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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. This kit provide 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 8-12 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 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 materials should be stored at 22-25°C.

Kit Contents

Product Number	M1256-00	M1256-01	M1256-02
Purifications	1 x 96Preps	4 x 96 Prpes	24 x 96 Preps
Mag-Bind Particle EX	4 ml	16 ml	96 ml
Process Plate (500µl)	1	4	24
Solution I	10 ml	35 ml	200 ml
Solution II	10 ml	30 ml	200 ml
Neutralization Buffer	10 ml	30 ml	200 ml
SPM Wash Buffer	15 ml	60 ml	300 ml
RNase A	50 ul	200 µl	1.2 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

Add a vial of RNase A to one bottle of Solution I provided. Store at 4°C.

SPM Wash Buffer has to be diluted with absolute ethanol as follows:

M1256-00	Add 35 ml ~ 96%-100% ethanol
M1256-01	Add 140 ml ~96%-100% ethanol
M1256-02	Add 700 ml ~96%-100% ethanol per bottle

PREPARE Mag-Bind Particle EX/Ethanol as follows:

M1256-00	Add 16 ml ~96%-100% ethanol
M1256-01	Add 64 ml ~96%-100% ethanol
M1256-02	Add 384 ml ~96%-100% ethanol

Store diluted SPM Wash Buffer at RT and Mag-Bind Particles EX/Ethanol at 4°C

Mag-Bind® Plasmid Isolation Protocol

Supplied By User:

- Centrifuge with swinging-bucket rotor at room temperature capable of 3000 x g (such as Eppendorf 5810 with MTP rotor)
- Adapter for 96-well deep-well plate
- Magnetic Separation Device (OBI# MSTND-01)
- Vacuum pump or vacuum aspirator capable of achieving a vacuum of 20-24 inches Hg (for vacuum protocol for clearing the cell lysate)
- Standard vacuum manifold (i.e: Omega Product #VAC-03) (for vacuum protocol for clearing the cell lysate)
- Sterile deionized water (or TE buffer)
- Absolute (96%-100%) ethanol
- Vacuum oven or incubator preset to 70°C
- Multiple Channel Pipettor

1. **Culture Volume: Inoculate 1.0-1.5 ml LB/antibiotic(s) medium placed in a 96-well 2ml culture plate and grow at 37°C with agitation fo plate/block with *E.coli* 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 the bacterial cells by centrifugation at 3000 x g in a swinging-bucket rotor at room temperature for 10-15 minutes at room temperature.**
3. **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 Neutralization Buffer** and mix by shaking or vortexing the plate for 1 minute until a flocculent white precipitate forms.

6. **Clear the cell lysates:** Centrifuge the plate contains bacterial lysate at 3500 x g for 15 minutes at room temperature.

Note: Omega Bio-tek's Lysate Clearance Plate can also be used to clear cell lysate without long centrifugation and avoid carryover of precipitates (Cat# FL9601)

7. **Transfer 200 µl cleared supernatant into the Process Plate (supplied).**

8. **Add equal volume of Mag-Bind[®] Particles EX/ethanol solution (200µl/well) and mix thoroughly by pipetting up and down for 20 times.**

NOTE: The Mag-Bind[®] Particles will settle 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. **Place the plate onto the magnetic separation stand to magnetite the magnetic particles.** 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.)

10. **Remove the plate from the magnetic separation stand, then wash the pelleted Mag-Bind[®] particles by adding 300µl SPM Wash Buffer.** Resuspend the particles by pipetting up and down for 20 times. Again place the plate on the magnet separation stand and remove the supernatant after Mag-Bind[®] particles have completely migrated to the walls of the plate.

NOTE: For better washing efficiency, Mag-Bind[™] particles should be fully resuspended.

11. **Remove the plate from magnetic separation stand and wash the**

Mag-Bind[®] particles by adding another 200 µl SPM Wash Buffer to each well. Resuspend Mag-Bind[®] particles by pipetting up and down for 10-20 times . Place the plate on the magnetic separation stand to magnetize the Mag-Binds[®] particles. Aspirate the supernatant.

12. **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.

13. **Leave the plate on the magnetic separation device.** Air dry the Mag-Binds[®] particles pellet for 5-10 minutes at room temperature.

14. **Elute DNA: Resuspend the Mag-Bind[®] particles pellet with 50-100µl water or TE buffer by pipetting up and down for 40 times.**

15. **Place the plate onto the magnetic separation stand to pellet the Mag-Binds[®] particles.**

16. **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 2 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.	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.

If the above suggestions fail to resolve any problems you are having with E-Z[®] 96 Mag-Bind[®] Plasmid Purification Kit, please feel free to contact our technical specialist:

United States customers: Tel: 800-832-8896

All other customers: 770-931-8400

Fax: 770-931-0230

Related Products

Product No.	Product Name	Description
M1260-01/02	Mag-Bind [®] Plasmidi Mini Kit	Isolation of plasmid with magnetic beads in single tube format
M1240-01/02	Mag-Bind [®] RX Plasmidi Mini Kit	Mag-Bind Plasmid kit using magnetic beads to clear cell lysate
D6943-01/02	Plasmid Miniprep Kit	Isolation of Plasmid in 15 minutes with mini-spin column .
D1097-01/02	96 well Fastfilter Plasmid Isolation Kit	Rapid method for isolating plasmid DNA with 96-well format.

* All OBI products available with size if 50 preps and 200 preps. Product number end with "-01" represent 50 preps kit and "-02" represent 200 preps kit.