omega BIO-TEK
innovations in nucleic acid isolation

# Product Manual

## Mag-Bind® Ultra-Pure Plasmid DNA 96 Kit

| | |
|---|---|
| M1258-00 | 1 x 96 preps |
| M1258-01 | 4 x 96 preps |

**Manual Date: March 2019**
**Revision Number: v4.0**

**For Research Use Only**


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# Mag-Bind® Ultra-Pure Plasmid DNA 96 Kit
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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® Ultra-Pure Plasmid DNA 96 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. A 1 mL overnight culture in LB medium typically produces 10-15 µg for high copy plasmids. 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:**

March 2019

- SPM Wash Buffer has been renamed SPM Buffer. This is a name change only. The fomulation has not changed.

December 2015

- A newly developed buffer system is used to lower endotoxin values, improve yields and improve A260/A230 readings.

# Kit Contents and Storage

| Product No. | M1258-00 | M1258-01 |
|---|---|---|
| Purification | 1 x 96 | 4 x 96 |
| Mag-Bind® Particles RQ | 2.2 mL | 8.8 mL |
| Solution I | 30 mL | 120 mL |
| Solution II | 30 mL | 120 mL |
| N3 Buffer | 15 mL | 60 mL |
| ETR Binding Buffer | 60 mL | 250 mL |
| ETR Wash Buffer | 60 mL | 250 mL |
| VHB Buffer | 66 mL | 264 mL |
| SPM Buffer | 15 mL | 60 mL |
| Elution Buffer | 15 mL | 60 mL |
| RNase A | 50 µL | 200 µL |
| User Manual | ✓ | ✓ |

## Storage and Stability

All of the Mag-Bind® Ultra-Pure Plasmid DNA 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles RQ, RNase A, and Solution I/RNase A mixture must be stored at 2-8°C. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

# Preparing Reagents

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

| Kit | 100% Ethanol to be Added |
|---|---|
| M1258-00 | 84 mL |
| M1258-01 | 336 mL |

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

| Kit | 100% Ethanol to be Added |
|---|---|
| M1258-00 | 60 mL |
| M1258-01 | 240 mL |

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

## Mag-Bind® Ultra-Pure Plasmid 96 Kit with Lysate Clearance via Centrifugation

**Materials and Equipment to be provided by user:**

- Centrifuge with swing-bucket rotor capable of 3,000 x *g*
- Rotor for 96-well deep-well plates
- Magnetic separation device (Recommend Alp Aqua Part# A000380)
- 2.0 mL 96-well deep-well plate for magnetic stand (Recommend Nunc Part No. 278752)
- 2.0 mL 96-deepwell Plate for bacterial growth
- 100% ethanol
- Multi-channel pipet
- Optional: Plate shaker (Recommend Eppendorf MixMate)

**Before Starting:**

- Prepare SPM Buffer, VHB Buffer, and Solution I according to the "Preparing Reagents" section on Page 4.

1. Culture Volume: Inoculate 1-1.5 mL LB/antibiotic(s) medium with *E. coli* in a 96-well deep-well plate and incubate at 37°C with agitation 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 250 µL Solution I/RNase A to the bacterial pellet in each sample. Resuspend the cells completely by vortexing. Complete resuspension of the cell pellet is vital for obtaining good plasmid yields.

   **Note:** RNase A must be added to Solution I before use. Please see the instructions in the Preparing Reagents section on Page 4.

5. Add 250 µL Solution II. Gently mix by 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 125 µL N3 Buffer. Mix by gently shaking 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 500 µL cleared cell lysate into a new 96-well deep-well plate (2.2 mL) not provided. Avoid transferring the white precipitate containing cell debris.

9. Add 500 µL ETR Binding Buffer and 20 µL Mag-Bind® Particles RQ. Mix thoroughly by pipetting up and down 10 times.

10. Let sit for 5 minutes at room temperature.

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

    **Note:** Magnetization time depends on the magnet used and plasticware but should take ~3-5 minutes. If Mag-Bind® Particles RQ are floating at top of surface after 5 minutes, pipet from the top of the liquid and slowly dispense to the bottom to move the Mag-Bind Particles RQ closer to the magnet.

12. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

13. Remove the plate from the magnetic separation device.

14. Add 500 µL ETR Wash Buffer. Resuspend the beads by pipetting up and down 10 times or shaking for 1 minute at 1,300 RPM.

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

16. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

17. Remove the plate from magnetic separation device.

18. Add 700 µL VHB Buffer. Resuspend the beads by pipetting up and down 10 times or shaking for 1 minute at 1,000 RPM.

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

20. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ

21. Remove the plate from magnetic separation device.

22. Repeat Steps 18-21 once for a second VHB Buffer wash step.

23. Add 700 µL SPM Buffer. Resuspend the beads by pipetting up and down 10 times or shaking for 1 minute at 1,000 RPM.

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

25. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ. **Leave the plate on the magnetic separation device.**

26. Let sit at room temperature for 10 minutes to dry the Mag-Bind® Particles RQ.

    **Note:** It is recommended to let sit for 1 minute then remove any remaining liquid from the wells then let sit for an additional 9 minutes.

27. Add 50-100 µL Elution Buffer. Resuspend the beads by pipetting up and down for 20 times or by shaking at 1,500 RPM for 2-5 minutes.

28. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles RQ.

29. Transfer the cleared supernatant containing the purified plasmid into a new 96-well microplate (not provided). Seal the plate and store at -20°C.

## Mag-Bind® Ultra-Pure Plasmid 96 Kit with Lysate Clearance via Magnetic Beads

**Materials to be provided by user:**

- Centrifuge with swing-bucket rotor capable of 3,000 x *g*
- Rotor for 96-well deep-well plates
- Magnetic separation device (Recommend Alp Aqua Part# A000380)
- 2.0 mL 96-well deep-well plate for magnetic stand (Recommend Nunc Part No. 278752)
- 2.0 mL 96-well deep-well plate for bacterial growth
- 100% ethanol
- Multi-channel pipet
- LC Beads
- Optional: Plate shaker (Recommend Eppendorf MixMate)

**Before Starting:**

- Prepare SPM Buffer, VHB Buffer, and Solution I according to the "Preparing Reagents" section on Page 4.

1. Culture Volume: Inoculate 1-1.5 mL LB/antibiotic(s) medium with *E. coli* in a 96-well deep-well plate and incubate at 37°C with agitation 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 250 µL Solution I/RNase A to the bacterial pellet in each sample. Resuspend the cells completely by vortexing. Complete resuspension of the cell pellet is vital for obtaining good plasmid yields.

   **Note:** RNase A must be added to Solution I before use. Please see the instructions in the Preparing Reagents section on Page 4.

5. Add 250 µL Solution II. Gently mix by 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 125 µL N3 Buffer and 30 µL LC Beads. Mix by gently shaking the plate until a flocculent white precipitate forms.

   **Note:** N3 Buffer and LC Beads can be made as a mastermixx

7. Place the plate on the magnetic separation device to magnetize the cell debris. Let sit at room temperature until the LC Beads are completely cleared from solution.

   **Note:** If LC Beads are floating at top of surface after 5 minutes, pipet from the top of the liquid and slowly dispense to the bottom to move the LC Beads closer to the magnet.

8. Transfer 500 µL cleared cell lysate into a new 96-well deep-well plate (2.2 mL) not provided. Avoid transferring the white precipitate containing cell debris.

9. Add 500 µL ETR Binding Buffer and 20 µL Mag-Bind® Particles RQ. Mix thoroughly by pipetting up and down 10 times.

10. Let sit for 5 minutes at room temperature.

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

    **Note:** Magnetization time depends on the magnet used and plasticware but should take ~3-5 minutes. If Mag-Bind® Particles RQ are floating at top of surface after 5 minutes, pipet from the top of the liquid and slowly dispense to the bottom to move the Mag-Bind Particles RQ closer to the magnet.

12. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

13. Remove the plate from the magnetic separation device.

14. Add 500 µL ETR Wash Buffer. Resuspend the beads by pipetting up and down 10 times or shaking for 1 minute at 1,300 RPM.

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

16. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

17. Remove the plate from magnetic separation device.

18. Add 700 µL VHB Buffer. Resuspend the beads by pipetting up and down 10 times or shaking for 1 minute at 1,000 RPM.

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

20. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ

21. Remove the plate from magnetic separation device.

22. Repeat Steps 18-21 once for a second VHB Buffer wash step.

23. Add 700 µL SPM Buffer. Resuspend the beads by pipetting up and down 10 times or shaking for 1 minute at 1,000 RPM.

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

25. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ. **Leave the plate on the magnetic separation device.**

26. Let sit at room temperature for 10 minutes to dry the Mag-Bind® Particles RQ.

    **Note:** It is recommended to let sit for 1 minute then remove any remaining liquid from the wells then let sit for an additional 9 minutes.

27. Add 50-100 µL Elution Buffer. Resuspend the beads by pipetting up and down for 20 times or by shaking at 1,500 RPM for 2-5 minutes.

28. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles RQ.

29. Transfer the cleared supernatant containing the purified plasmid into a new 96-well microplate (not provided). 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. Do not use more than 1.2 mL.<br><br>Cells may not be dispersed adequately prior to addition of Solution II. Vortex cell suspension(after solution I addition) to completely disperse.<br><br>Increase incubation time with Solution II to obtain a clear lysate.<br><br>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 resuspended 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 or N3 Buffer | Do not vortex or mix aggressively after adding Solution II or N3 Buffer. 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 | Use TE Buffer instead of Elution Buffer. |
| Problem | Cause | Solution |
| RNA visible on agarose gel | RNase A not added to Solution I | Add 1 vial of RNase A to Solution I. |

Notes:

Notes:

For more purification solutions, visit **www.omegabiotek.com**

## AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

## SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

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