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## Introduction

The E.Z.N.A.<sup>®</sup> Magbead<sup>®</sup> 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 Magbead<sup>®</sup> 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 E.Z.N.A.<sup>®</sup> Magbead<sup>®</sup> Plasmid Purification Kit combines the power of MagBead<sup>®</sup> 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<sup>®</sup> 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. 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 E.Z.N.A.<sup>®</sup> Magbead<sup>®</sup> 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 Magbead<sup>®</sup> particle solution should be stored at 4°C; all other material at 22-25°C.

## E.Z.N.A<sup>®</sup> Magbead<sup>®</sup> Plasmid Purification Kit

Product Number	D1256-01	D1256-02
Purifications	4 x 96	24 x 96
MagBead <sup>®</sup> Particle Solution E	4.2 ml	25 ml
96 well Binding Plate (500µl)	4	24
Lysate Clearance Plate	4	24
MGC Binding Buffer	20	2 x 50 ml
Solution I	40 ml	2 x 125 ml
Solution II	40 ml	2 x 125 ml
Neutralization Buffer	40 ml	2 x 125 ml
SPM Wash Buffer	60 ml	3 x 150 ml
RNase A Concentrate	400 µl	2 x 1.2 ml
Instruction Booklet	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.

<b>IMPORTANT</b>	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:	
	D1256-01	Add 140 ml ~96%-100% ethanol
	D1256-02	Add 350 ml ~96%-100% ethanol per bottle
	3. MGC Binding Buffer has to be diluted with absolute ethanol as follows:	
	D1256-01	Add 80 ml ~96%-100% ethanol
	D1256-02	Add 200 ml ~96%-100% ethanol
	<b>Store diluted MGW Wash Buffer &amp; MGC Binding Buffer at room temperature</b>	

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

### E.Z.N.A<sup>®</sup> MagBead<sup>®</sup> Plasmid Isolation Protocol

#### 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# 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<sup>®</sup> and JM109<sup>®</sup>.**

2. Seal the plate with tape or film 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 70 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 70 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 70 µl 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 with Centrifugation:** plate the Lysate Clearance Plate (supplied) on top of the 500µl Binding plate. Transfer the lysate from step 5 into the Lysate Clearance Plate. Allow the cell lysate to stand for 5 minutes. The white precipitate should float to the top. Centrifuge at 2000 x g for 5 minutes.

**Clear the cell lysates with vacuum manifold:** Assemble the vacuum manifold by placing the 500µl Binding plate (supplied) or a 2 ml collection plate (not supplied) into the base of the vacuum manifold and then place the E-Z 96<sup>®</sup> Lysate Clearance plate atop the manifold. (Some manifolds might require internal height adjustment by using small plate.) Apply the vacuum until all the lysate passes through the membrane.

7. Add 10µl/well of MagBeads<sup>®</sup> Particles Solution E and follow by addition of 180µl/well of MGC Binding Buffer. Mix well with pipetting few times.

**NOTE: The MagBeads<sup>®</sup> 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 redispersed in solution. (IMPORTANT)**

**Tip:** MGC Binding Buffer and MagBeads<sup>®</sup> Particles Solution E can be combined in appropriate proportions to make a master mix before starting the procedure. Add 190 µl per well of MagBeads<sup>®</sup> Particle Solution E/MGC Binding Buffer master mix to each well of the 96-well microplate.

8. Incubate for 5 minutes at room temperature, mixing once by pipetting or briefly vortexing. Use fresh tips to avoid cross-contamination.
9. 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.)
10. Remove the plate from the Magnetic Separation stand, then wash the pelleted MagBeads<sup>®</sup> particles by adding 250µl 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 MagBeads<sup>®</sup> particles have completely migrated to the walls of the plate.

**NOTE: For better washing efficiency, MagBeads<sup>®</sup> particles should be fully resuspended. Resuspension can be performed by pipetting or by vortexing.**

11. Remove the plate from magnetic separation stand and wash the MagBeads<sup>®</sup> particles by adding 250 µl SPM Wash Buffer to each well. Resuspend the MagBeads<sup>®</sup> particles by pipetting. Place the plate on the magnetic separation stand to pellet the MagBeads<sup>®</sup> particles. Aspirate the supernatant.
12. Optional: Remove the plate from magnetic separation stand and wash the MagBeads<sup>®</sup> particles by adding 200 µl absolute ethanol to each well. Resuspend the MagBeads<sup>®</sup> particles by pipetting. Place the plate on the magnetic separation stand to pellet the MagBeads<sup>®</sup> particles. Aspirate the supernatant
13. Air dry the MagBeads<sup>®</sup> particles pellet for 5-10 minutes at room temperature.
14. Elute DNA: Resuspend the MagBeads<sup>®</sup> particles pellet with 50-100µl water or TE buffer.
15. Place the plate onto the magnetic separation stand to pellet the MagBeads<sup>®</sup> 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 0.1µg DNA from a 1 ml overnight culture.
	Lost Magbead 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 magbead 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<sup>®</sup> 96 Magbead<sup>®</sup> 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.