Mag-Bind® Endo-Free Plasmid 96 Kit
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OMEGA bio-tek

Innovations in nucleic acid isolation

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 Endo-Free Purification Kit combines the power of Mag-Bind® technology with the innovative ETR technology to deliver high quality endotoxin free plasmid DNA in high throughput format . 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 for high-copy plasmid. The purified plasmid can be used directly for automated fluorescent DNA sequencing, such as with BigDye sequencing chemistry, transfection as well as for other standard molecular biology techniques including restriction enzyme digestion.

New in this Edition:

The latest manual has been redesigned to enhance readability and layout.

Kit Contents and Storage

Product No.	M1258-00	M1258-01	M1258-02
Purification	1 x 96	4 x 96	24 x 96
Mag-Bind® Particles CND	1.1 mL	4.4 mL	23.5 mL
ETR Binding Beads	2.7 mL	11 mL	66 mL
Solution I	12 mL	45 mL	250 mL
Solution II	12 mL	45 mL	250 mL
NFC Buffer	12 mL	45 mL	250 mL
PFC Binding Buffer	10 mL	40 mL	240
SPM Wash Buffer	15 mL	50 mL	2 x 150 mL
96-well Microplate (500 µL)	2	8	48
Endotoxin-free Water	15 mL	60 mL	360 mL
RNase A	50 µL	200 µL	1200 µL
Instruction Manual	1	1	1

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® Particles CND should be stored at 2-8°C; all other materials at 22-25°C.

Preparing Reagents

SPM Wash Buffer must be diluted with ethanol(96-100%) prior to use:

Kit	Ethanol to be Added
M1258-00	60 mL
M1258-01	200 mL
M1258-02	600 mL per bottle

ETR Binding Beads must be prepared freshly before each isolation. Prepare the ETR Binding Beads as following:

1. For each 96-well plate, transfer 2.7 mL ETR Binding Beads into a 15 mL centrifuge tube.
2. Centrifuge at 3,000 x g at room temperature for 5 minutes. Completely aspirate and discard the supernatant.
3. Add 10 mL water and resuspend the beads thoroughly by vortexing for 30 seconds.
4. Centrifuge at 3,000 x g at room temperature for 5 minutes. Completely aspirate and discard the supernatant.
5. Add 2.7 mL water and resuspend the beads.
6. The ETR Binding Beads are ready to use.

Solution I must be combined with RNase A as follows:

1. Add vial of RNase A to the bottle of Solution I before use. Store solution at 4°C.

Mag-Bind® Endo-Free Plasmid 96 Kit

Materials to be provided by user:

- Centrifuge with swinging-bucket rotor capable of 3,000 x *g*
- Rotor for 96-well deep-well plate
- 96-well deep-well plate
- Magnetic Separation Device (OBI# MSD-01)
- 96-well microplate
- 100% Ethanol
- Pipettor
- Isopropanol
- 15 mL centrifuge tube or 24-well deep-well plate

Before Starting

- Prepare SPM Wash Buffer, ETR Binding Beads and Solution 1/RNase A according "Preparing Reagents" section

1. Culture Volume: Inoculate 1-1.5 mL LB/antibiotic(s) medium in a 96-well deep-well plate grown at 37°C with agitation with *E. coli* for 12-16 hours.

   Note: 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. Centrifuge at 2,000-3,000 x *g* at room temperature for 10 minutes to collect bacteria.

3. Discard supernatant. Dry the plate by inverting the plate on a absorbent paper towel to remove excess media.

4. Add 100 µL Solution I/RNase A to the bacterial pellet in each sample. Resuspend the cells completely by vortexing. Complete re-suspension of the cell pellet is vital for obtaining good plasmid yields.

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

6. Add 100 µL NFC Buffer and mix by vortexing the plate until a flocculent white precipitate forms.

7. Centrifuge at 2,000-3,000 x *g* at room temperature for 10 minutes.

8. Transfer 200 µL cleared cell lysate into a new 96-well Microplate (500 µL) (provided). Avoid transferring the white precipitate containing cell debris.

9. Add 25 µL ETR Binding Beads and mix thoroughly by pipetting up and down for 20 times. Incubate at room temperature for 5 minutes.

   Note: The ETR Binding Beads must be freshly prepared before each isolation. See "Before Starting" section on Page 4 for details.

10. Place the plate onto a magnetic separation device (MSD-01) to collect the beads. It normally takes 2-3 minutes to clear all the beads from solution.

11. Transfer the cleared supernatant into a new 96-well Microplate (500 µL) (provided). Avoid transferring any ETR Binding Beads since they contain endotoxins.

12. Add 75 µL PFC Binding Buffer followed by 10 µL of Mag-Bind® Particles CND. Mix the sample thoroughly by pipetting up and down 10 times. Incubate at room temperature for 5 minutes.

13. Place the plate onto a magnetic separation device to collect the beads. It normally take 2-3 minutes to clear all the beads from solution. Aspirate and discard the supernatant.

14. Remove the plate from magnet, add 300 µL SPM Wash Buffer. Resuspend the beads by pipetting up and down 10 times.

    Note: The SPM Wash Buffer must be diluted with ethanol before use. See "Before Starting" section on Page 4 for details.

15. Place the plate onto a magnetic separation device to collect the beads. Aspirate and discard the supernatant.

16. Remove the plate from magnet, add another 300 µL SPM Wash Buffer. Resuspend the beads by pipetting up and down 10 times.

17. Place the plate onto a magnetic separation device to collect the beads. Aspirate and discard the supernatant.

18. Leave the plate on the magnet and wait 5-7 minutes to air dry the beads. Remove any liquid drops from the plate with pipette.

19. Add 50-100 µL Endotoxin-free Water or TE Buffer and resuspend the beads by pipetting up and down for 20 times.

20. Place the plate onto a magnetic separation device to collect the beads.

21. Transfer the cleared supernatant that contains purified plasmid into a new 96-well microplate. Seal the plate and store at -20°C.

Troubleshooting Guide

Please use this guide to solve any problems that may arise. We hope that it will aid in clearing up any questions for you. If for any reason you need further assistance, please contact our technical support staff at our **Toll Free Number, 1-800-832-8896.**

Possible Problems and Suggestions

Problem	Cause	Solution
Low DNA yield	Poor cell lysis	Only use LB or YT medium containing ampicillin. Do not use more than 1.2 mL 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.
	Bacterial clone is not fresh.	Use fresh glycerol cultures and avoid repeated freeze/thaw cycles of clones. Always make enough replica plates and use precultures for inoculation. The remainder of the precultures can be used to set up fresh glycerol stocks.
Problem	Cause	Solution
No DNA eluted.	Lysate prepared incorrectly.	Check the stock of buffers and age of the buffers. Make sure the correct volume of buffer is added to the samples.
	Cells are not resuspend-ed completely.	Pelleted cells should be completely resuspended with Solution I. Do not add Solution II until an even cell suspension is obtained.

Troubleshooting Guide

Problem	Cause	Solution
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. Reduce the culture volume if lysate is too viscous for gentle mixing.
	Culture overgrown	Overgrown culture contains lysed cells and degraded DNA. Do not grow cells for longer than 16 hours
Problem	Cause	Solution
DNA degraded after the storage	High level of endonuclease activity	Heat the cell lysate at 6°C for 5 minutes after step 6.
Problem	Cause	Solution
RNA visible on agarose gel.	RNase A not added to Solution I	Add 1 vial of RNase to each bottle of Solution I
	Cause	Solution
Endotoxin level is too high	ETR Beads is not freshly prepared	Prepare ETR Binding Beads freshly by following the instruction on page 4.
	ETR Beads is transferred with cell lysate	Avoid the ETR beads during transfer after ETR removing step (step 11).

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