omega BIO-TEK

innovations in nucleic acid isolation

Product Manual

Mag-Bind® DNA/RNA/miRNA Kit

M6932-00	**1 x 96 preps**
M6932-01	**4 x 96 preps**

Manual Date: August 2024
Revision Number v1.0

For Research Use Only

Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

omega-bio-tek
omegabiotek
omegabiotek

Mag-Bind® DNA/RNA/miRNA Kit

Table of Contents

Manual Date: August 2024
Revision Number v1.0

omega BIO-TEK

Intended Use/Intended User

Intended Use

For professional research use.

The Mag-Bind® DNA/RNA/miRNA Kit is intended for simultaneous purification of DNA and total RNA including miRNA from cells and tissues into two separate eluates from the same sample without the need for sample splitting. The Kit is also capable of separate purification of miRNA fraction from the total RNA using an optional protocol.

The Mag-Bind® DNA/RNA/miRNA 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® DNA/RNA/miRNA 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. This product is intended for use in a laboratory environment.

Product Description

The Mag-Bind® DNA/RNA/miRNA Kit is designed for simultaneous purification of DNA and total RNA including miRNA from up to 1 x 10^6 cells and 5-10 mg tissue samples into two separate eluates from the same sample. The Kit is also capable of separate purification of miRNA fraction from the total RNA using an optional protocol. DNA and RNA yield is maximized and allows for comprehensive molecular analysis since there is no need for sample splitting.

The protocol uses an innovative Lysis/Binding master mix that conveniently lyses the sample first and upon addition of Mag-Bind® Particles CH binds the DNA to the particles. The RNA-containing supernatant is saved, and an RNA binding step is completed in the presence of binding buffer and isopropanol to bind total RNA including miRNA to the second set of Mag-Bind® Particles CH. This results in separation of DNA and total RNA into two fractions. The RNA-fraction is further treated with DNase I enzyme to digest the residual DNA and rebound to the same set of Mag-Bind® Particles CH by adjusting the binding conditions. Mag-Bind® Particles CH bound to DNA and RNA are individually washed and nucleic acids are eluted in two different tubes/microplates for further analyses. The purified DNA and RNA is ready for use in a wide range of downstream applications such as NGS, PCR, qPCR, RT-qPCR, etc.

The Mag-Bind® DNA/RNA/miRNA 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. Please follow the instructions outlined in the manual for tissues stored in preservation reagents such as DNA/RNA Shield™ and RNAlater™.

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. Please visit the product page at www.omegabiotek.com for more details and ordering information.

Kit Contents

Product No	M6932-00	M6932-01
Purifications	1 x 96	4 x 96
RNA-Lock Reagent	50 mL	150 mL
GTC Lysis Buffer	40 mL	150 mL
DRT Buffer	5 mL	20 mL
KWB Buffer	3 x 30 mL	9 x 30 mL
PCL Buffer	75 mL	250 mL
DNase I Digestion Buffer	25 mL	25 mL
PHM Buffer	20 mL	65 mL
Elution Buffer	15 mL	50 mL
Nuclease-free Water	40 mL	150 mL
Proteinase K Solution	2.2 mL	8.8 mL
Mag-Bind® DNase I	220 µL	900 µL
Mag-Bind® Particles CH	4.4 mL	20 mL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® DNA/RNA/miRNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. DNase I Digestion Buffer, PHM Buffer, and Mag-Bind® DNase I are cold components for which long-term storage is at -20°C ± 10°C. Mag-Bind® Particles CH is a cold component stored at 2-8°C. Store PHM Buffer at room temperature after addition of ethanol. Proteinase K Solution can be stored at room temperature for up to 12 months. For longer-term storage, store Proteinase K Solution at 2-8°C. Store all other components at recommended temperatures as mentioned on the bottle label 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

Please take a few minutes to read this manual thoroughly to become familiar with the protocol before beginning the procedure. To minimize RNA degradation, prepare all required materials before starting. Wear gloves/protective goggles/and take great care when working with chemicals.

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

Kit	100% Ethanol to be Added
M6932-00	40 mL
M6932-01	130 mL

2. Prepare a stock of 3 mL of 1 M Dithiothreitol (DTT). This can be made fresh or frozen in aliquots wrapped in foil and thawed prior to use.

3. Prepare enough stock of 80% ethanol and store at room temperature.

4. Shake or vortex the Mag-Bind® Particles CH to fully resuspend the particles before use. The particles must be fully suspended 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® DNA/RNA/miRNA 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
RNA-Lock Reagent 	Contains: Guanidine thiocyanate. Danger! Harmful if swallowed. Causes severe skin burns and eye damage. Harmful to aquatic life with long lasting effects. Wear protective gloves, protective clothing, eye protection, and face protection. Do not breathe mist/vapors/spray. Do not eat, drink, or smoke when using this product. 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. DO NOT induce vomiting. Call a POISON CENTER/doctor/physician/first aider/if you feel unwell. INHALED: Remove person to fresh air and keep comfortable for breathing. ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. Wash contaminated clothing before use.
GTC Lysis Buffer 	Contains: Guanidine thiocyanate. Danger! Harmful if swallowed. Causes severe skin burns and eye damage. May cause an allergic skin reaction. Harmful 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. Avoid breathing mist/vapors/spray. Wash all exposed external body areas thoroughly after handling. Contaminated work clothing must not be allowed out of the workplace. Avoid release to the environment. IN EYES: Tinse cautiously with water for serval minutes. Remove contact lenses, if present and easy to do. Continue rinsing. SWALLOWED: Rinse mouth. DO NOT induce vomiting. Immediately call a POISON CENTER/doctor/physician/first aider/if you feel unwell. ON SKIN (or hair): Wash with plenty of water and soap. Take off contaminated clothing and wash it before reuse. If skin irritation or rash occurs: Get medical advice/attention. INHALED: Remove person to fresh air and keep comfortable for breathing.

Precautions

Component	Description
KWB Buffer 	Contains: Guanidine hydrochloride, sodium perchlorate, and ethanol. Danger! Flammable liquid and vapor. Harmful if swallowed. Causes skin irritation. Causes serious eye irritation. May cause fire or explosion; strong oxidizer. May cause damage to organs through prolonged or repeated exposure (Oral, Dermal). Do not eat, drink, or smoke when using this product. Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. Take any precaution to avoid mixing with combustibles/organic material. Keep container tightly closed. Do not breathe mist/vapors/spray. Wear protective gloves, protective clothing, eye protection, and face protection. Wash all exposed external body areas thoroughly after handling. In case of fire: Use water spray/fog to extinguish. In case of major fire and large quantities: Evacuate area. Fight the fire remotely due to the risk of explosion. IN EYES: Rinse cautiously with water for serval minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention if irritation persists. SWALLOWED: Rinse mouth. Call a POISON CENTER/doctor/physician/first aider/if you feel unwell. ON SKIN (or hair): Wash with plenty of water. Take off immediately all contaminated clothing. Rinse skin with water/shower. Get medical advice/attention if irritation persists. ON CLOTHING: Rinse immediately contaminated clothing and skin with plenty of water before removing clothes. Take off contaminated clothing and wash it before use.
Proteinase K Solution 	Contains: Proteinase K. Danger! Causes mild skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. Wear respirator protection. If exposed or concerned: Call a poison center or doctor/physician. Remove victim to fresh air and keep at rest in position comfortable for breathing.

Precautions

Component	Description
PCL Buffer 	Contains: Guanidine thiocyanate and ammonium acetate. Warning! Harmful if inhaled, swallowed, or in contact with skin. May cause an allergic skin reaction. Causes serious eye irritation. May cause damage to organs through prolonged or repeated exposure (Oral, Dermal, Inhalation). Harmful 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. Do not breathe mist/vapors/spray. Use only in a well-ventilated area. Wash all exposed external body areas thoroughly after handling. Contaminated work clothing must not be allowed out of the workplace. IN EYES: Rinse cautiously with water for serval minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention. SWALLOWED: Call a POISON CENTER/doctor/physician/first aider/if you feel unwell. Rinse mouth. ON SKIN: Wash with plenty of water. Take off contaminated clothing and wash it before reuse. If skin irritation or rash occurs: Get medical advice/attention. INHALED: Remove person to fresh air and keep comfortable for breathing.

Tissue Protocol

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 capable of 4,000*g* with adapter for 96-well plates
- Magnetic separation device (Recommend Alpaqua® Magnum™ EX, Part #A000380)
- Vortexer
- Ice bucket containing ice
- 96-well plate for homogenization with capacity of at least 2.0 mL (Recommend Nunc, Part #95040452)
- 96-well plate with a capacity of at least 2.0 mL for sample processing (Recommend Nunc, Part #278752)
- 96-well microplate for nucleic acid storage
- Silicone mat for homogenization plate (Recommend Nunc, Part #9503230)
- 2.8 mm ceramic beads (Omni International, Part #19-646)
- 100% ethanol
- 100% isopropanol
- 80% ethanol
- **Required:** 1 M Dithiothreitol (DTT)
- Optional: Mixer mill such as a SPEX CertiPrep Geno/Grinder® 2010 or Omni's Bead Ruptor 96
- Optional: RNase A (25 mg/mL)

Before Starting:

- Prepare PHM Buffer and 80% ethanol according to the "Preparing Reagents" section on Page 5.
- Prepare an ice bucket for RNA sample storage.
- **Required:** Prepare a 3 mL stock of fresh 1 M Dithiothreitol (DTT) to process 96 samples. For all other sample amounts, prepare only what is needed for immediate use.

DNA Purification Procedure

1. Add 5-10 mg tissue to a 96-well homogenizer plate with a well capacity of at least 2.0 mL (not provided). Add two 2.8 mm ceramic beads (not provided) to each well. Continue to Step 3.

2. For tissue samples stored in RNAlater® Solution or DNA/RNA Shield follow the modified protocols below:

   A. RNAlater® Solution: Aspirate and discard RNAlater® from tissue. Retrieve tissue with sterile forceps and blot with absorbent paper towel. Transfer tissue to a new 96-well homogenizer plate with well capacity of at least 2.0 mL. Add 300 µL RNA-Lock Reagent and two 2.8 mm ceramic beads. Continue to Step 4.

   OR

   B. DNA/RNA Shield: Bring the total volume to 300 µL with additional RNA-Lock Reagent (i.e., Add 200 µL RNA-Lock Reagent to tissue sample containing 100 µL DNA/RNA Shield) and transfer everything to a 96-well homogenizer plate. Add two 2.8 mm ceramic beads. Continue to Step 4.

3. Add 300 µL RNA-Lock Reagent.

4. Seal the 96-well homogenizer plate with a silicone mat (not provided).

5. Vortex at maximum speed for 2 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Omni's Bead Ruptor 96, should be used.

   Note:
   - Parameters for complete lysis vary depending on homogenization method. Adjust the parameters accordingly for your method.
   - Visually inspect that the sample is completely homogenized after lysis as incomplete homogenization may affect yields.

6. Remove the silicone mat from the 96-well processing plate. Save the silicone mat for use in Step 8.

7. Freshly prepare a master mix of GTC Lysis Buffer, DRT Buffer, and 1 M DTT (required, not provided) only for samples to be extracted according to the table below:

Component	Amount per Purification	Total Amount per 96-well Plate
GTC Lysis Buffer	300 µL	31.7 mL*
DRT Buffer	45 µL	4.75 mL*
1 M DTT	25 µL	2.64 mL*

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

Important: 1 M DTT is not stable in solution and should be made fresh each time or immediately used once thawed. Only prepare as much GTC Lysis Buffer/DRT Buffer/1 M DTT master mix that will be used within 8 hours of preparation.

8. Add 370 µL GTC Lysis Buffer/DRT Buffer/1 M DTT master mix. Seal the plate with the silicone mat removed from Step 6. Vortex at maximum speed for 30 seconds to mix.

9. Centrifuge at 4,000*g* for 5 minutes.

10. Transfer 600 µL cleared lysate to new 96-well processing plate (not provided). Avoid transferring the debris as it can reduce yield and purity.

11. Add 20 µL Mag-Bind® Particles CH. Pipet up and down 20 times or vortex on high speed for 30 seconds to resuspend the Mag-Bind® Particles CH.

    Note: Mag-Bind Particles CH must be fully suspended to ensure proper binding.

12. Vortex at minimum speed for 10 minutes. Keep the Mag-Bind® Particles CH suspended in solution by adjusting vortex speed.

    Note:
    - If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.
    - If using a vortex with settings 1-10, use setting 1 for minimum speed. Briefly increase the speed setting if necessary to maintain Mag-Bind® Particles CH in suspension.

13. Place the 96-well processing plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

    Note: If excess foaming occurred during vortexing, briefly centrifuge the plate at maximum speed to remove excess foam before placing the plate on the magnetic separation device.

14. **Aspirate the supernatant and transfer 550 µL to a new 96-well processing plate with a well capacity of at least 2.0 mL. The supernatant will be used for RNA Purification on Page 15.**

    Note: Please complete the RNA extraction within 2 hours. For longer times, RNA-containing supernatant can be stored on ice until DNA extraction is completed.

15. Aspirate and discard remaining supernatant from the 96-well processing plate on the magnetic separation device.

16. Remove the 96-well processing plate from the magnetic separation device.

    Optional RNase A Treatment:
    - Add 100 µL GTC Lysis Buffer and 5 µL RNase A (25 mg/mL, not provided).
    - Pipet up and down 20 times to mix.
    - Incubate at room temperature for 5 minutes.
    - Place the 96-well processing plate on the magnetic separation device.
    - Aspirate and discard the supernatant.
    - Continue to Step 17.

17. Add 600 µL KWB Buffer. Vortex at minimum speed for 2 minutes.

18. Place the 96-well processing plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

20. Remove the 96-well processing plate from the magnetic separation device.

21. Add 600 µL 80% ethanol (not provided). Vortex at minimum speed for 2 minutes.

    Note: Prepare enough 80% ethanol for all wash steps.

22. Place the 96-well processing plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

24. Repeat Steps 20-23 for a second 80% ethanol wash step.

25. Leave the 96-well processing plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipette. Air dry the Mag-Bind® Particles CH for an additional 10 minutes.

26. Remove the 96-well processing plate from the magnetic separation device.

27. Add 50-100 µL Elution Buffer.

28. Vortex at minimum speed for 5 minutes. Keep the Mag-Bind® Particles CH suspended in solution by adjusting vortex speed.

    Note: If constant vortexing for 5 minutes is not possible, vortex for 15 seconds every 1-2 minutes for 5 minutes.

29. Place the 96-well processing plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

30. Transfer the cleared supernatant containing purified DNA to a clean 96-well microplate (not provided).

31. Store DNA at -20°C.

RNA Purification Procedure

1. Freshly prepare a master mix of PCL Buffer, Mag-Bind® Particles CH, and 100% isopropanol (not provided) only for samples to be extracted according to the table below:

Component	Amount per Purification	Total Amount per 96-well Plate
PCL Buffer	550 µL	58.0 mL*
Mag-Bind® Particles CH	20 µL	2.11 mL*
100% isopropanol	195 µL	20.6 mL*

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

2. Start with the supernatant from Step 14 in the DNA Purification Protocol. Add 765 µL PCL Buffer/Mag-Bind® Particles CH/100% isopropanol master mix.

3. Vortex for 5 minutes. Keep particles suspended in solution by adjusting vortex speed.

   Note:
   - If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.
   - If using a vortex with settings 1-10, use setting 1 for minimum speed. Briefly increase the speed setting if necessary to maintain Mag-Bind® Particles CH in suspension.

   Important: Please skip to Step 6 if miRNA fraction is not required in the total RNA purification. Otherwise proceed with Step 4.

   Optional: This is the starting point of the optional miRNA Separation Protocol. If additional RNA separation is required, please continue to the miRNA Separation Protocol found on Page 21. (Additional Mag-Bind® Particles CH, Part #MBPCH-50, will need to be purchased separately). If miRNA separation is not required, proceed to Step 4.

4. Add 665 µL 100% isopropanol and 20 µL Proteinase K Solution.

5. Vortex at minimum speed for 5 minutes. Keep the Mag-Bind® Particles CH suspended in solution by adjusting vortex speed.

   Note: The 96-well processing plate will be near full during this vortex step. Take care when vortexing to prevent spillage.

6. Place the 96-well processing plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

7. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

8. Remove the 96-well processing plate from the magnetic separation device.

9. Add 600 µL 80% ethanol. Vortex at minimum speed for 2 minutes.

   Note: Prepare enough 80% ethanol for all wash steps.

10. Place the 96-well processing plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

11. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

12. Leave the 96-well processing plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipette.

13. Remove the 96-well process plate from the magnetic separation device.

14. Add 100 µL Nuclease-free Water. Vortex for 30 seconds to mix.

15. Freshly prepare a master mix of DNase Digestion Buffer and Mag-Bind® DNase I according to the table below:

Component	Amount per Purification	Total Amount per 96-well Plate
DNase Digestion Buffer	50 µL	5.28 mL
Mag-Bind® DNase I	2 µL	211 µL

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

16. Add 52 µL DNase Digestion Buffer/Mag-Bind® DNase I master mix. Pipet up and down 20 times to mix.

    Note:
    - DO NOT vortex samples once Mag-Bind® DNase I has been added as this enzyme is sensitive to excessive agitation
    - Prepare only what is needed for each run.

17. Let sit at room temperature for 10 minutes.

18. Add 455 µL PHM Buffer. Vortex for 30 seconds to mix.

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

    Important: Order of reagent addition is critical to prevent precipitation from occurring. PHM Buffer must be added prior to the addition of 100% ethanol in the next step (Step 19).

19. Add 195 µL 100% ethanol. Vortex at minimum speed for 10 minutes. Keep the Mag-Bind® Particles CH suspended in solution by adjusting vortex speed.

    Note: If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

20. Place the 96-well processing plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

21. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind Particles CH. Remove the 96-well processing plate from the magnetic separation device.

22. Add 600 µL 80% ethanol. Vortex at minimum speed for 2 minutes.

23. Place the 96-well processing plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

25. Leave the 96-well processing plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipette. Air dry the Mag-Bind® Particles CH for an additional 10 minutes.

26. Remove the 96-well processing plate from the magnetic separation device.

27. Add 100 µL Nuclease-free Water.

28. Vortex at minimum speed for 5 minutes. Keep the Mag-Bind® Particles CH suspended in solution by adjusting vortex speed.

    Note: if constant vortexing for 5 minutes is not possible, vortex for 15 seconds every 1-2 minutes for 5 minutes.

29. Place the 96-well processing plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

30. Transfer the cleared supernatant containing purified RNA to a clean 96-well microplate.

31. Store the RNA at -80°C.

Cultured Cells Protocol

Important: If automating this procedure on a liquid handler or a magnetic processor, please contact your Omega Bio-tek representative for instrument-specific instructions.

Materials and Equipment to be provided by user:

- Centrifuge with swing bucket capable of 4,000*g* with adapter for 96-well plates
- Magnetic separation device (Recommend Alpaqua® Magnum™ EX, Part #A000380)
- Vortexer
- Ice bucket containing ice
- 96-well plates with a capacity of at least 2.0 mL (Recommend Nunc, Part #278752)
- 96-well microplates for nucleic acid storage
- 100% ethanol
- 100% isopropanol
- 80% ethanol
- **Required:** 1 M Dithiothreitol (DTT)
- Optional: Mixer mill such as a SPEX CertiPrep Geno/Grinder® 2010 or Omni's Bead Ruptor 96
- Optional: RNase A (25 mg/mL)

Before Starting:

- Prepare PHM Buffer and 80% ethanol according to the "Preparing Reagents" section on Page 5.
- Prepare an ice bucket for RNA sample storage.
- **Required:** Prepare a 3 mL stock of fresh 1 M Dithiothreitol (DTT).

DNA Purification Procedure

Collect cells as a cell pellet prior to lysis. Each cell pellet can contain up to 1 x 10^6 cells. For frozen cell pellets, thaw the cell pellet and immediately resuspend the pellet in the lysis/bind master mix as described in Step 1.

1. Freshly prepare a master mix of RNA-Lock Reagent, GTC Lysis Buffer, DRT Buffer, and 1 M DTT (not provided) according to the table below:

Component	Amount per Purification	Total Amount per 96-well Plate
RNA-Lock Reagent	300 µL	31.7 mL*
GTC Lysis Buffer	300 µL	31.7 mL*
DRT Buffer	45 µL	4.75 mL*
1 M DTT	25 µL	2.64 mL*

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

2. Add 670 µL RNA-Lock Reagent/GTC Lysis Buffer/DRT Buffer/1 M DTT master mix. Vortex or pipet up and down to fully resuspend the cell pellet.

3. Centrifuge at 4,000*g* for 5 minutes.

4. Transfer 600 µL cleared lysate to new 96-well processing plate (not provided). Avoid transferring the debris as it can reduce yield and purity.

5. Process to Step 11 of the Tissue Protocol on Page 12 to continue with the remainder of the DNA Purification Procedure and start the RNA Purification Procedure.

miRNA Separation Protocol

Follow the protocol below to further separate the miRNA fraction (<200 nt) from the total RNA fraction. **This protocol requires purchase of additional Mag-Bind® Particles CH (Part # MBPCH-1).**

After completing Steps 1-3 of the RNA Purification Procedure (Page 15), proceed with the following protocol.

User Supplied Materials:

- Magnetic separation device (Recommend Alpaqua® Magnum™ EX, Part #A000380)
- Vortexer
- Ice bucket containing ice
- 96-well plates with a capacity of at least 2.0 mL (Recommend Nunc, Part #278752)
- 96-well microplates for nucleic acid storage
- Mag-Bind® Particles CH (Omega Bio-tek, Part #MBPCH-50)
- 100% ethanol
- 100% isopropanol
- 80% ethanol

miRNA Purification Procedure

1. Place the 96-well processing plate containing Total RNA from Step 3 on Page 15 on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

2. Transfer all the cleared supernatant containing the miRNA to a new 96-well processing plate.

   Note: Aspirate and discard any residual supernatant if not all are transferred as it will interfere with the DNase Digestion in Step 16 on Page 17.

3. **Add 600 µL 80% ethanol to the RNA-bound Mag-Bind® Particles CH from Step 1. Vortex for 2 minutes. This processing plate will be used for purification of large RNA fraction (>200 nt) on Page 24.**

   Note:
   - Store the large RNA fraction-bound Mag-Bind® Particles CH resuspended in 80% ethanol on ice until miRNA extraction is completed.
   - Prepare enough 80% ethanol for all wash steps.

4. Add 665 µL 100% isopropanol, 20 µL Proteinase K Solution, and 20 µL Mag-Bind® Particles CH to the 96-well processing plate from Step 2.

5. Vortex at minimum speed for 5 minutes. Keep the Mag-Bind® Particles CH suspended in solution by adjusting vortex speed.

   Note: The 96-well processing plate will be near full during this vortex step. Take care when vortexing to prevent spillage.

6. Place the 96-well processing plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

7. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

8. Remove the 96-well processing plate from the magnetic separation device.

9. Add 600 µL 80% ethanol. Vortex at minimum speed for 2 minutes.

10. Place the 96-well processing plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

11. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

12. Repeat Steps 8 – 11 for a second 80% ethanol wash step.

13. Leave the 96-well processing plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipette. Air dry the Mag-Bind® Particles CH for an additional 10 minutes.

14. Remove the 96-well processing plate from the magnetic separation device.

15. Add 100 µL Nuclease-free Water.

16. Vortex at minimum speed for 5 minutes. Keep the Mag-Bind® Particles CH suspended in solution by adjusting vortex speed.

    Note: If constant vortexing for 5 minutes is not possible, vortex for 15 seconds every 1-2 minutes for 5 minutes.

17. Place the 96-well processing plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

18. Transfer the cleared supernatant containing the purified miRNA to a new 96-well microplate.

19. Store at -80°C.

Large Fraction RNA (>200 nt) Purification Procedure

1. Place the 96-well processing plate from Step 3 on Page 22 on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completed cleared from solution.

2. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

3. Leave the 96-well processing plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipette.

4. Remove the 96-well processing plate from the magnetic separation device.

5. Continue with the Large Fraction RNA (>200 ng) Purification Procedure starting on Step 14 of the RNA Purification Protocol on Page 16.

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 Yields	Incomplete resuspension of magnetic particles	Resuspend the magnetic particles by vortexing before use.
	Loss of magnetic particles during operation	Increase particle collection/ magnetization time
	Too much starting material	Use only the amounts listed in the Tissue Protocol (5 mg – 10 mg) or Cultured Cell Protocol (≤ 1 x 10^6 cells) on Page 11 and Page 19, respectively.
	Magnetic particles falling out of solution	• Visually inspect that magnetic particles remain suspended in solution during vortex steps. • Start with a brief vortex at high speed to suspend magnetic particles in solution. • Lower the speed to minimum and continue with vortex step. • If magnetic particles begin falling out of solution, increase vortex speed.
Problem	**Cause**	**Solution**
Low RNA Yields	PHM Buffer was not prepared correctly	Prepare PHM Buffer according to the instructions on Page 5.
	Sample not processed immediately	Homogenize and process samples immediately once RNA-Lock Reagent (Page 3) or master mix (Page 20) is added.
	Improper order for adding 100% isopropanol	Add regents as written in order for the RNA Purification Procedure (Page 15) for Steps 1-4.

Troubleshooting Guide

Problem	Cause	Solution
Degraded RNA	Source	• Proper storage of sample at -80°C prior to extraction. • Proper storage using a preservative (i.e., RNA-Lock Reagent) for sample not processed immediately. • Prepare 1 M DTT fresh or freshly thawed and use as described in the lysis master mix in the Tissue Protocol (Page 11) and Cultured Cell Protocol (Page 20).
	RNA supernatant held at room temperature for extend time	Complete the RNA extraction within 2 hours. For longer extraction times, the RNA-containing supernatant can be stored on ice.
	Heat caused by homogenization	Reduce the homogenization settings for speed and time. Homogenize the tissue in short bursts or cycles and visually inspect that tissue is digested.
RNA contamination	RNA contamination	Perform the optional RNase A Treatment after Step 16 of the DNA Purification Protocol: • Add 100 µL GTC Lysis Buffer and 5 µL RNase A (25 mg/mL). • Pipet up and down 20 times to mix. • Incubate at room temperature for 5 minutes. • Place the 96-well processing plate on the magnetic separation device. • Aspirate and discard the supernatant. • Continue to Step 17 of the DNA Purification Protocol.

Troubleshooting Guide

Problem	Cause	Solution
Problem with downstream application	Ethanol carryover	Completely dry and remove any residual liquid from the magnetic particles before eluting nucleic acids.
DNA contamination	Incomplete digestion of DNA during DNase Digestion Step	Remove any residual ethanol (RNA Purification Procedure, Step 12 or Large Fraction RNA Purification Procedure, Step 3) before addition of Nuclease-free Water.
Sample forms gel-like substance	1 M Dithiothreitol (DTT) missing or prepared incorrectly	Fresh 1 M DTT is required as part of lysis in the Tissue Protocol (Page 11) and Cultured Cell Protocol (Page 20). If missing or prepared incorrectly, some samples may form a gel-like substance when mixed. This can affect yields and purity.
Excess foaming in sample	Vortex speed too fast	Briefly centrifuge the plate at maximum speed for 2 minutes to reduce excess foam before placing the plate on the magnetic separation device.

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

Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

omega-bio-tek
omegabiotek
omegabiotek