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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. Up to 5 ml overnight culture can be processed in one single tube. Yields vary according to plasmid copy number, E.coli strain, and conditions of growth, 1 ml of overnight culture in LB medium typically produces 10-15 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 material at 22-25°C.

Kit Contents

Product No.	M1260-00	M1260-01	M1260-02
Purification	5	50	200
Mag-Bind Particle F	110 µl	1.1 ml	4.2 ml
Solution I	5 ml	15 ml	60 ml
Solution II	5 ml	15 ml	60 ml
Neutralization Buffer	5 ml	15 ml	60 ml
MGC Binding Buffer	1 ml	6 ml	25 ml
SPM Wash Buffer	3 ml	36 ml	150 ml
Elution Buffer	1.5 mL	15 mL	45 mL
RNase A	50 µl	150 µl	600 µl
Instruction Manual	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 provided. Store at 4°C.
2. SPM Wash Buffer has to be diluted with absolute ethanol as follows:

M1260-00	Add 7 ml ~ 96%-100% ethanol
M1260-01	Add 84 ml ~96%-100% ethanol
M1260-02	Add 350 ml ~96%-100% ethanol per bottle

3. MGC Binding Buffer has to be diluted with absolute ethanol as follows:

M1260-00	Add 4 ml ~96%-100% ethanol
M1260-01	Add 24 ml ~96%-100% ethanol
M1260-02	Add 100 ml ~96%-100% ethanol

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

Note: All steps must be carried out at room temperature.

Mag-Bind® Plasmid Isolation Protocol

Supplied By User:
- Centrifuge capable at least 13,000 x g
- Magnetic Separation Device (OBI# MSD-02)
- 1.5 or 2 ml centrifuge tube
- Absolute (96%-100%) ethanol
- Heating block or incubator preset to 65°C
- Pipettor

1. Culture Volume: Innoculate 1-5 ml LB/antibiotic(s) medium placed in a culture tube 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. Centrifugation at 13,000 x g at room temperature for 5 minutes at room temperature.

3. Discard supernatant into a waste container. Dry the plate by inverting the tube on a paper towel to remove excess media. Add **160 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 **160 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 **160 µl** Neutralization Buffer and mix by inverting the tube 10-20 times or until a flocculent white precipitate forms.

6. **Centrifuge at 13,000 x g for 10 minutes at room temperature.**

7. Transfer cleared supernatant into a new 1.5 ml centrifuge tube.

8. Add 500µl of MGC Binding Buffer followed by well-mixed 20µl Mag-

Binds Particles Solution F.

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 5-10 minutes at room temperature with gently shaking or inverting.

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

11. Remove the tube from the Magnetic Separation device, then wash the pelleted Mag-Binds® particles by adding 1ml SPM Wash Buffer. Resuspend the particles in SPM Wash Buffer by pipetting or briefly vortexing. Again place the tube on the magnet separation device 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 1 ml 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. Air dry the Mag-Binds® particles pellet for 5-10 minutes at room temperature. If necessary, remove any liquid drop from the tube with pipettor.

14. Elute DNA: Adding 100-200µl Elution Buffer(10 mM Tris Hcl pH 8.5) into the tube, mix throughly by vortexing for 20 seconds. Incubate at room temperature for 10 minutes.

    Note: Incubate at 60°C for 10 minutes may slightly increase the DNA yield.

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 1.5 ml tube.

17. Store the DNA at -20°C

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 0.1µg 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.

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
D6942-01/02 D6943-01/02 D6944-01/02	Plasmid Miniprep Kit	Isolation of Plasmid in 15 minutes with mini-spin column .
D6904-01/02	Plasmid Midiprep Kit	Isolating up to 200ug plasmid with spin column format
D6905-01/02	Fastfilter Plasmid Midiprep Kit	Isolating up to 200ug plasmid in 30 minutes
D6924-01/02	Fastfilter Plasmid Maxiprep Kit	Isolating up to 1.5mg plasmid in 30 minutes
D6915-01/02	Endo-free Plasmid Midiprep Kit	Isolate up to 200ug endotoxin free plasmid DNA in less than 60 minutes
D6926-01/02	Endo-free Plasmid Maxiprep Kit	Isolate up to 1.5mg endotoxin free plasmid DNA in less than 60 minutes
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.