Contents

Revised July 2007

Introduction

The 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 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. By using 96-well format, up to 96 samples can be simultaneously processed in less than 60 minutes. The new E-Z 96® Lysate Clearance Plate obviates time-consuming centrifugation for clearing of the bacterial alkaline lysates. It also has an average DNA recovery rate 10 to 30% higher than the manual centrifuge method. 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 10-15 µg high-copy plasmid DNA. By Integrating the one endotoxin extraction procedure, the , Mag-Bind® Endo-free Plasmid Purification Kit provides an efficient method for high throughput endotoxin-free plasmid isolation. The purified plasmid can be used directly for most downstream applications include transfection, automated fluorescent DNA sequencing, and other standard molecular biology techniques.

Storage and Stability

All Mag-Bind® Endo-free 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	M1258-00	M1258-01	M1258-02
Purification times	1 x 96	4 x 96	24 x 96
Mag-Bind® Particle Solution E	1ml	4.2 ml	25 ml
96 well Binding Plate (500µl)	1	4	24
MGC Binding Buffer	5	20	2 x 60 ml
Solution I	10 ml	40 ml	250 ml
Solution II	10 ml	40 ml	250 ml
Neutralization Buffer	10 ml	40 ml	250 ml
ETR-L Reagent	4 ml	15 ml	60 ml
SPM Wash Buffer Concentrate	15 ml	60 ml	3 x 100 ml
RNase A, Concentrate	100 ul	400 µl	2 x 1.2 ml
Instruction Booklet	1	1	1

Before Starting

Brieflyexamine 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 a bottle of Solution I and Store at 4°C.
2. **SPM Wash Buffer** Concentrate has to be diluted with absolute

M1258-00 Add 60 ml ~ 96%-100% ethanol

M1258-01 Add 240 ml ~96%-100% ethanol

M1258-02 Add 400 ml ~96%-100% ethanol per bottle

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

Endo-Free Plasmid Isolation Protocol (For clearing lysate with centrifugation)

Supplied By User

- Centrifuge with swinging-bucket rotor at room temperature capable of 4000 x g (such as Eppendorf 5810 with MTP rotor)
- Adapter for 96-well deep-well plate
- Magnetic Separation Device (OBI# MSD-01)
- Sterile deionized water (or TE buffer)
- Absolute (96%-100%) ethanol
- Vacuum oven or incubator preset to 70°C
- Multiple Channel Pipettor
- 96 deep-well plate
- Optional: E-Z Lysate Clearance Plate

1. **Culture Volume:** Innoculate 1.0-1.5 ml LB/antibiotic(s) medium placed in a 96-well 2 mL culture plate and grow at 37°C with agitation plate/block with *E.coli* for 16-20 hours.

   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. Seal the plate with tape or film (not supplied) and pellet bacteria by centrifugation at 3000 x g in a swinging-bucket rotor at room temperature for 10-15 minutes at room temperature.

3. Remove the tape and discard supernatant into a waste container. Dry the plate by placing upside-down on a paper towel to remove excess media. **Add 100 µl Solution I/RNase A to the bacterial pellet in each well of the deep well plate.** Resuspend cells completely by shaking or pipetting.

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

4. **Add 100 µl Solution II and mix by gently shaking and rotating the plate for 1 minute to obtain a cleared lysate.** A 5 min 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.)

5. **Add 100 µl chilled (4°C) Neutralization Buffer and mix by gently shaking and rotating the plate for 1 minute until a flocculent white precipitate forms**.

6. **Clear the cell lysates**: Centrifuge at 3000 x g for 20 minutes at 4°C. Transfer 200µl cleared supernatant into a new 96- deep well plate (not supplied).

   Note: Cleared cell lysate can also obtained by using E-Z 96 Lysate Cleared plate (Cat# FL9601/FL9602). Plate the E- Z 96 Lysate Clearance Plate (supplied) on top of the 500 µl collection plate. Transfer the lysate from step 5 into the Lysate Clearance Plate. Allow the cell lysate to stand for 3 minutes. The white precipitate should float to the top. Centrifuge at 2,000 x g for 5 minutes.

7. **Add 50µl/well of ETR-L Reagent and pipetting up and down for 5 times. Incubate the plate on ice for 10 minutes.** Centrifuge at 3000 x g for 5 minutes. Transfer the supernatant into a new 96 deep well plate.

8. **Add 108µl/well of isopropanol and followed by addition of 10µl/well of Mag-Binds™ Particles Solution E.** Mix well with pipetting few 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 re-dispersed in solution. (IMPORTANT)

9. **Incubate for 2 minutes at room temperature, mixing once by pipetting or briefly vortexing.** Use fresh tips to avoid cross-contamination.

10. Place the plate onto the magnetic separation stand and remove the supernatant after the magnetic particles have completely migrated to the walls of each well adjacent to the magnets. (Supernatant should be clear when migration is complete.)

11. Remove the plate from the Magnetic Separation stand, then wash the pelleted Mag-Binds® particles by adding 200µl SPM Wash Buffer. **Resuspend the particles in SPM Wash Buffer by pipetting or briefly vortexing plate.** Again place the plate on the magnet separation stand and remove the supernatant after Mag-Binds® particles have completely migrated to the walls of the plate.

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

12. **Remove the plate from magnetic separation stand and wash the Mag-Binds® particles by adding 200 µl SPM Wash Buffer to each well.** Resuspend the Mag-Binds® particles by pipetting. Place the plate on the magnetic separation stand to pellet the Mag-Binds® particles. Aspirate the supernatant.

13. **Optional: Remove the plate from magnetic separation stand and wash the Mag-Binds® particles by adding 200 µl absolute ethanol to each well.** Resuspend the Mag-Binds® particles by pipetting. Place the plate on the magnetic separation stand to pellet the Mag-Binds® particles. Aspirate the supernatant

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

15. **Elute DNA: Resuspend the Mag-Binds® particles pellet with 50-100µl water or TE buffer.**

16. Place the plate onto the magnetic separation stand to pellet the Mag-Binds® particles.
17. Transfer the supernatant containing the purified plasmid into a clean 96-well microplate.

Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	Do not use more than 1 ml with **high copy** plasmids. Cells may not be dispersed adequately prior to addition of Solution I. 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