omega BIO-TEK

innovations in nucleic acid isolation

Product Manual

Mag-Bind® Endo-Free Plasmid Midi Kit

M1272-00	**1 x 24 preps**
M1272-01	**4 x 24 preps**

Manual Date: November 2024
Revision Number v1.0

For Research Use Only

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Mag-Bind® Endo-Free Plasmid Midi Kit

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Intended Use/Intended User

Intended Use

For professional research use.

The Mag-Bind® Endo-free Plasmid Midi Kit is intended for isolation and purification of endotoxin-free (<0.1 EU/µg) plasmid DNA from up to 50 mL overnight culture of *E.* coli in LB medium or up to 10 mL overnight of *E.* coli in TB medium. Please ensure that the cell density does not exceed an OD_{600} of 3.0.

The Mag-Bind® End-free Plasmid Midi 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 Midi 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 Midi Kit is designed to deliver large scale purification of high-quality plasmid DNA with endotoxins 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 50 mL bacterial culture in LB or up to 10 mL bacterial culture in TB or other suitable growth medium. The Mag-Bind® Endo-free Plasmid DNA Kit can be processed either manually or automated on most open-ended liquid handling platforms as well as magnetic processors. Yields may vary depending on the copy number of the plasmid, *E. coli* strain used, culture media, and condition of growth.

The Mag-Bind® Endo-free Plasmid Midi 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 Lysate Clearance Filter Syringe (sold separately) or centrifugation. The novel paramagnetic lysate clearance beads bind to cell debris and pull 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 the 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

Product No	M1272-00	M1272-01
Purifications	1 x 24	4 x 24
Solution I	70 mL	270 mL
Solution II	70 mL	270 mL
N3 Buffer	60 mL	250 mL
IRD Buffer	100 mL	320 mL
VHB Buffer	44 mL	176 mL
Endo-free Water	60 mL	2 x 60 mL
Mag-Bind® Particles RQ	6 mL	22 mL
RNase A	300 µL	1.2 mL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® Endo-free Plasmid Midi 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. Store all other components at recommended temperatures as mentioned on the bottle label. Once bottle is opened, continue to maintain the product in accordance with labeled instructions. 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
M1272-00	56 mL
M1272-01	224 mL

2. Prepare a stock of 100 mL of 70% ethanol and store at room temperature.

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

4. Shake or vortex the Mag-Bind® Particles RQ and Mag-Bind® Particles 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 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. Use appropriate decontaminations and disposal methods in adherence to all applicable local state/provincial, and/or national regulations.

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 Midi 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 serval minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If irritation persist: 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 contact 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 not 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 of 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 you 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 use.

Protocol for 5 mL Bacterial Culture

Important: 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.

Materials and Equipment to be provided by user:

- Centrifuge with swing-bucket rotor capable of 4,000*g*
- Shaker or rocker for Step 8
- Magnetic separation device compatible with 15 mL centrifuge tubes and 1.5/2.0 microcentrifuge tubes
- 15 mL centrifuge tubes and 1.5/2.0 mL microcentrifuge tubes compatible with magnetic separation device used
- 100% ethanol
- 70% ethanol
- 100% isopropanol
- *Optional:* Mag-Bind® Particles LC (sold separately if not using lysate clearance via centrifugation, Part #MBPLC-50)
- *Optional:* Lysate clearance filter syringe (sold separately if not using lysate clearance via centrifugation, Part #FLMIDI-03)

Before Starting:

- Prepare Solution I/RNase A, VHB Buffer, and 70% ethanol 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	Amount per Prep
IRD Buffer	1.57 mL
100% isopropanol	1.93 mL

1. Culture Volume: Inoculate 5 mL *E. coli* cultured in LB or suitable growth medium containing the appropriate selective antibiotic in a culture 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 15 mL centrifuge 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 4,000*g* for 10 minutes at room temperature to collect the bacteria.

3. Decant or aspirate and discard the culture media.

4. Add 1.25 mL Solution I/RNase A to the bacteria 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 1.25 mL Solution II. Invert and rotate the tube gently 8-10 times to obtain a cleared lysate. Incubate for 2-3 minutes at room temperature.

   Note: Avoid vigorous mixing as this 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 <u>one</u> of the following lysate clearance methods:

   A. Lysate Clearance via Centrifugation:

      i. Add 1 mL N3 Buffer. Gently invert 10 times or until a flocculent white precipitate forms.
      ii. Centrifuge at 4,000*g* for 10 minutes at room temperature.
      iii. Transfer 2.5 mL cleared cell lysate to a new 15 mL centrifuge tube. Avoid transferring the precipitate containing cell debris.
      iv. Continue to Step 7 on Page 12.

B. Lysate Clearance via Syringe:

Important: Lysate Clearance Filter Syringes 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. Add 1 mL N3 Buffer. Gently invert 10 times or until a flocculent white precipitate forms.
ii. Prepare a Lysate Clearance Filter Syringe (not provided, Part #FLMIDI-03) by removing the plunger. Place the barrel in a tube rack to keep upright. Make sure the end cap is attached to the syringe tip.
iii. Immediately transfer the lysate from Step B-i into the barrel of the Lysate Clearance Filter Syringe.
iv. Hold the Lysate Clearance Filter Syringe barrel over a new 15 mL centrifuge tube and remove the end cap from the syringe tip.
v. Gently insert the plunger into the barrel to expel the cleared lysate into the 15 mL centrifuge tube.

Note: Some of the lysate may remain in the white flocculent. DO NOT force this residual lysate through the filter.

vi. Continue to Step 7 on Page 12.

C. 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 master mix of N3 Buffer and Mag-Bind® Particles LC (not provided, Part #MBPLC-50) according to the table below. Only prepare as much N3/Mag-Bind® Particles LC master mix that will be used within 72 hours.

Component	Amount per Prep	Total Amount per 24 Preps
N3 Buffer	1.0 mL	26.4 mL*
Mag-Bind® Particles LC	200 µL	5.28 mL*

*10% excess volume has been calculated for 24 preps.

ii. Add 1.2 mL N3 Buffer/Mag-Bind® Particles LC master mix. 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 2.5 mL cleared cell lysate to a new 15 mL centrifuge tube. Avoid transferring the precipitate containing cell debris.

v. Continue to Step 7 on Page 12.

7. Add 2.5 mL IRD Buffer and 50 µL Mag-Bind® Particles RQ to the cleared lysate from Step 6.

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 9.

8. Invert the sample 10 times or pipet up and down to mix. Incubate for 10 minutes at room temperature with continuous mixing. The sample must be mixed throughout the 10-minute incubation period by shaking or rocking. Avoid vortexing.

9. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until all 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.

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

11. Remove the tube from the magnetic separation device.

12. Add 1 mL IRD Buffer. 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 instructions in the "Before Starting" section on Page 9.

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

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

15. Remove the tube from the magnetic separation device.

16. Add 1 mL VHB Buffer. Vortex at maximum speed briefly to resuspend the Mag-Bind® Particles RQ.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see instructions on Page 5.

17. 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.

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

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

20. Remove the tube from the magnetic separation device.

21. Add 1 mL VHB Buffer. Vortex at maximum speed for 1 minute.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see instructions on Page 5.

22. 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.

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

24. Remove the tube from the magnetic separation device.

25. Add 1 mL 70% ethanol (not provided). Vortex at maximum speed for 1 minute.

26. 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.

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

28. Leave the tube on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipette. Air dry the Mag-Bind® Particles RQ for an additional 10 minutes.

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

30. 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 are completely cleared from solution.

31. Transfer the cleared supernatant containing the purified plasmid DNA to a new 1.5 mL microcentrifuge tube.

32. Store plasmid DNA at -20°C.

Protocol for up to 50 mL Bacterial Culture or up to 10 mL TB Culture

Important: 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.

Materials and Equipment to be provided by user:

- Centrifuge with swing-bucket rotor capable of 4,000*g*
- Shaker or rocker for Step 8
- Magnetic separation device compatible with 15 mL centrifuge tubes and 1.5/2.0 microcentrifuge tubes
- 15 mL centrifuge tubes and 1.5/2.0 mL microcentrifuge tubes compatible with magnetic separation device used
- 100% ethanol
- 70% ethanol
- 100% isopropanol
- *Optional:* Mag-Bind® Particles LC (sold separately if not using lysate clearance via centrifugation, Part #MBPLC-50)
- *Optional:* Lysate clearance filter syringe (sold separately if not using lysate clearance via centrifugation, Part #FL96Maxi-03)

Before Starting:

- Prepare Solution I/RNase A, VHB Buffer, and 70% ethanol 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	Amount per Prep
IRD Buffer	3.0 mL
100% isopropanol	3.75 mL

1. Culture Volume: Inoculate up to 50 mL bacterial culture grown in LB or up to 10 mL bacteria culture grown in TB or other suitable growth medium containing the appropriate selective antibiotic in a culture 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 50 mL centrifuge 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 4,000*g* for 10 minutes at room temperature to collect the bacteria.

3. Decant or aspirate and discard the culture media.

4. Add 2.5 mL Solution I/RNase A to the bacteria 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 2.5 mL Solution II. Invert and rotate the tube gently 8-10 times to obtain a cleared lysate. Incubate for 2-3 minutes at room temperature.

   Note: Avoid vigorous mixing as this 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 <u>one</u> of the following lysate clearance methods:

   A. Lysate Clearance via Centrifugation:

      i. Add 2 mL N3 Buffer. Gently invert 10 times or until a flocculent white precipitate forms.
      ii. Centrifuge at 4,000*g* for 10 minutes at room temperature.
      iii. Transfer 5.5 mL cleared cell lysate to a new 15 mL centrifuge tube. Avoid transferring the precipitate containing cell debris.
      iv. Continue to Step 7 on Page 18.

B. Lysate Clearance via Syringe:

Important: Lysate Clearance Filter Syringes 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. Add 2 mL N3 Buffer. Gently invert 10 times or until a flocculent white precipitate forms.
ii. Prepare a Lysate Clearance Filter Syringe (not provided, Part #FLMIDI-03) by removing the plunger. Place the barrel in a tube rack to keep upright. Make sure the end cap is attached to the syringe tip.
iii. Immediately transfer the lysate from Step B-i into the barrel of the Lysate Clearance Filter Syringe.
iv. Hold the Lysate Clearance Filter Syringe barrel over a new 50 mL centrifuge tube and remove the end cap from the syringe tip.
v. Gently insert the plunger into the barrel to expel the cleared lysate into the 50 mL centrifuge tube.

Note: Some of the lysate may remain in the white flocculent. DO NOT force this residual lysate through the filter.

vi. Continue to Step 7 on Page 18.

C. 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 master mix of N3 Buffer and Mag-Bind® Particles LC (not provided, Part #MBPLC-50) according to the table below. Only prepare as much N3/Mag-Bind Particles LC master mix that will be used within 72 hours.

Component	Amount per Prep	Total Amount per 24 Preps
N3 Buffer	2.0 mL	52.8 mL*
Mag-Bind® Particles LC	600 µL	15.8 mL*

*10% excess volume has been calculated for 24 preps.

ii. Add 2.6 mL N3 Buffer/Mag-Bind® Particles LC master mix. 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 5.5 mL cleared cell lysate to a new 15 mL centrifuge tube. Avoid transferring the precipitate containing cell debris.

v. Continue to Step 7 on Page 18.

7. Add 5.5 mL IRD Buffer and 200 µL Mag-Bind® Particles RQ to the cleared lysate from Step 6.

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. Invert the sample 10 times or pipet up and down to mix. Incubate for 10 minutes at room temperature with continuous mixing. The sample must be mixed throughout the 10-minute incubation period by shaking or rocking. Avoid vortexing.

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

Note: Magnetization time depends on the magnet used and plasticware but should take ~5-7 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 to move the Mag-Bind® Particles RQ closer to the magnet.

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

11. Remove the tube from the magnetic separation device.

12. Add 1.25 mL IRD Buffer. 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 instructions in the "Before starting" section on Page 15.

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

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

15. Remove the tube from the magnetic separation device.

16. Add 1.25 mL VHB Buffer. Vortex at maximum speed briefly to resuspend the Mag-Bind® Particles RQ.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see instructions on Page 5.

17. 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.

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

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

20. Remove the tube from the magnetic separation device.

21. Add 1.25 mL VHB Buffer. Vortex at maximum speed for 1 minute.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see instructions on Page 5.

22. 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.

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

24. Remove the tube from the magnetic separation device.

25. Add 1.25 mL 70% ethanol (not provided). Vortex at maximum speed for 1 minute.

26. 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.

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

28. Leave the tube on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipette. Air dry the Mag-Bind® Particles RQ for an additional 10 minutes.

29. Add 0.5-1 mL Endo-free Water. Vortex at maximum speed for 3 minutes.

    Note: For culture volumes up to 25 mL, 250 µL elution volume can be used without compromising plasmid DNA yield.

30. 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 are completely cleared from solution.

31. Transfer the cleared supernatant containing the purified plasmid DNA to a new 1.5 mL microcentrifuge tube.

32. Store plasmid DNA at -20°C.

Protocol for 24-well Plate for Magnetic Processor

Follow this protocol for performing upfront alkaline lysis steps in a 24-well plate before moving to a magnetic processor for subsequent steps for up to 50 mL bacterial culture or up to 10 mL TB culture. 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 magnetic processor instrument-specific instruction. It is the responsibility of the user to validate any automated method for any particular use.

Materials and Equipment to be provided by user:

- Centrifuge with swing-bucket rotor capable of 4,000*g*
- Orbital shaker with 3 mm amplitude
- Magnetic separation device (Recommend Alpaqua Part# A000380)
- 24-well plate compatible with magnetic separation device used
- 100% ethanol
- 70% ethanol
- 100% isopropanol
- *Optional:* Mag-Bind® Particles LC (sold separately if not using lysate clearance via centrifugation, Part #MBPLC-50)

Before Starting:

- Prepare Solution I/RNase A, VHB Buffer, and 70% ethanol 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 once purification. Adjust volume accordingly based on the number of purifications to be performed.

Component	Amount per Prep
IRD Buffer	3.0 mL
100% isopropanol	3.75 mL

1. Culture Volume: Inoculate up to 50 mL bacterial culture grown in LB or up to 10 mL bacterial culture grown in TB or other suitable growth medium containing the appropriate selective antibiotic in a culture 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 50 mL centrifuge 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 4,000*g* for 10 minutes at room temperature to collect the bacteria.

3. Decant or aspirate and discard the culture media.

4. Add 2.5 mL Solution I/RNase A to the bacteria 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. Transfer the 2.5 mL resuspended cells into a 24-well plate (not provided). Discard the 50 mL centrifuge tube.

6. Shake at 1250 rpm for 10 minutes.

7. Add 2.5 mL Solution II. Shake at 400 rpm for 3 minutes.

   Note: Avoid vigorous mixing as this 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.

8. Prepare a master mix of N3 Buffer and Mag-Bind® Particles LC (not provided, Part #MBPLC-50) according to the table below. Only prepare as much N3/LC beads master mix that will be used within 72 hours.

Component	Amount per Prep	Total Amount per 24 Preps
N3 Buffer	2.0 mL	52.8 mL*
Mag-Bind® Particles LC	600 µL	15.8 mL*

*10% excess volume has been calculated for 24 preps.

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.

9. Add 2.6 mL N3 Buffer/Mag-Bind® Particles LC master mix. Shake at 800 rpm for 3 minutes or until a flocculent precipitate forms.

10. 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 LC particles closer to the magnet.

11. Transfer 5.5 mL cleared cell lysate to a new 24-well plate (not provided). Avoid transferring the precipitate containing cell debris.

12. Add 5.5 mL IRD Buffer and 200 µL 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 22.

13. At this point, the plate can be placed on a magnetic processor for the remainder of the extraction. Contact your Omega Bio-tek representative for an automated procedure for magnetic processor-specific instructions.

    Note: For liquid handler specific instructions, contact your Omega Bio-tek representative for more information.

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.
		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 viscous solution.
		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.
	Alkaline lysis is prolonged	Reduce the lysis time (Solution II) to 3 minutes or until the suspended cells for a clear viscous solution.
	Too many or too few cells were used	Confirm the cell density by measuring OD. To calculate the volume of culture to use, take the desired cell mass and divide by the absorbance of the overnight culture at 600 nm.
	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. See Page 5 for instructions on preparing reagents.

Troubleshooting Guide

Problem	Cause	Solution
No Eluted DNA	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 completed resuspended with Solution I. Do not add Solution II until an even cell suspension is obtained.
	VHB Buffer not diluted with ethanol	Prepare VHB Buffer according to the instructions on Page 5.
	IRD Buffer not diluted with isopropanol	Prepare IRD Buffer according to the "Before Starting" section on Page 9 of the Protocol for 5 mL Bacterial Culture, Page 15 of the Protocol for up to 50 mL Bacterial Culture or up to 10 mL TB Culture, or Step 21 of the Protocol for 24-well Plate.
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 according to the instructions on Page 5.
Plasmid DNA floats out of well while loading agarose gel	Ethanol has not been removed completely from RQ particles following wash steps	• Air dry the RQ particles as instructed in Step 28 of the Protocol for 5 mL Bacterial Culture and 20-50 mL Bacterial Culture. • Remove residual liquid from the RQ particles then air dry them for an additional 10 minutes.

Troubleshooting Guide

Problem	Cause	Solution
Absorbance of purified DNA does not accurately reflect quality of the plasmid (A_{260}/A_{280} ratio is too high or too low)	70% ethanol is diluted with ethanol containing impurities	• Check that the absorbance of ethanol is between 250 nm and 300 nm. • Do not use ethanol with high absorbance. Trace impurities may remain on the column after washing and can contribute to the absorbance.
	Plasmid DNA is contaminated with RNA; RNase A treatment is insufficient	• Confirm that the RNase A was added to Solution I prior to first use. • RNase A Solution may degrade due to high temperatures (>65°C) or prolonged storage (>6 months) at room temperature.
	Tinted eluate caused by fine silica particulates	• Increase the elution magnetization time to clear the Mag-Bind® Particles RQ from eluate. • Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings.
	Plasmid DNA is contaminated with chromosomal DNA	• Do not use cultures that have grown for more than 24 hours or are in the cell death phase. • Do not vortex or vigorously shake the cells during the lysis reaction or after adding N3 Buffer.

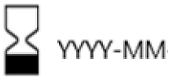
Contact Information

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

	**Manufacturer** Omega Bio-tek, Inc. 400 Pinnacle Way Suite $450 Norcross, GA 30097 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. Also see hazardous symbols as defined in the Precautions Section
	Website
	Telephone
	Fax
	Email
	LinkedIn
	Twitter
	Facebook

Document Revision History

Revision	Description
v1.0	Initial release.

Notices and Disclaimers

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Notes

For more purification solutions, visit www.omegabiotek.com

AVAILABLE FORMATS


Spin Columns

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