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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 Maxi 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. Cultured bacterial cells are pelleted by centrifugation, cells are then suspended and lysed in a alkaline-SDS buffer. By addition of neutralization buffer, genomic DNA, proteins are removed. The cleared cell lysate is mixed with magnetic particles on which the DNA binds. With two wash steps, the purified DNA was eluted with lower salt buffer or water. Yields vary according to plasmid copy number, E.coli strain, and conditions of growth, but 200 ml of overnight culture in LB medium typically produces 0.8-2mg high-copy 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 isolation 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 all other material at 22-25°C.

E.Z.N.A.® Mag-Bind® Plasmid Maxi Kit

Product Number	M1257-01	M1257-02
Purifications times	5 Preps	20 Preps
Mag-Bind® Particles	0.3 ml	1.05 ml
MGC Binding Buffer	25 ml	2 x 50 ml
Solution I	50 ml	200 ml
Solution II	50 ml	200 ml
Neutralization Buffer	50 ml	200 ml
RNase A Concentrate	300 µl	1.2 ml
Instruction Booklet	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 RNase A to bottle of Solution I provided. Store at 4°C. 2. MGC Binding Buffer has to be diluted with absolute ethanol (96-100%)as follows: D1257-01 Add 100 ml absolute ethanol D1257-02 Add 200 ml absolute ethanol to each bottle ***Store diluted MGW Wash Buffer & MGC Binding Bufefr at room temperature***

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

This Protocol is designed to isolate 1-10 mg of high Copy-Number plasmids or 0.2 - 1mg of low Copy-Number Plasmid from 2000 ml overnight cultures using E.Z.N.A.® Mag-Bind Plasmid Mega Kit.

Materials to be Provided by By User

- Centrifuge Capable of >15,000 x g
- 50 ml centrifuge tubes capable of >15,000 x g
- 50 ml centrifuge tube.
- Sterile deionized water (or TE buffer)
- Absolute (96%-100%) ethanol

■ **Growth of bacterial culture**

1. **Inoculate 200 ml LB/ampicillin (50 µg/ml) medium placed in a 1 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 (0.2~0.4 ml) 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 a 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}. We recommend a bacterial density of between 2.0 and 3.0 at $OD_{600.}$ When using untrient-rich media, care should be taken ensure that the cell density does not exceed an OD_{600} of 3.0.

If using a frozen glycerol stock as inoculum, streak it onto an agar plate containing the appropriate antibiotic for single colony isolation. Then picking a single colony and inoculate the 2-5ml starter culture as described above.

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

2. Pellet up to 200 ml bacteria in appropriate vessels by centrifugation at 3,000x 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 8 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* result.

4. Transfer cell suspension to a 50 ml centrifuge tube capable of withstanding >15,000 x g (screw-cap polycarbonate or Corex® glass tubes will suffice). **Add 8 ml Solution II and gently mix by inverting and rotating the tube 10-15 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. (Store Solution II tightly capped when not in use.)

5. **Add 8 ml ice chilled Neutralization Buffer, cover, and gently mix by inverting several 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.

■ **Remove KDS-Proteins and Genomic DNA By Spin at high Speed**

6. **Incubate the lysis solution on ice for 10 min. Centrifuge at > 15,000 x g for 20 minutes at 4°C**. Increasing centrifugation speed is helpful to completely remove the precipitated bacterial cell material. A tightly packed cell debris pellet indicates efficient lysis.
   Note: For fast removing the precipitated bacteiral cell materials, one may order OBI Lysate Clearance filter syringe to replace this centrifugation step. This filter cartridge completely removes SDS precipitates and clears bacterial lysates using

filter syringe.

■ **Purify Plasmid by Mag-Bind™ Silica-Particles Beads**

7. Transfer 20 ml of the supernatant contains plasmid DNA promptly to a 50ml new tube.

8. **Add 50 µl of Mag-Binds® Particles Solution into the tube and follow by addition of equal volume of MGC Binding Buffer.** Mix well by inverting the tube few times.

   NOTE: The Mag-Binds® 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)

9. Incubate for 10 minutes at room temperature, mixing few times by inverting the tube.

10. **Separate the Magnetic particles by centrifugation or Magnetic strand:**
    Centrifugation: Centrifuge at 3,000 x g for 10 minutes to pellet the magnetic particles. Discard the supernatant.
    Magnetic Strand: Place the tube onto the magnetic separation stand and remove the supernatant after the magnetic particles have completely migrated to the walls of the tube adjacent to the magnets. (Supernatant should be clear when migration is complete.)

11. Add 15 ml of 70% ethanol into each tube. Resuspend the magnetic particles by vortexing at maxi speed for 15 seconds.

12. **Separate the Magnetic particles by centrifugation or Magnetic strand:**
    Centrifugation: Centrifuge at 3,000 x g for 10 minutes to pellet the magnetic particles. Discard the supernatant.
    Magnetic Strand: Place the tube onto the magnetic separation stand and remove the supernatant after the magnetic particles have completely migrated to the walls of the tube adjacent to the magnets. (Supernatant should be clear when migration is complete.)

13. Completely to remove the supernatant and air dry for 10 min.

14. Elute DNA: Resuspend the Mag-Binds® particles pellet with 0.2-1ml Elution Buffer or TE buffer (depending on desired concentration of final product and plasmid copy-number). Incubate at RT for 10 minutes.

15. **Separate the Magnetic particles by centrifugation or Magnetic strand:**
Centrifugation: Centrifuge at 3,000 x g for 10 minutes to pellet the magnetic particles.
Magnetic Strand: Place the tube onto the magnetic separation stand for 15 minutes. (Supernatant should be clear when migration is complete.)

16. Transfer the Solution into a new 1.5 or 2.0ml microcentrifuge tube.

Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	Do not use more than 200 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.
	Lost Mag-Bind Particles during operation	careful 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.
Optical densities do not agree with DNA yield on agarose gel.	Trace contaminants eluted from column increase A_{260}.	Make sure to wash Mag-Bind pellet as instructed. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantization.
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 column prior to elution.	The DNA plate must be washed with absolute ethanol and dried before elution. Ethanol precipitation may be required following elution**.**