Mag-Bind® Plasmid DNA 96 Kit

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Manual Revision: September 2013

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® Particles 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 DNA 96 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 a 96-well format, up to 96 samples can be simultaneously processed in less than 60 minutes. The new E-Z 96® Lysate Clearance Plate eliminates time-consuming centrifugation for clearing bacterial alkaline lysates. It also has an average DNA recovery rate 10-30% higher than the manual centrifuge method. Yields vary according to plasmid copy number, *E. coli* strain, and conditions of growth. A 1 mL overnight culture in LB medium typically yields 10-15 µg high-copy plasmid DNA. The purified plasmid DNA can be used directly for automated fluorescent DNA sequencing, as well as for other standard molecular biology techniques including restriction enzyme digestion.

New in this Edition: PFC Binding Buffer replaces isopropanol for binding group of DNA to the Mag-Bind® Particles.

Kit Contents

Product Number	M1256-00	M1256-01	M1256-02
Purifications	1 x 96 preps	4 x 96 preps	24 x 96 preps
Mag-Bind® Particles CNR	2.2 mL	8.8 mL	50 mL
96-well Microplate (500 µL)	1	4	24
E-Z 96 Lysate Clearance Plate	1	4	24
Solution I	30 mL	110 mL	3 x 200 mL
Solution II	30 mL	110 mL	3 x 200 mL
Neutralization Buffer	30 mL	110 mL	3 x 200 mL
Elution Buffer	15 mL	40 mL	250 mL
PFC Binding Buffer	30 mL	110 mL	3 x 200 mL
EWR Buffer	30 mL	110 mL	3 x 200 mL
PFW Buffer	30 mL	110 mL	3 x 200 mL
SPM Wash Buffer	15 mL	50 mL	6 x 50 mL
RNase A	100 µL	400µL	3 x 0.8 mL
User Manual	✓	✓	✓

Storage and Stability

All Mag-Bind® Plasmid DNA 96 Kit components are guaranteed for at least 24 months from the date of purchase when stored as follows. Store the Mag-Bind® Particles CNR at 2-8°C. Store Solution I/RNase A at 2-8°C after being combined (see Page 4). All remaining components should be stored at room temperature.

Preparing Reagents

1. Dilute SPM Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M1256-00	60 mL
M1256-01	200 mL
M1256-02	200 mL per bottle

2. Add the vial of RNase A to the bottle of Solution I. Store at 2-8°C.

Guidelines for Vacuum Manifold

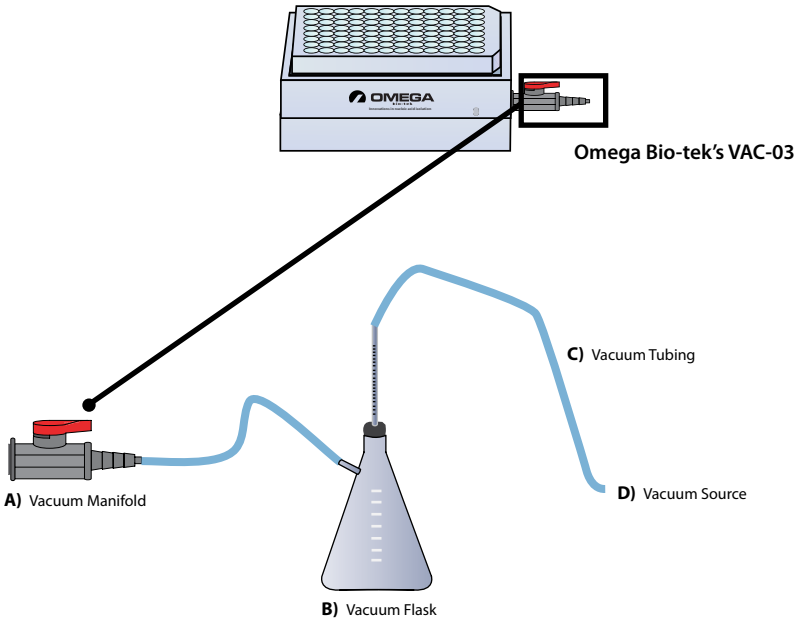
The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-03)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

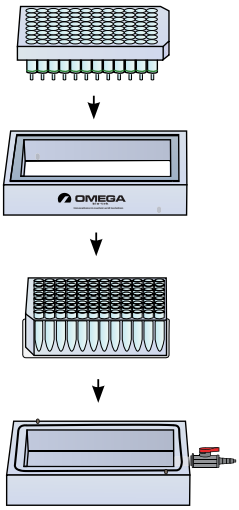
Manifold	Recommended Pressure (mbar)
VAC-03	-200 to -600

Conversion from millibars:	Multiply by:
millimeters of mercury (mmHg)	0.75
kilopascals (kPa)	0.1
inches of mercury (inHg)	0.0295
Torrs (Torr)	0.75
atmospheres (atm)	0.000987
pounds per square inch (psi)	0.0145

Illustrated Vacuum Setup:



Lysate Clearance Setup with 96-well Round-well Plate (1.2 mL)



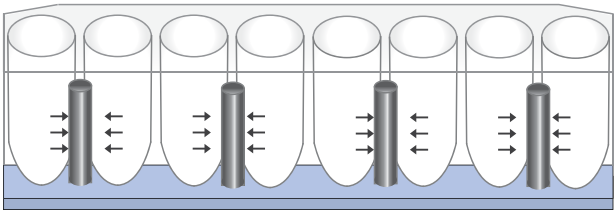
E-Z 96 Lysate Clearance Plate

Vacuum Manifold Top

96-well Round-well Plate (1.2 mL)

Vacuum Manifold Bottom

Radial Magnet (MSD-01B)



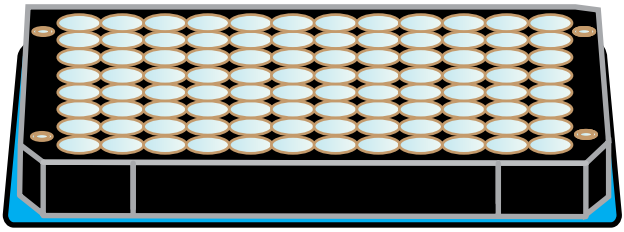
Radial magnets can be used with 1.2 mL or 2 mL 96-well deep-well plates where the posts of the magnets can fit between the wells. The magnetic beads will form a line along the inside of the wells, making it ideal for washing by plate shakers, or for larger volume extractions.

Note: Check the volumes used in the protocol and the speed of the plate shaker to ensure cross-contamination will not occur.

Alp Aqua 96R Magnetic Stand (A001219)

Alp Aqua's 96R Magnetic Stand is ideal for customers looking to automate their DNA extractions. An integrated spring allows for easy and complete supernatant removal without creating air suction which can lead to cross-contamination or sample loss. The spring can accommodate up to 1.25 mm of flexibility on the Z axis. This magnetic stand contains 96 individual N48 NdFeB rings for fast magnetization response times. The stand works with PCR plates, microtiter plates, and round-bottom deep-well plates.

The SBS footprint is designed to work with multiple automated liquid handlers and gripper grooves allow for easy placement on and off the magnetic stand. Compatible with Corning Costar 3795, Abgene AB1127 (1.2 mL square well), Abgene AB-0661 (2.2 mL square well, and Nunc 260251 (1.0 mL round well).



Mag-Bind® Plasmid DNA 96 Kit Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge with swinging-bucket rotor at room temperature capable of 4,000 x *g*
- Adapter for 96-well deep-well plates
- Magnetic separation device
- Refrigerator capable of 2-8°C
- 100% ethanol
- Multi-channel pipettor and tips
- Sealing film (Cat# AC1200-01)
- Multi-channel reservoirs (Cat# AC1331-01)
- 96-well microplates (Cat# EZ9603-01/-02)
- 1-2.2 mL 96-well round-well plates compatible with magnetic stand
- Optional: Vacuum pump or vacuum aspirator capable of achieving a vacuum of 20-24 inHg (for vacuum protocol for clearing the cell lysate)
- Optional: Standard vacuum manifold (Cat# VAC-03; for vacuum protocol for clearing the cell lysate)
- Optional: Incubator capable of 60°C

Before Starting:

- Prepare SPM Wash Buffer and Solution I/RNase A according to instructions on Page 4.
- Chill Neutralization Buffer to 4°C.
- Vortex the Mag-Bind® Particles CNR thoroughly before use.
- Optional: Heat the Elution Buffer to 60°C.

Note: If you choose to vortex the samples in during the protocol below, make sure to seal the plate completely to avoid any loss of sample or cross-contamination.

1. Grow 1-1.5 mL *E. coli* LB cultures in a 2 mL 96-well culture plate at 37°C with agitation with for 16-20 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. Seal the plate with sealing film.

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

4. Remove the sealing film. Discard supernatant.

5. Dry the plate by placing upside-down on a paper towel to remove excess media.

6. Add 250 µL Solution I. Vortex or pipet up and down to completely resuspend the cell pellet.

   Note: RNase A must be added to Solution I prior to use. Please see Page 4 for instructions.

7. Add 250 µL Solution II. 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.

   Note: Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity. Store Solution II tightly capped when not in use.

8. Add 250 µL chilled (4°C) Neutralization Buffer. Mix by gently shaking and rotating the plate for 1 minute until a flocculent white precipitate forms.

9. Choose one of the following methods for lysate clearance:

   A. Clear the cell lysates with centrifugation:
      1. Place the E-Z 96 Lysate Clearance Plate on top of the 96-well Microplate a 1.0-2.2 mL 96-well round-well plate (not provided).
      2. Transfer the lysate from Step 8 to the E-Z 96 Lysate Clearance Plate.
      3. Let sit for 1 minute. The white precipitate should float to the top.
      4. Centrifuge at 2,000 x *g* for 5 minutes.

   B. Clear the cell lysates with vacuum manifold:
      1. Place the 1.2 mL 96-well round-well plate (not provided) into the base of the vacuum manifold. See Page 6 for setup guidelines.
      2. Place the E-Z 96 Lysate Clearance Plate on top of the manifold.
      3. Switch on vacuum source to draw the lysate through the membrane.

10. Add 20 µL Mag-Bind® Particles CNR and 235 µL PFC Binding Buffer to the cleared lysate. Vortex or pipet up and down 20 times to mix thoroughly.

    Important: The Mag-Bind® Particles CNR will settle and clump together at the bottom of the bottle during storage. Vortex the Mag-Bind® Particles CNR thoroughly before use.

11. Let sit for 12 minutes at room temperature. Vortex or pipet up and down 20 times to mix thoroughly.

    Note: For low copy number plasmid isolation a 25 minute or overnight incubation at room temperature may increase yields.

12. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

13. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

14. Remove the plate from the magnetic separation device.

15. Add 250 µL PFW Buffer to each sample.

16. Vortex or pipet up and down 20 times to resuspend the Mag-Bind® Particles CNR.

17. Let sit for 1 minute at room temperature.

18. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

19. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

20. Add 250 µL EWR Wash Buffer to each sample.

21. Vortex or pipet up and down 20 times to resuspend the Mag-Bind® Particles CNR.

22. Let sit for 2 minutes at room temperature.

23. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

24. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

25. Add 250 µL SPM Wash Buffer to each sample.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

26. Vortex or pipet up and down 20 times to resuspend the Mag-Bind® Particles CNR.

27. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

28. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

29. Leave the plate on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

30. Remove the plate from the magnetic separation device.

31. Add 50-100 µL Elution Buffer.

32. Vortex or pipet up and down 20 times to resuspend the Mag-Bind® Particles CNR.

33. Let sit 2 minutes at room temperature.

    Note: Incubation at 60°C rather than at room temperature may give a modest increase in DNA yield.

34. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

35. Transfer the cleared supernatant containing purified DNA to a clean 96-well microplate (not provided). Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896.**

Possible Problems and Suggestions

Problem	Cause	Solution
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.
	Low copy number plasmid used	Such plasmids may yield as little as 0.1 µg DNA from a 1 mL overnight culture.
	Insufficient EWR Buffer wash time	EWR Buffer must be used for a minimum of 2 minutes.
	Insufficient bind time	Mag-Bind® Particles CNR, lysate, and PFC Binding Buffer must be incubated for a minimum of 10 minutes.
Problem	**Cause**	**Solution**
No DNA eluted	SPM Wash Buffer is not diluted with ethanol	Prepare SPM Wash Buffer as instructed on the label.
High-molecular weight DNA contamination	Over mixing of cell lysate upon addition of Solution II	Do not vortex or aggressively mix after adding Solution II. 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 A260	Make sure to wash Mag-Bind® pellet as instructed. Alternatively, rely on agarose gel/ethidium bromide electrophoresis or dye based method for quantification.
RNA visible on agarose gel	RNase A not added to Solution I	Add 1 vial of RNase to each bottle of Solution I.
DNA floats out of well while loading agarose gel	Ethanol not completely removed before elution	Increase air dry time before elution step.
Problems in downstream applications	Traces of ethanol remain Mag-Bind® Particles CNR prior to elution	The Mag-Bind® Particles CNR must be dried before elution. Ethanol precipitation may be required.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Alp Aqua Magnetic Stand 96R	A001322
E-Z 96 Magnetic Separation Device, Radial Magnetizing	MSD-01B
E-Z 96 Vacuum Manifold	VAC-03
SealPlate Film, 100/box	AC1200-01
96-well Microplate (500 µL), 5/pk	EZ9604-01
96-well Microplate (500 µL), 25/pk	EZ9604-02
Solution I, 250 mL	PS001
Solution II, 250 mL	PS002
Neutralization Buffer, 250 mL	PS004
SPM Wash Buffer, 40 mL	PS014
Elution Buffer, 100 mL	PDR048
RNase A, 400 µL	AC117
RNase A, 5 mL	AC118

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