omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

Mag-Bind® Endo-Free Plasmid Mini Kit

M1261-00	1 x 96 preps
M1261-01	4 x 96 preps

Manual Date: November 2024
Revision Number: v1.0

For Research Use Only

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Mag-Bind® Endo-Free Plasmid Mini Kit

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Intended Use

For professional research use.

The Mag-Bind® Endo-Free Plasmid Mini Kit is intended for isolation and purification of endotoxin-free (<0.1 EU/µg) plasmid DNA from up to 1.5 mL overnight culture of *E. coli* in LB or TB media. Please ensure that the cell density does not exceed an OD_{600} of 3.0.

The Mag-Bind® Endo-Free Plasmid Mini Kit utilizes magnetic bead-based technology and can be processed either manually or automated on most open-ended liquid handling platforms as well as magnetic processors.

Intended User

The Mag-Bind® Endo-Free Plasmid Mini Kit is intended for professional use and to be used by or under the supervision of professional users, such as laboratory personnel, technicians, researchers, and physicians specifically instructed and trained in molecular biology techniques and familiar with magnetic bead-based purification, either manual or automated.

Product Description

The Mag-Bind® Endo-free Plasmid Mini Kit is designed to deliver high-quality, plasmid DNA with endotoxin levels <0.1 EU/µg for use in eukaryotic transfections and other sensitive *in vitro* applications. The kit follows magnetic bead-based technology to purify plasmid DNA from up to 1.5 mL overnight bacterial culture in LB or TB or other suitable growth medium. The Mag-Bind® Endo-free Plasmid Mini Kit can be processed either manually or automated on most open-ended liquid handling platforms as well as magnetic processors. Purified plasmid DNA is endotoxin free (<0.1 EU/µg), and yields may vary depending on the copy number of the plasmid, *E. coli* strain used, culture media, and conditions of growth.

The Mag-Bind® Endo-free Plasmid Mini Kit follows a modified alkaline lysis method forming a bacterial lysate. The lysate is then cleared using either Mag-Bind® Particles LC (sold separately) or centrifugation. The novel paramagnetic lysate clearance beads bind to cell debris and pulls them down when magnetized. This eliminates the need for centrifugation or vacuum manifold for lysate clearance, making this suitable for high-throughput, automation workflows. Post lysate clearance, plasmid DNA is then bound to Mag-Bind® Particles RQ through a specially formulated binding buffer and the Mag-Bind® particles are subjected to a wash routine to remove endotoxins, salt, and other contaminants. High-quality plasmid DNA is then eluted in Endo-free Water and is ready for use in a wide range of downstream applications such as transfection, routine screening, restriction enzyme digestion, transformation, PCR, and DNA sequencing etc.

Important:

1. If automating this procedure on a liquid handler or a magnetic processor, please contact your Omega Bio-tek representative for instrument-specific instructions. It is the responsibility of the user to validate any automated method for any particular use.

2. This kit includes enough reagents for the specified number of preparations plus at least an additional 10% overage to ensure there is sufficient volume. Please be aware that the actual number of preparations may be lower due to pre-aliquoting of reagents, processing partial plates, and automation platform used etc. Additional reagents are available for purchase separately. Please visit the product page at www.omegabiotek.com for more details and ordering information.

Kit Contents and Storage

Product No.	M1261-00	M1261-01
Purifications	1 x 96	4 x 96
Solution I	30 mL	120 mL
Solution II	30 mL	120 mL
N3 Buffer	40 mL	85 mL
IRD Buffer	50 mL	200 mL
VHB Buffer	66 mL	2 x 176 mL
Endo-free Water	15 mL	60 mL
Mag-Bind® Particles RQ	2.2 mL	8.8 mL
RNase A	100 µL	400 µL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® Endo-Free Plasmid Mini 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 and away from bright light. 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 Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M1261-00	84 mL
M1261-01	224 mL each bottle

2. Prepare enough stock of 70% ethanol needed and store at room temperature.

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

4. Dilute IRD Buffer with 100% isopropanol according to the instruction in the "Before Starting" section of each protocol.

5. Shake or vortex the Mag-Bind® Particles RQ and LC to fully resuspend the particles before use. The particles must be fully resuspended during use to ensure proper binding.

Warnings

This kit is for professional research use.

Please read all instructions carefully before using the kit.

Please decontaminate and dispose of all potentially infectious materials in accordance with applicable local, state/provincial, and/or national regulations. For any assistance, please contact Omega Bio-tek at info@omegabiotek.com.

If you use this kit following an automated extraction workflow, the surface of the automated platform is considered a biohazard. Use appropriate decontamination and disposal methods in adherence to all applicable local state/provincial, and/or national regulations.

Safety Information

All chemicals and biological materials are potentially hazardous.

Biological samples such as plasma, serum, tissues, body fluids, blood etc. are potentially infectious and must be treated as biohazardous materials. Conduct all work in properly equipped facilities following universal precautions and using appropriate personal safety equipment such as disposable gloves, lab coats, safety glasses etc. as required by policies and procedures outlined by your facility.

Please refer to safety data sheets (SDSs) for information on safe handling, transport and disposal of different reagents included in this kit. SDSs are made available in PDF format on the product page at www.omegabiotek.com. Discard all waste in accordance with the local safety regulations.

Precautions

Some of the buffers included in the Mag-Bind® Endo-Free Plasmid Mini Kit contain guanidine-based chaotropic agents, which can form highly reactive compounds when combined with bleach. **DO NOT add bleach or acidic solutions to guanidine containing sample-preparation waste.** Please access the SDSs online for detailed information on the reagents.

Component	Description
Solution II 	Contains: Sodium hydroxide. Warning! Causes serious eye irritation. Wear protective gloves, protective clothing, eye protection and face protection. Wash all exposed external body areas thoroughly after handling. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If irritation persists: Get medical advice/attention.
N3 Buffer 	Contains: Acetic acid glacial. Danger! Causes severe skin burns and eye damage. Do not breathe mist/vapors/spray. Wash all exposed external body areas thoroughly after handling. Wear protective gloves, protective clothing, eye protection and face protection. INHALED: Remove person to fresh air and keep comfortable for breathing. SWALLOWED: Rinse mouth. DO NOT induce vomiting. ON SKIN (or hair): Take off immediately all contaminated clothing. Wash all contaminated clothing before reuse. Rinse skin with water/shower. IN EYES: Rinse cautiously with water for several minutes. Remove ocntact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor/physician/first aider.
IRD Buffer   	Contains: Guanidine hydrochloride. Danger! Causes serious eye damage. Causes skin irritation. Harmful if swallowed. Toxic to aquatic life with long lasting effects. Wear protective gloves, protective clothing, eye protection and face protection. Do no eat, drink, or smoke when using this product. Wash all exposed external body areas thoroughly after handling. Avoid release to the environment. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. SWALLOWED: Rinse mouth. Call a POISON CENTER/doctor/physician/first aider/if you feel unwell. ON SKIN: Wash with plenty of water and soap. If skin irritation occurs: Get medical advice/attention. Take off contaminated clothing and wash it before reuse. Collect spillage.

Precautions

Component	Description
VHB Buffer 	Contains: Guanidine hydrochloride. Warning! Causes serious eye irritation. Causes skin irritation. May cause an allergic skin reaction. Harmful if swallowed. Avoid breathing mist/vapors/ spray. Do not eat, drink or smoke when using this product. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/protective clothing/ eye protection/face protection. If exposed or concerned: call a poison center or doctor/physician. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention if eye irritation persists. Take off contaminated clothing and wash before reuse. ON SKIN: Wash with plenty of water and soap. Get medical advice/attention if skin irritation or rash occurs. SWALLOWED: Rinse mouth. Call a poison center or doctor/physician if you feel unwell.
RNase A 	Contains: Ribonuclease A. Danger! May cause allergy or asthma symptoms or breathing difficulties when inhaled. May cause an allergic skin reaction. Avoid breathing mist/vapors/ spray. Wear protective gloves and protective clothing. [In case of inadequate ventilation] wear respiratory protection. Contaminated work clothing must not be allowed out of the workplace. INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor/ physician/first aider if experience respiratory symptoms. ON SKIN: Wash with plenty of water and soap. Get medical advice/attention if skin irritation or rash occurs. Take off contaminated clothing and wash it before reuse.

Protocol for Microcentrifuge Tube

Recommend: Use this protocol when performing extractions by hand or processing a few samples.

Materials and Equipment to be provided by user:

- Microcentrifuge capable of at least 13,000*g*
- Vortexer
- Magnetic separation device compatible with 1.5 mL or 2 mL microcentrifuge tubes
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- 100% isopropanol
- 100% ethanol
- 70% ethanol
- *Optional:* Mag-Bind® Particles LC (sold separately if not using lysate clearance via centrifugation, Part #MBPLC-05)

Before Starting:

- Prepare Solution I/RNase A and VHB Buffer according to the "Preparing Reagents" section on Page 5.
- Freshly prepare IRD Buffer by adding 100% isopropanol for as much as needed. Do not reconstitute the entire IRD Buffer and only prepare as much IRD Buffer as needed for the number of purifications to be performed.
- Follow the table below for IRD Buffer preparation for the bind step and first wash step required for one purification. Adjust volume accordingly based on the number of purifications to be performed.

Component	Total Amount per Prep
IRD Buffer	450 µL
100% isopropanol	550 µL

Recommended steps to achieve low endotoxin levels:

- Clean work surfaces and pipettes with 70% ethanol before starting.
- Change gloves frequently during the procedure.
- Use endotoxin-free or pyrogen-free plastic pipet tips and plasticwares for endotoxin-sensitive applications.
- Aliquot and store Endo-free Water at 4°C.

1. Culture Volume: Inoculate 1-1.5 mL *E. coli* cultured in LB or suitable growth medium containing the appropriate selective antibiotic in a culture tube or flask with a volume of at least 4 times the volume of the culture and incubate at 37°C with agitation for 12-16 hours. Transfer to a 1.5 mL or 2 mL microcentrifuge tube (not provided) after incubation.

   Note: Please ensure that the cell density does not exceed an OD_{600} of 3.0. 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 13,000*g* for 1 minute at room temperature to collect bacteria.

3. Decant or aspirate and discard the culture media.

4. Add 250 µL Solution I/RNase A to the bacterial pellet. 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 prior to use. Please see the "Preparing Reagents" section on Page 5 for instructions.

5. Add 250 µL Solution II. Invert and rotate the tube gently 8-10 times to obtain a cleared lysate. Incubate for 3 minutes at room temperature.

   Note: Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO_2 in the air.

6. Select one of the following lysate clearance steps:

   A. Lysate Clearance via Centrifugation:

      i. Add 200 µL N3 Buffer. Gently invert 10 times or until a flocculent white precipitate forms.
      ii. Centrifuge at 13,000*g* for 10 minutes at room temperature.
      iii. Transfer 500 µL cleared cell lysate to a new microcentrifuge tube. Avoid transferring the precipitate containing cell debris.
      iv. Continue to Step 7 on Page 11.

OR

B. Lysate Clearance via Magnetic Beads:

Important: Mag-Bind® Particles LC are not provided and will need to be purchased separately. Please visit the product page at www.omegabiotek.com for more details and ordering information.

i. Prepare a mastermix of N3 Buffer and Mag-Bind® Particles LC (not provided, Part #MBPLC-05) according to table below. Only prepare as much N3 Buffer/ Mag-Bind® Particles LC mastermix that will be used within 72 hours.

Component	Amount per Prep	Total Amount per 96-well Plate
N3 Buffer	200 µL	21.12 mL*
Mag-Bind® Particles LC	30 µL	3.17 mL*

*10% excess volume has been calculated for a 96-well plate.

ii. Add 230 µL N3 Buffer/Mag-Bind® Particles LC mastermix. Invert 20 times or until a flocculent precipitate forms.

iii. Place the tube on the magnetic separation device to magnetize the cell debris. Let sit at room temperature until the Mag-Bind® Particles LC are completely cleared from solution.

Note: If Mag-Bind® Particles LC are floating at the top of the surface after 5 minutes, pipet from the top of the liquid and slowly dispense to the bottom of the tube to move the Mag-Bind® Particles LC closer to the magnet.

iv. Transfer 500 µL cleared cell lysate to a new microcentrifuge tube. Avoid transferring the precipitate containing cell debris.

v. Continue to Step 7 on Page 11.

7. Add 500 µL IRD Buffer/Isopropanol master mix and 20 µL Mag-Bind® Particles RQ. Vortex at maximum speed for 5 minutes.

Note: IRD buffer must be freshly diluted with 100% isopropanol prior to use. Do not reconstitute the entire IRD Buffer in the bottle. **Mix well immediately before use.** Please see the "Before Starting" section on Page 9 for instructions.

8. Place the tube 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 the top of the surface after 5 minutes, pipet from the top of the liquid and slowly dispense to the bottom of the tube to move the Mag-Bind Particles RQ closer to the magnet.

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

10. Remove the tube from the magnetic separation device.

11. Add 500 µL IRD Buffer/Isopropanol master mix. Vortex at maximum speed for 1 minute.

    Note: IRD buffer must be freshly diluted with 100% isopropanol prior to use. Do not reconstitute the entire IRD Buffer in the bottle. **Mix well immediately before use.** Please see the "Before Starting" section on Page 9 for instructions.

12. Place the tube 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.

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

14. Remove the tube from the magnetic separation device.

15. Add 700 µL VHB Buffer. Vortex at maximum speed for 1 minute.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see the "Preparing Reagents" section on Page 5 for instructions.

16. Transfer the solution with particles to a new 1.5 mL microcentrifuge tube (not provided). Vortex at maximum speed for 1 minute. Discard used tube.

    Note: Transfer to a new tube is critical for extraction of high-quality plasmid DNA.

17. Place the tube 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.

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

19. Remove the tube from magnetic separation device.

20. Add 700 µL VHB Buffer. Vortex at maximum speed for 1 minute.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see the "Preparing Reagents" section on Page 5 for instructions.

21. Place the tube 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.

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

23. Remove the tube from magnetic separation device.

24. Add 700 µL 70% ethanol (not provided). Vortex at maximum speed for 1 minute.

25. Place the tube 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.

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

27. Let sit for 10 minutes at room temperature 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.

28. Remove the tube from magnetic separation device.

29. Add 50-100 µL Endo-free Water. Vortex at maximum speed for 3 minutes.

30. Place the tube 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.

31. Transfer the cleared supernatant containing the purified plasmid DNA into a new microcentrifuge tube.

32. Store at -20°C.

Protocol for 96-well Deep-well Plate

Important: Follow this protocol for performing upfront alkaline lysis steps in a 96-well deep-plate before moving to a magnetic processor for subsequent steps. It is possible to automate the alkaline lysis steps using Mag-Bind® Particles LC Beads when starting with a bacterial pellet depending on the automation platform used. Please contact your Omega Bio-tek representative for instrument-specific instruction. It is the responsibility of the user to validate any automated method for any particular use.

Materials to be provided by user:

- Centrifuge with swing-bucket rotor capable of 3,000*g*
- Rotor for 96-well deep-well plates
- Orbital shaker with 3 mm orbital diameter
- Magnetic separation device (Recommend Alpaqua Part# A000380)
- 2.2 mL 96-well deep-well plate for magnetic stand (Recommend Nunc Part No. 278752)
- 2.2 mL 96-well deep-well plate for bacterial growth
- 96-well microplate
- 100% isopropanol
- 100% ethanol
- 70% ethanol
- Multichannel pipet
- *Optional:* Mag-Bind® Particles LC (sold separately if not using lysate clearance via centrifugation, Part #MBPLC-05)
- *Optional:* Plate shaker (Recommend Eppendorf MixMate)

Before Starting:

- Prepare Solution I/RNase A, IRD Buffer, and VHB Buffer according to the "Preparing Reagents" section on Page 5.
- Freshly prepare IRD Buffer by adding 100% isopropanol for as much as needed. Do not reconstitute the entire IRD Buffer and only prepare as much IRD Buffer as needed for the number of purifications to be performed.
- Follow the table below for IRD Buffer preparation for the bind step and first wash step required for one purification. Adjust volume accordingly based on the number of purifications to be performed.

Component	Total Amount per Prep
IRD Buffer	450 µL
100% isopropanol	550 µL

Recommended steps to achieve low endotoxin levels:

- Clean work surfaces and pipettes with 70% ethanol before starting.
- Change gloves frequently during the procedure.
- Use endotoxin-free or pyrogen-free plastic pipet tips and plasticwares for endotoxin-sensitive applications.
- Aliquot and store Endo-free Water at 4°C.

1. Culture Volume: Inoculate 1-1.5 mL *E. coli* cultured in LB or suitable growth medium containing the appropriate selective antibiotic in a 2.2 mL 96-well deep-well plate (not provided) and incubate at 37°C with agitation for 12-16 hours.

   Note: Please ensure that the cell density does not exceed an OD_{600} of 3.0. 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*g* for 10 minutes at room temperature 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 shaking on orbital shaker at 400 rpm. Complete resuspension of the cell pellet is vital for obtaining good plasmid yields.

   Note: RNase A must be added to Solution I prior to use. Please see the "Preparing Reagents" section on Page 5 for instructions.

5. Add 250 µL Solution II. Gently mix by shaking and rotating the plate or use an orbital shaker at 400 rpm for 1 minute to obtain a cleared lysate. Remove from orbital shaker and incubate for 3 minutes at room temperature.

   Note: Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO_2 in the air.

6. Select one of the following lysate clearance steps:

   A. Lysate Clearance via Centrifugation:

      i. Add 200 µL N3 Buffer. Gently mix by shaking at 1,250 rpm for 1 minute until a flocculent precipitate forms.
      ii. Centrifuge at 2,000-3,000*g* for 10 minutes at room temperature.
      iii. Transfer 450 µL cleared cell lysate into a 2.2 mL 96-well deep-well plate. Avoid transferring the precipitate containing cell debris.
      iv. Continue to Step 7 on Page 18.

   OR

   B. Lysate Clearance via Magnetic Beads:

   Important: Mag-Bind® Particles LC are not provided and will need to be purchased separately. Please visit the product page at www.omegabiotek.com for more details and ordering information.

      i. Prepare a mastermix of N3 Buffer and Mag-Bind® Particles LC (not provided) according to the table below. Only prepare as much N3 Buffer/LC beads mastermix that will be used within 72 hours.

Component	Amount per Prep	Total Amount per 96-well Plate
N3 Buffer	200 µL	21.12 mL*
LC Beads	30 µL	3.17 mL*

      *10% excess volume has been calculated for a 96-well plate.

      ii. Add 230 µL N3 Buffer/Mag-Bind® Particles LC mastermix. Shake at 1,250 rpm for 1 minute until a flocculent precipitate forms.
      iii. Place the plate on the magnetic separation device to magnetize the cell debris. Let sit at room temperature until the Mag-Bind® Particles LC are completely cleared from solution.

      Note: If Mag-Bind® Particles LC are floating at the top of the surface after 5 minutes, pipet from the top of the liquid and slowly dispense to the bottom of the well to move the Mag-Bind® Particles LC closer to the magnet.

      iv. Transfer 500 µL cleared cell lysate into a 2.2 mL 96-well deep-well plate. Avoid transferring the precipitate containing cell debris.
      v. Continue to Step 7 on Page 18.

7. Add 500 µL IRD Buffer/Isopropanol master mix and 20 µL Mag-Bind® Particles RQ. Shake at 1,500 rpm for 5 minutes at room temperature to resuspend Mag-Bind® Particles RQ.

   Note: IRD Buffer must be freshly diluted with 100% isopropanol prior to use. Do not reconstitute the entire IRD Buffer in the bottle. **Mix well immediately before use.** Please see instructions in the "Before Starting" section on Page 15.

8. 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 the top of the surface after 5 minutes, pipet from the top of the liquid and slowly dispense to the bottom of the well to move the Mag-Bind® Particles RQ closer to the magnet.

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

10. Remove the plate from the magnetic separation device.

11. Add 500 µL IRD Buffer/Isopropanol master mix. Shake at 1,500 rpm for 3 minutes to resuspend Mag-Bind® Particles RQ.

    Note: IRD Buffer must be freshly diluted with 100% isopropanol prior to use. Do not reconstitute the entire IRD Buffer in the bottle. **Mix well immediately before use.** Please see instructions in the "Before Starting" section on Page 15.

12. 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.

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

14. Remove the plate from magnetic separation device.

15. Add 700 µL VHB Buffer. Shake at 1,500 rpm for 3 minutes to resuspend Mag-Bind® Particles RQ.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see the "Preparing Reagents" section on Page 5 for instructions.

16. 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.

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

18. Remove the plate from magnetic separation device.

19. Repeat Steps 15-18 for a second VHB Buffer wash step.

20. Add 700 µL 70% ethanol (not provided). Shake at 1,500 rpm for 3 minutes to resuspend the Mag-Bind® Particles RQ.

21. 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.

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

23. Let sit for 10 minutes at room temperature 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.

24. Remove the plate from magnetic separation device.

25. Add 50-100 µL Endo-free Water. Shake at 1,500 rpm for 5 minutes to resuspend the Mag-Bind® Particles RQ.

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

27. Transfer the cleared supernatant containing the purified plasmid DNA into a new 96-well microplate (not provided).

28. 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 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	Do not exceed an OD_{600} of 3.0. Do not use more than 1.5 mL.
		Cells may not be dispersed adequately prior to addition of Solution II. Vortex cell suspension (after Solution I addition) to completely disperse and resuspend.
		Increase incubation time with Solution II to obtain a clear lysate.
		Solution II, if not tightly closed, may need to be replaced.
	Bacterial colony 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.
	IRD Buffer not made fresh	Only make enough IRD Buffer that will be used within 72 hours. Evaporation of isopropanol can lead to lower DNA yields.
	Incomplete mixing	Use shaker with 3 mm orbital distance and adjust shake speeds if needed to ensure good mixing after Solution 2 and N3 Buffer addition.
Problem	Cause	Solution
Low Purity	Residual IRD Buffer	Transfer to a new tube after VHB Buffer wash step on Step 16 Page 13.

Troubleshooting Guide

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.
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
RNA visible on agarose gel	RNase A not added to Solution I	Add 1 vial of RNase A to Solution I.
Problem	Cause	Solution
High endotoxin levels	OD_{600} exceeds 3.0	Reduce the input volume to ensure the cell density does not exceed OD_{600} of 3.0.

Contact Information

To reorder supplies, report a device failure or complaint, please contact:

	**Manufacturer** Omega Bio-tek, Inc. 400 Pinnacle Way Suite #450 Norcross, GA 30071, USA Website: www.omegabiotek.com Email: info@omegabiotek.com SRN: US-MF-000024148

Symbols

The following symbols may appear in the instructions for use or on the packaging and labeling:

Picture	Description
	Use-by date
	Check components for storage conditions
	Lot number
	Manufacturer
	No additional hazards or not classified as hazardous according to GHS
	Website
	Telephone
	Fax
	Email
	LinkedIn
	Twitter
	Facebook

Document Revision History

Revision	Description
v1.0, November 2024	Initial Release.

Notices and Disclaimers

Trademarks and Licenses

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Qiagen®, QIAvac®, and Vacman® are all trademarks of their respective companies.
PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.

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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