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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.

Applications such as transfection normally require a large amount of plasmid DNA that meets a number of stringent criteria, including a high degree of purity and a high percentage of supercoiling. The most common difficulty for large scale plasmid isolation is endotoxin contamination, which can have a negative effect on downstream applications such as transfection. The E.Z.N.A.™ Endo-Free Mag-Bind® Plasmid Mega 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 N3 buffer, genomic DNA, proteins are removed. Following the N3 step, the cleared lysate is extracted with ETR Buffer, which will almost completely remove the endotoxins. 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 1500 mL of overnight culture in LB medium typically produces 5 g of 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 OB protease mixtures at -20°C after received, all other material at 22-25°C.

Kit Contents

Product Number	M1253-00	M1253-01	M1253-02
Purification times	2 Preps	5 Preps	20 Preps
Mag-Bind® Particle Solution	1.2 mL	2.75 mL	11 mL
MGC Binding Buffer	140 mL	2 x 140 mL	5 x 200 mL
Solution I	170 mL	450 mL	2 x 900 mL
Solution II	170 mL	450 mL	2 x 900 mL
N3 Buffer	170 mL	450 mL	2 x 900 mL
ETR Reagent	50 mL	120 mL	500 mL
SPM Wash Buffer Concentrate	50 mL	200 mL	4 x 200 mL
RNase A Concentrate	1.5 mL	3 mL	2 x 6 mL
Instruction Booklet	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 a vial of RNase A to one 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:

   M6202-00 Add 200 mL ~ 96%-100% ethanol

   M6202-01 Add 800 mL ~ 96%-100% ethanol

   M6202-02 Add 800 mL 96%-100% ethanol per bottle
3. **MGC Binding Buffer** has to be diluted with absolute ethanol as follows:

   M6202-00 Add 560 mL ~96%-100% ethanol

   M6202-01 Add 560 mL ~96%-100% ethanol per bottle

   M6202-01 Add 800 mL ~96%-100% ethanol per bottle

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

Mag-Bind® Endo-Free Plasmid Mega Protocol

Supplied By User:

- Centrifuge Capable of 12,000 x g
- Tubes or vessel capable of 12,000 x g
- 50 mL tube
- 250 mL centrifuge bottle.
- Magnetic Separation Device or Centrifuge that holds 50mL tubes
- Centrifuge capable of holding 250 mL Bottles
- Sterile deionized water (or TE buffer)
- Absolute (96%-100%) ethanol
- waterbath or heat block preset to 70°C
- Pipettor
- Pre Chill N3 Buffer to 4°C
- Pre Chill ETR Reagant to 4 °C

1. Isolate a single colony from freshly streaked selective late and inoculate a starter culture of 2-5mL LB medium containing proper antibiotic. Incubate st 37C for about 8 hours with vigorously shaking. Dilute 2.0-3.0 mL starter culture into a 1500 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 10^9 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 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 80 mL of Solution I/RNase A to the bacterial pellet Resuspend cells completely by vortexing or pipetting. **Complete resuspension of cell pellet is vital for obtaining good plasmid yields.**

4. Add 80 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 the plasmid DNA.

5. Add 80 mL pre chllled 4°C N3 Buffer and mix by gently shaking and rotating the

tubefor 1 minute until a flocculent white precipitate forms.

6. **Clear cell lysate by centrifugation: Centrifuge at > 6,000 x g for 30 minutes at 4°C**. Remove the supernatant contains plasmid DNA and filter the lysate through a folded filter paper prewetted with distilled water. Note: it might be necessary to divide the lysate into few centrifugal tubes or bottles to proceed this step

7. Divide the cleared cell lysate into 2 of 250 mL centrifuge tubes or bottles.

8. Add 1/10 volume of pre chlllded ETR reagent and mix by vortexing. Incubate on ice for 10 minutes. **Note: After addition of ETR reagent, the lysate should appear turbid and it should become clear after the incubation on ice.**

9. Incubate the lysate on 37°C for 5 minutes, the lysate should appear turbid again. Centrifuge at 6000 x g for 15 minutes at 25°C. The ETR reagent will form a blue layer at bottom of the tube.

10. Transfer the top aqueous phase (top layer) into a new centrifugal bottle or tube.

11. Add 250µl of of Mag-Binds® Particles Solution into each tube and follow by adding an equal volume of MGC Binding Buffer as cleared lysate. (Note: If you have 75 mL cleared lysate add 75 mL 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)

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

13. Centrifuge at 6000 x g for 10 minutes to pellet the magnetic particles. Discard the supernatant.

14. Aspirate the supernatant. Add 50mL of SPM Wash Buffer into each tube. Resuspend the magnetic particles by pipetting or vortexing.

15. Combine the magnetic particles into one tube. Centrifuge at 6000 x g for 10 minutes to pellet the magnetic particles. Discard the supernatant.
    NOTE: For better washing efficiency, Mag-Binds® particles should be fully resuspended. Resuspension can be performed by pipetting or by vortexing.

16. Add 50 mL of SPM Wash Buffer. Resuspend the Mag-Binds® particles by vortexing.

Transfer the suspended Mag-Bind particles into a 50 mL centrifuge tube.

17. Centrifuge at 6000 x g for 10 minutes to pellet the magnetic particles. Discard the supernatant and remove any liquid by invert the tube on a absorbent paper.
18. Air dry the Mag-Binds® particles pellet for 5-10 minutes at room temperature.

19. Elute DNA: Resuspend the Mag-Binds® particles pellet with 2-5 mL Elution Buffer (Elution Buffer is Tris-HCl pH 8.5) or TE buffer. Incubate at 37°C for 10 minutes.

20. Centrifuge at 6000 x g for 10 minutes to pellet the Mag-Binds® particles.

21. Transfer the supernatant containing the purified plasmid into a new tube.

Trouble Shooting

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	Do not use more than1500 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.

Ordering Information

Product No.	Product Name	Description
E.Z.N.A.™ Plasmid Mini System		
D6942-01/02 D6943-01/02	Plasmid Mini Kit I	Isolation of up to 30µg plasmid in 15 minutes
D6945-01/02	Plasmid Mini Kit II	Isolation of up to 70µg plasmid in 15 minutes
D7042-01/02 D7043-01/02	HP Plasmid Mini Kit I	Isolation of up to 30µg plasmid from end A+ bacterial in 25 minutes
D7045-01/02	HP Plasmid Mini Kit II	Isolation of up to 70µg plasmid from end A+ bacterial in 25 minutes
D6948-01/02	Endo-free Plasmid Kit I	Isolation of up to 30µg endotoxin free plasmid
D6950-01/02	Endo-free Plasmid Kit II	Isolation of up to 70µg endotoxin free plasmid
D3476-01/02 D3376-01/02	Yeast Plasmid Kit	Isolation of plasmid from yeast
D6900-01/02	M13 isolation kit	Isolation of M13 DNA from culture
E.Z.N.A.™ Plasmid Midi/Maxi Isolation System		
D6904-01/02	Plasmid Midi Kit	Isolation of ≥200µg plasmid with midi column
D6905-03/04	Fastfilter Plasmid Midi kit	Isolation of ≥200µg plasmid under 30 min
D6915-01/03/04	Endo-free Fastfilter Plasmid Midi kit	Isolation of up to 200µg endotoxin-free plasmid in less than 60 minutes
D6922-01/02	Plasmid Maxi Kit	Isolation ≥200µg plasmid with maxi column
D6924-01/03/04	Fastfilter Plasmid Maxi kit	Isolation of ≥1.5 mg plasmid under 30 min.
D6926-01/03/04	Endo-free Fastfilter Plasmid Maxiprep kit	Isolation of up to 1.5 mg endotoxin-free plasmid in less than 60 minutes
E-Z 96® Plasmid Isolation System		
D1097-01/02	E-Z 96® Fastfilter Plasmid Isolation Kit	Isolation of plasmid in 96 well format with lysate clearance plate
D1900-01	E-Z 96 M13 Isolation Kit	Isolation of M13 DNA in 96 well format