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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 200-500mL of overnight culture in LB medium typically produces 800-1500 µg 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 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, all other material at 22-25°C.

## Kit Contents

Product Number	M1257-01	M1257-02
Purification times	5 Preps	20 Preps
Lysate Clearance Filter Syringe	5	20
Mag-Bind® Particle Solution E	850 µL	3.5 mL
MGC Binding Buffer	50 mL	200 mL
Solution I	90 mL	350 mL
Solution II	90 mL	350 mL
Neutralization Buffer	90 mL	350 mL
SPM Wash Buffer Concentrate	75 mL	200 mL
RNase A Concentrate	500 µL	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 vial of RNase A to bottle of Solution I and store at 4°C.
2. **SPM Wash Buffer concentrate** has to be diluted with absolute ethanol (~96-100%) as follows:  
  
D1257-01 Add 300 mL ~ 96%-100% ethanol  
  
D1257-02 Add 800 mL ~96%-100% ethanol
3. **MGC Binding Buffer** has to be diluted with isopropanol as follows:  
  
D1257-01 Add 200 mL ~96%-100% ethanol  
  
D1257-02 Add 800 mL ~96%-100% ethanol

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

## Mag-Bind® Plasmid Maxi Magnetic/Spin Protocol

### Materials Supplied By User

- Centrifuge Capable of 20,000 x g
- 50 mL centrifuge tubes capable of 20,000 x g
- Magnetic Separation Device that hold 50mL tube
- Sterile deionized water (or TE buffer)
- Absolute (96%-100%) ethanol
- waterbath or heat block preset to 70°C

1. Isolate a single colony from freshly streaked selective antibiotic plate and inoculate a starter culture of 2-5 mL LB medium containing proper antibiotic. Incubate at 37°C for about 8 hours with vigorously shaking. Dilute 0.5-1.0 mL starter culture into a 200-500 mL selective LB/antibiotic(s) medium and grow at 37°C with agitation for 12-16 h. The culture density should reach 3-4 x 10<sup>9</sup> per mL.

**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. Harvest 200-500 mL bacterial cells by centrifugation at 4,000-6,000 x g for 10 minutes at 4°C.

**Note: If you want to stop the protocol and continue later, discard the medium and freeze the cell pellet at -20°C.**

3. Discard supernatant into a waste container. Dry the pellet by placing centrifuge tube upside-down on a paper towel to remove excess media. **Add 16 mL Lof Solution I/RNase A to the bacterial pellet.** Resuspend cells completely by shaking or pipetting.

**Complete resuspension of cell pellet is vital for obtaining good plasmid yields.**

4. **Add 16 mL Solution II and mix by gently shaking and rotating the tube for 1 minute to obtain a cleared lysate.** A 2-3 minutes incubation at room temperature may be necessary. Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity.

(Store Solution II tightly capped when not in use.) Note: do not incubate the lysate over 5 minutes since it can cause permanently denature plasmid.

5. **Add 16 mL chilled (4°C) Neutralization Buffer and mix by gently shaking and rotating the plate for 1 minute until a flocculent white precipitate forms.**
6. **Centrifuge at ≥15,000 x g for 30 minutes at 4°C.**
7. Pour the Supernatant into the barrel of the Lysate Clearance Filter Syringe. Use a new 50 ml tube 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 50 ml tube and gently insert the plunger to expel the cleared lysate to the tube. Divide the cleared cell lysate into 2 of the 50 mL centrifuge tubes.

9. **Add 80 µl of Mag-Binds® Particles Solution into each 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)

10. **Incubate for 10 minutes at room temperature. Mix the tube few times by inverting the tube during incubation.**

11. 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.)

12. Aspirate the cell lysate. Remove the tube from the Magnetic Separation stand, then Add 10 mL of SPM Wash Buffer diluted with absolute ethanol into each tube. Resuspend the magnetic particles by pipetting or vortexing.

13. Combine the magnetic particles into one tube. Again place the tube on the magnet separation stand and remove the supernatant after Mag-Binds® particles have completely migrated to the walls of the tube.

**NOTE: For better washing efficiency, Mag-Binds® particles should be fully resuspended. Resuspension can be performed by pipetting or by vortexing.**

14. Remove the tube from magnetic separation stand and **wash the Mag-Binds® particles by adding 25 mL of SPM Wash Buffer.** Resuspend the Mag-Binds® particles by pipetting or vortexing. Place the tube on the magnetic separation stand to pellet the Mag-Binds® particles. Aspirate the supernatant.

15. **Optional:** Remove the plate from magnetic separation stand and wash the Mag-Binds® particles by adding 25 mL of absolute ethanol. Resuspend the Mag-Binds® particles by pipetting or vortexing. Place the tube on the magnetic separation stand to pellet the Mag-Binds® particles. Aspirate the supernatant

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

17. Elute DNA: **Resuspend the Mag-Binds® particles pellet with 1-2 mL Elution Buffer or TE buffer.** Incubate at 37°C for 10 minutes.

18. Place the plate onto the magnetic separation stand to pellet the Mag-Binds® particles. Transfer the supernatant containing the purified plasmid into a clean 50 mL centrifuge tube.

## Mag-Bind® Plasmid Maxi Spin Protocol

1. Isolate a single colony from freshly streaked selective antibiotic plate and inoculate a starter culture of 2-5mL LB medium containing proper antibiotic. Incubate at 37°C for about 8 hours with vigorously shaking. Dilute 1.0-1.5 mL starter culture into a 200-500 mL selective LB/antibiotic(s) medium and grow at 37°C with agitation for 12-16 h. The culture density should reach 3-4 x 10<sup>9</sup> per mL.

2. Harvest the bacterial cells by centrifugation at 6000 x g for 15 minutes at 4°C.

**Note: If you want to stop the protocol and continue later, discard the medium and freeze the cell pellet at -20°C.**

3. Discard supernatant into a waste container. Dry the pellet by placing centrifuge tube upside-down on a paper towel to remove excess media. **Add 16 mL of Solution I/RNase A to the bacterial pellet.** Resuspend cells completely by shaking or pipetting.

**Complete resuspension of cell pellet is vital for obtaining good plasmid yields.**

4. **Add 16 mL Solution II and mix by gently shaking and rotating the tube for 1 minute to obtain a cleared lysate.** A 2-3 minutes incubation at room temperature may be necessary. Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity. (Store Solution II tightly capped when not in use.) Note: do not incubate the lysate over 5 minutes since it can cause permanently denature plasmid.

5. **Add 16 mL chilled (4°C) Neutralization Buffer** and mix by gently shaking and rotating the tube for 1 minute until a flocculent white precipitate forms.

**Note: Precipitate will be enhanced by using chilled (4°C) Neutralization Buffer.**

6. **Centrifuge at ≥15,000 x g for 30 minutes at 4°C.**

7. **Pour the supernatant into the barrel of the Lysate Clearance Filter Syringe.** Use a new 50 ml tube 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 50 ml tube and gently insert the plunger to expel the cleared lysate to the tube.** Divide the cleared cell lysate into 2 of the 50 mL centrifuge tubes.

9. **Add 80µl of Mag-Binds® Particles Solution into each tube and follow by addition of equal volume of MGC Binding Buffer.** Mix well by inverting the tube few times.

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

11. **Centrifuge at 3,000-5,000 x g for 10 minutes to pellet the magnetic particles.** Discard the supernatant.

12. **Add 25 mL of SPM Wash Buffer diluted with absolute ethanol into each tube.**  
Resuspend the magnetic particles by pipetting or vortexing.
13. **Combine the magnetic particles into one tube. Centrifuge at 3,000-5,000 x g for 5 minutes to pellet the magnetic particles.**
14. **Discard the supernatant and add 25 mL of SPM Wash Buffer to the tube.**  
Resuspend the pellet by vortexing.
15. **Centrifuge at 3,000-5,000 x g for 5 minutes to pellet the magnetic particles.**  
Aspirate the supernatant. Remove any drop of liquid with pipettor. Air dry the Mag-Binds® particles pellet for 5-10 minutes at room temperature.
16. **Elute DNA: Resuspend the Mag-Binds® particles pellet with 1-2 mL Elution Buffer or TE buffer.** Incubate at RT for 5 minutes.
17. **Centrifuge at 3,000-5,000 x g for 5 minutes to pellet the magnetic particles.**  
Transfer the supernatant containing the eluted DNA into a new tube.

## Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	Do not use more than 500 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 0.1 µg 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 or isopropanol.	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.