend the Mag-Bind® particles by shaking or vortexing the tube. Place the tube on the magnetic separation stand to pellet the Mag-Bind® particles. Aspirate the supernatant

13. Air dry the Mag-Bind® particles pellet for 15-30 minutes at room temperature.

14. Elute DNA: Resuspend the Mag-Bind® particles pellet with 20-50µl water or TE buffer.

15. Place the tube into the magnetic separation stand to pellet the Mag-Bind® particles.

16. Transfer the supernatant containing the purified plasmid into a clean centrifuge tube.

E.Z.N.A.® Mag-Bind® Plasmid Kit Spin Protocol

1. Follow Mag-Bind Plasmid Magnetic Protocol step 1-8 on page 4.

2. Centrifuge at 10,000 x g for 2 min at room temperature to collect Mag-Bind® Particles. Discard the supernatant.

3. Add 1 ml SPM Wash Buffer, vortex to completely resuspend the Mag-Bind® particles.

4. Centrifuge at 10,000 x g for 2 minutes. Discard the supernatant.

5. Air dry the Mag-Bind® particles pellet for 5-10 minutes at room temperature.

6. Resuspend the Mag-Bind® particles pellet with 20-50 µl water or buffer TE.

7. Centrifuge at 10,000 x g for 2 minutes. Transfer the DNA solution in a new tube.

Yield and Quality of DNA

Determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

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

High copy number plasmids generally yield up to 25 µg of DNA from 5 ml culture. 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.

Troubleshooting Guide

Problem	Likely Cause	Suggestions

Low DNA yields	Poor cell lysis	Do not use more than 2 ml with high copy number 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.
	Lost Mag-Bind® Particles during operation	carefully remove the supernatant when aspirating the supernatant during process.
No DNA eluted.	SPM Wash Buffer or MGC Binding Buffer is not diluted with absolute ethanol.	Prepare SPM Wash Buffer and MGC Binding Buffer 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 before elution.	Increase air dry time before elution step
Plasmid DNA will not perform in downstream application	Traces of ethanol remain on tubes prior to elution.	The tubes must be washed with absolute ethanol and dried before elution.

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	Isolation of up to 250 µg Plasmid DNA, featuring filter syringes for lysate clearance	D6905
Fastfilter Plasmid Maxi Kit	Isolation of up to 1.5 mg Plasmid DNA, featuring filter syringes for lysate clearance	D6924
E-Z 96 Fastfilter Plasmid	Isolation of Plasmid DNA using a 96-well format	D1097
E-Z 96 SE Plasmid Kit	Isolation of plasmid DNA 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	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 DNA, featuring filter syringes for lysate	D6915
EndoFree Plasmid Maxi Kit	Isolation of up to 1.5 mg Endotoxin free Plasmid DNA, featuring filter syringes for lysate	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	D7022
E.Z.N.A.™Single Strand Phage DNA Isolation Kits		
M13 Isolation Kit	Isolation of up to 15µg of single stranded	D6900
E-Z 96 M13 Isolation Kit	Isolation of up to 15µg of M-13 DNA using a 96-	D1900
E.Z.N.A.™ Large Construct DNA Isolation Kits		
BAC/PAC DNA Isolation Kit	Effective purification of BAC or PAC DNA	D2156