omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

Mag-Bind® FFPE DNA/RNA 96 Kit

M6955-00	1 x 96 preps
M6955-01	4 x 96 preps

Manual Date: October 2025
Revision Number: v5.0

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® FFPE DNA/RNA 96 Kit

Table of Contents

Manual Date: October 2025
Revision Number: v5.0

omega BIO-TEK
innovations in nucleic acid isolation

Product Description

Mag-Bind® FFPE DNA/RNA 96 Kit is designed for the sequential isolation of DNA and RNA in separate eluates from the same formalin-fixed, paraffin-embedded (FFPE) tissue sample. The purification of DNA and RNA from the same sample enables a more comprehensive analysis from a precious sample source. The protocol utilizes a specially formulated buffer system that not only partially reverses the formaldehyde-induced crosslinking but also ensures DNA and RNA are differentially purified with no cross-contamination. The kit can also be used for purification of only DNA or only RNA from FFPE samples if sequential isolation is not desired. Magnetic bead-based extraction makes it suitable for both manual as well as automated processing. Purified DNA and RNA are suitable for a variety of downstream applications including SNP analysis, next generation sequencing, and genotyping.

The Mag-Bind® FFPE DNA/RNA 96 Kit integrates a unique buffer system with the highly efficient binding properties of Mag-Bind® technology to isolate total DNA and RNA in separate eluates from the same FFPE sample. The protocol utilizes non-toxic mineral oil in combination with heat for efficient deparaffinization of the FFPE sample eliminating the use of hazardous xylene.

Samples are first lysed in FDR Buffer aided by the presence of Proteinase K enzyme. The lysate is then heated to denature the proteinase and reverse the chemical crosslinking of the nucleic acids. Post-heating, the lysate is mixed with MB4 Buffer and Mag-Bind® Particles CH to bind DNA to the particles. The RNA-containing supernatant is saved and a second binding step is completed with addition of isopropanol to bind RNA to the Mag-Bind® Particles CH. This results in separation of DNA and RNA into two fractions. Mag-Bind® Particles bound to DNA and RNA are individually washed and nucleic acids are eluted in two different tubes for further analyses. Only DNA or RNA can be isolated following the appropriate protocols outlined in the manual.

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.

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.

Starting Materials

Standard formalin-fixation, paraffin embedding procedures cause significant fragmentation of nucleic acids. We recommend the following guidelines to limit the extent of DNA/RNA fragmentation: 1) Use 4-10% formalin to fix tissue samples; 2) Limit the fixation time to 14-24 hours; 3) Completely dehydrate samples before embedding. Always use freshly cut sections of FFPE tissue. For first time users, we recommend using 1-3 FFPE sections of 10 µm thickness. Depending on the yield and purity obtained, it may be possible to increase the starting material.

Kit Contents

Product	M6955-00	M6955-01
Purifications	1 x 96	4 x 96
FDR Buffer	50 mL	180 mL
MB4 Buffer	75 mL	275 mL
RMP Buffer	25 mL	100 mL
Elution Buffer	30 mL	125 mL
Nuclease-free Water	30 mL	125 mL
DNase Digestion Buffer	25 mL	2 x 25 mL
PHM Buffer	10 mL	50 mL
Proteinase K Solution	2.2 mL	8.8 mL
Mag-Bind® DNase I	220 µL	4 x 220 µL
Mag-Bind® Particles CH	2.2 mL	10 mL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® FFPE DNA & RNA 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® DNase I, DNase Digestion Buffer, and PHM Buffer should be stored at -20°C. Store PHM Buffer at room temperature after addition of ethanol. Mag-Bind® Particles CH should be stored at 2-8°C for long-term use. Proteinase K Solution can be stored at room temperature for up to 12 months. For long-term storage, store Proteinase K Solution at 2-8°C. Store all other components at room temperature and away from bright light. During shipment or storage in cool ambient conditions, precipitates may form. 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 familar 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 and store at room temperature.

Kit	100% Ethanol to be Added
M6955-00	20 mL
M6955-01	100 mL

2. Dilute RMP Buffer with 100% isopropanol and store at room temperature.

Kit	100% Isopropanol to be Added
M6955-00	25 mL
M6955-01	100 mL

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 components 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.

Some of the buffers included in the product 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 waste. Please access the SDSs online for detailed information on the reagents.

Precautions

Some of the buffers included in the Mag-Bind® FFPE DNA/RNA 96 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
FDR Buffer 	Contains: Anionic detergent. Warning! Causes serious eye irritation. May cause an allergic skin reaction. Wear protective gloves, protective clothing, eye protection and face protection. Avoid breathing mist/vapors. 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 several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If irritation persists: Get medical advice/attention. ON SKIN: Wash with plenty of water and soap. If skin irritation or rash occurs: Get medical advice/attention. Take off contaminated clothing and wash it before reuse.
RMP Buffer 	Contains: Guanidine hydrochloride. Warning! Causes serious eye irritation. Causes skin 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 eye irritation persists: Get medical advice/attention. ON SKIN: Wash with plenty of water and soap. If skin irritation or rash occurs: Get medical advice/attention. Take off contaminated clothing and wash it before reuse.
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 respiratory protection. If exposed or concerned: Call a poison center or doctor/physician. Remove victim to fresh air and keep at rest in a position comfortable for breathing.

Component	Description
MB4 Buffer  	Contains: Guanidine hydrochloride and non-ionic detergent. Danger! Harmful if swallowed. Causes severe skin burns and eye damage. May cause an allergic skin reation. Wear protective gloves, protective clothing, eye protection and face protection. Wash all exposed external body areas thoroughly after handling. Contaminated work clothing must not be worn outside of the workplace. Do not eat, drink or smoke when using this product. Do not breathe mist/vapors/spray. 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. Immediately call a POISON CENTER/doctor/ physician/first aider. 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 with plenty of water and soap. Wash contaminated clothing before reuse.

Sequential 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.

Note:
- For DNA purification only, please follow this procotol until the end of Step 31 of the DNA Purification Procedure.
- Order of purification does not have to follow as written. Once the DNA and RNA fractions have been separated, customer can proceed to the RNA Purification Procedure before completing the DNA Purification Procedure.

Materials and Equipment to be Supplied by User:

- Centrifuge with swing bucket rotor capable of 2,000*g*
- Rotor adaptor for 96-well deep-well plates
- Magnetic separation device
- Vortexer
- Incubator capable of 90°C
- 96-well processing plates with at least a 2 mL capacity
- 96-well microplate (for eluted RNA)
- 1.5 mL or 2.0 mL microcentrifuge tube
- 80% ethanol
- 100% ethanol
- 100% isopropanol
- Mineral oil (Recommend VWR, Cat# 97064-128)
- Sealing film (Recommend Omega Bio-tek, Cat# AC1200)

Before Starting:

- Prepare PHM Buffer and RMP Buffer according to the Preparing Reagents section on Page 5.
- Prepare 80% ethanol.
- Set incubator to 90°C.

Important: For optimal yields, incubation must be carried out at the recommended temperature.

DNA Purification Procedure

1. Transfer the FFPE samples to a 96-well processing plate with a well capacity of at least 2.0 mL (not provided) or a 2.0 mL microcentrifuge tube (not provided).

2. Add 300 µL mineral oil (not provided) to the 96-well processing plate or tube. Seal the processing plate with sealing film (not provided) or close the cap on the tube.

   Note: Other non-xylene solvent alternatives such as HistoChoice, HistoClear, HistoClear II, or hexadecane can be used for deparaffinization in place of mineral oil. Add 900 µL of the solvent if following the mineral oil-free option. Follow Safety Data Sheet (SDS) guidelines for safe handling of the solvent used.

3. Incubate at 90°C for 3 minutes. Remove the sealing film from the 96-well processing plate or open the tube.

   Note: It is important for incubation to be carried out at recommended temperatures for optimal yields.

4. Add 400 µL FDR Buffer. Seal the 96-well processing plate with sealing film or close the tube.

5. Centrifuge at 2,000*g* for 1 minute to create two phases within the solution: an upper oil phase and a lower aqueous phase. Ensure the tissue is in contact with FDR Buffer in the lower aqueous phase.

   Note: The upper mineral oil layer may turn opaque or cloudy but this will not affect DNA or RNA extraction.

6. Incubate at 90°C with constant shaking at 300 rpm for 30 minutes. Remove the sealing film from the 96-well processing plate or open the tube.

7. Add 20 µL Proteinase K Solution to the lower aqueous layer. Seal the 96-well processing plate with sealing film or close the tube.

8. Shake the 96-well processing plate at 600 rpm or vortex the tube for 30 seconds to mix thoroughly.

9. Incubate at room temperature for 30 minutes.

10. Incubate at 90°C with constant shaking at 300 rpm for 1 hour.

11. Centrifuge at 2,000*g* for 1 minute to create two phases within the solution: an upper oil phase and a lower aqueous phase. Remove and discard the sealing film from the 96-well processing plate.

12. Transfer 325 µL of the lower aqueous layer to a new 96-well processing plate capable of at least 2.0 mL or a new 2.0 mL microcentrifuge tube. Avoid disturbing the mineral oil layer as much as possible.

13. Add 650 µL MB4 Buffer and 10 µL Mag-Bind® Particles CH. Vortex for 10 minutes.

    Note:
    - MB4 Buffer and Mag-Bind® Particles CH can be prepared as a mastermix. Prepare only what is needed for each run.
    - If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

14. Place the 96-well processing plate or tube 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.

15. **Aspirate the supernatant and transfer to a new 96-well processing plate with a well capacity of at least 2.0 mL or 2.0 mL microcentrifuge tube. The supernatant will be used for RNA Purification on Page 13!**

    Note:
    - If desired, RNA can be processed immediately prior to completing DNA purification. Please see Page 13 to continue with the RNA Purification Procedure. Once RNA purification is completed, continue with DNA Purification Procedure on Page 11 from Step 16 onwards.
    - If proceeding with DNA Purification Procedure first, RNA-containing supernatant can be stored at room temperature up to 2 hours or on ice until DNA extraction is completed.

    Optional: If only DNA is desired, do not save the supernatant for RNA extraction.

16. Remove the 96-well processing plate or tube containing the Mag-Bind® Particles CH from the magnetic separation device.

17. Add 400 µL RMP Buffer to the 96-well processing plate or tube (containing DNA-bound magnetic beads). Vortex for 2 minutes.

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

18. Place the 96-well processing plate or tube 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 or tube containing the Mag-Bind® Particles CH from the magnetic separation device.

21. Add 400 µL 80% ethanol (not provided). Vortex for 2 minutes.

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

22. Place the 96-well processing plate or tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles 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 or tube 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 or tubecontaining the Mag-Bind® Particles CH from the magnetic separation device.

27. Add 50-100 µL Elution Buffer to elute DNA from the Mag-Bind® Particles CH.

28. Vortex for 5 minutes to mix.

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

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

31. Store DNA at -20°C.

    Optional: If only DNA is desired, skip the RNA Purification Procedure.

RNA Purification Procedure

1. Start with the supernatant (~845 µL) from Step 15 in DNA Purification Protocol. Add 845 µL 100% isopropanol and 10 µL Mag-Bind® Particles CH.

   Note: Isopropanol and Mag-Bind® Particles CH can be prepared as a mastermix. Prepare only what is needed for each run.

2. Vortex or tip mix for 10 minutes.

   Note: If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes. Proper mixing is crucial to efficiently bind RNA to Mag-Bind® Particles CH.

3. Place the 96-well processing plate or tube 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.

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

5. Remove the 96-well processing plate or tube containing the Mag-Bind® Particles CH from the magnetic separation device.

6. Add 400 µL 80% ethanol. Vortex for 2 minutes.

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

7. Place the 96-well processing plate or tube 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.

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

9. Leave the 96-well processing plate or tube on the magnetic separation device for 3 minutes to air dry the Mag-Bind® Particles CH. Remove any residual liquid with a pipette.

10. Remove the 96-well processing plate or tube containing the Mag-Bind® Particles CH from the magnetic separation device.

11. Add 73.5 µL DNase Digestion Buffer and 1.5 µL Mag-Bind® DNase I. Pipet up and down 20 times to mix.

    Note: A mastermix of DNase Digestion Buffer and Mag-Bind® DNase I can be made. Prepare only what is needed for each run.

12. Let sit at room temperature for 15 minutes.

13. Add 225 µL PHM Buffer. Vortex for 5 minutes.

    Note:
    - PHM Buffer must be diluted with 100% ethanol prior to use. Please see instructions on Page 5.
    - If constant vortexing for 5 minutes is not possible, vortex for 30 seconds every 2 minutes for 5 minutes.

14. Place the 96-well processing plate or tube 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.

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

16. Remove the 96-well processing plate or tube containing the Mag-Bind® Particles CH from the magnetic separation device.

17. Add 400 µL 80% ethanol. Vortex for 2 minutes.

18. Place the 96-well processing plate or tube on the magnetic separation device for 3 minutes to air dry the Mag-Bind® Particles CH. Remove any residual liquid with a pipette.

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

20. Repeat Steps 16-19 for a second 80% ethanol wash step.

21. Leave the 96-well processing plate or tube 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.

22. Remove the 96-well processing plate or tube containing the Mag-Bind® Particles CH from the magnetic separation device.

23. Add 50-100 µL Nuclease-free Water.

24. Vortex for 5 minutes to mix.

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

25. Place the 96-well processing plate or tube 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.

26. Transfer the cleared supernatant containing purified RNA to a clean 96-well microplate (not provided) or new microcentrifuge tube.

27. Store RNA at -80°C.

DNA Only 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 Supplied by User:

- Centrifuge with swing bucket rotor capable of 2,000*g*
- Rotor adaptor for 96-well deep-well plates
- Magnetic separation device
- Vortexer
- Incubator capable of 90°C
- 96-well processing plates with at least a 2 mL capacity
- 1.5 mL or 2.0 mL microcentrifuge tube
- 80% ethanol
- 100% ethanol
- Mineral oil (Recommend VWR, Cat# 97064-128)
- Sealing film (Recommend Omega Bio-tek, Cat# AC1200)

Before Starting:

- Prepare PHM Buffer and RMP Buffer according to the Preparing Reagents section on Page 5.
- Prepare 80% ethanol.
- Set incubator to 90°C.

Important: For optimal yields, incubation must be carried out at the recommended temperature.

1. Transfer the FFPE samples to a 96-well processing plate with a well capacity of at least 2.0 mL (not provided) or a 2.0 mL microcentrifuge tube (not provided).

2. Add 300 µL mineral oil (not provided) to the 96-well processing plate or tube. Seal the processing plate with sealing film (not provided) or close the cap on the tube.

   Note: Other non-xylene solvent alternatives such as HistoChoice, HistoClear, HistoClear II, or hexadecane can be used for deparaffinization in place of mineral oil. Add 900 µL of the solvent if following the mineral oil-free option. Follow Safety Data Sheet (SDS) guidelines for safe handling of the solvent used.

3. Incubate at 90°C for 3 minutes. Remove the sealing film from the 96-well processing plate or open the tube.

   Note: It is important for incubation to be carried out at recommended temperatures for optimal yields.

4. Add 400 µL FDR Buffer. Seal the 96-well processing plate with sealing film or close the tube.

5. Centrifuge at 2,000*g* for 1 minute to create two phases within the solution: an upper oil phase and a lower aqueous phase. Ensure the tissue is in contact with FDR Buffer in the lower aqueous phase.

   Note: The upper mineral oil layer may turn opaque or cloudy but this will not affect DNA or RNA extraction.

6. Incubate at 90°C with constant shaking at 300 rpm for 30 minutes. Remove the sealing film from the 96-well processing plate or open the tube.

7. Add 20 µL Proteinase K Solution to the lower aqueous layer. Seal the 96-well processing plate with sealing film or close the tube.

8. Shake the 96-well processing plate at 600 rpm or vortex the tube for 30 seconds to mix thoroughly.

9. Incubate at room temperature for 30 minutes.

10. Incubate at 90°C with constant shaking at 300 rpm for 1 hour.

11. Centrifuge at 2,000*g* for 1 minute to create two phases within the solution: an upper oil phase and a lower aqueous phase. Remove and discard the sealing film from the 96-well processing plate.

12. Transfer 325 µL of the lower aqueous layer to a new 96-well processing plate capable of at least 2.0 mL or a new 2.0 mL microcentrifuge tube. Avoid disturbing the mineral oil layer as much as possible.

13. Add 650 µL MB4 Buffer and 10 µL Mag-Bind® Particles CH. Vortex for 10 minutes.

    Note:
    - MB4 Buffer and Mag-Bind® Particles CH can be prepared as a mastermix. Prepare only what is needed for each run.
    - If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

14. Place the 96-well processing plate or tube 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.

15. Aspirate and discard the supernatant.

16. Remove the 96-well processing plate or tube containing the Mag-Bind® Particles CH from the magnetic separation device.

17. Add 400 µL RMP Buffer to the 96-well processing plate or tube (containing DNA-bound magnetic beads). Vortex for 2 minutes.

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

18. Place the 96-well processing plate or tube 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 or tube containing the Mag-Bind® Particles CH from the magnetic separation device.

21. Add 400 µL 80% ethanol (not provided). Vortex for 2 minutes.

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

22. Place the 96-well processing plate or tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles 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 or tube 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 or tubecontaining the Mag-Bind® Particles CH from the magnetic separation device.

27. Add 50-100 µL Elution Buffer to elute DNA from the Mag-Bind® Particles CH.

28. Vortex for 5 minutes to mix.

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

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

31. Store DNA at -20°C.

RNA Only 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 Supplied by User:

- Centrifuge with swing bucket rotor capable of 2,000*g*
- Rotor adaptor for 96-well deep-well plates
- Magnetic separation device
- Vortexer
- Incubator capable of 90°C
- 96-well processing plates with at least a 2 mL capacity compatible with the magnetic separation device used
- 96-well microplate (for eluted RNA)
- 1.5 mL or 2.0 mL microcentrifuge tube
- 80% ethanol
- 100% ethanol
- 100% isopropanol
- Mineral oil (Recommend VWR, Cat# 97064-128)
- Sealing film (Recommend Omega Bio-tek, Cat# AC1200)

Before Starting:

- Prepare PHM Buffer according to the Preparing Reagents section on Page 5.
- Prepare 80% ethanol.
- Set incubator to 90°C.

Important: For optimal yields, incubation must be carried out at the recommended temperature.

1. Transfer the FFPE samples to a 96-well processing plate with a well capacity of at least 2.0 mL (not provided) or a 2.0 mL microcentrifuge tube (not provided).

2. Add 300 µL mineral oil (not provided) to the 96-well processing plate or tube. Seal the 96-well processing plate with sealing film (not provided) or close the tube.

   Note: Other non-xylene solvent alternatives such as HistoChoice, HistoClear, HistoClear II, or hexadecane can be used for deparaffinization in place of mineral oil. Add 900 µL of the solvent if following the mineral oil-free option. Follow Safety Data Sheet (SDS) guidelines for safe handling of the solvent used.

3. Incubate at 90°C for 3 minutes. Remove the sealing film from the 96-well processing plate or open the tube.

4. Add 400 µL FDR Buffer. Seal the 96-well processing plate with sealing film or close the tube.

5. Centrifuge at 2,000*g* for 1 minute to create two phases within the solution: an upper oil phase and a lower aqueous phase. Ensure the tissue is in contact with FDR Buffer in the lower aqueous phase.

   Note: The upper mineral oil layer may turn opaque or cloudy but this will not affect RNA extraction.

6. Incubate at 90°C with constant shaking at 300 rpm for 30 minutes. Remove the sealing film from the 96-well processing plate or open the tube.

   Note: It is important for incubation to be carried out at recommended temperatures for optimal yields

7. Add 20 µL Proteinase K Solution to the lower aqueous layer. Seal the 96-well processing plate with sealing film or close the tube.

8. Shake the 96-well processing plate at 600 rpm or vortex the tube for 30 seconds to mix thoroughly.

9. Incubate at room temperature for 30 minutes.

10. Incubate at 90°C with constant shaking at 300 rpm for 1 hour.

11. Centrifuge at 2,000*g* for 1 minute to create two phases within the solution: an upper oil phase and a lower aqueous phase.

12. Transfer 325 µL of the lower aqueous layer to a new 96-well processing plate capable of at least 2.0 mL or a new 2.0 mL microcentrifuge tube. Avoid disturbing the mineral oil layer as much as possible.

13. Add 650 µL MB4 Buffer, 10 µL Mag-Bind® Particles CH, and 845 µL 100% isopropanol. Vortex or tip mix samples for 10 minutes.

    Note:
    - MB4 Buffer and Mag-Bind® Particles CH can be prepared as a mastermix. Prepare only what is needed for each run.
    - If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

14. Place the 96-well processing plate or tube 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.

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

16. Remove the 96-well processing plate or tube containing the Mag-Bind® Particles CH from the magnetic separation device.

17. Add 400 µL 80% ethanol (not provided). Vortex for 2 minutes.

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

18. Place the 96-well processing plate or tube 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. Leave the 96-well processing plate or tube on the magnetic separation device for 3 minutes to air dry the Mag-Bind® Particles CH. Remove any residual liquid with a pipette.

21. Remove the 96-well processing plate or tube containing the Mag-Bind® Particles CH from the magnetic separation device.

22. Add 73.5 µL DNase Digestion Buffer and 1.5 µL Mag-Bind® DNase I. Pipet up and down 20 times to mix.

    Note: A mastermix of DNase Digestion Buffer and Mag-Bind® DNase I can be made. Prepare only what is needed for each run.

23. Let sit at room temperature for 15 minutes.

24. Add 225 µL PHM Buffer. Vortex for 5 minutes.

    Note:
    - PHM Buffer must be diluted with 100% ethanol prior to use. Please see instructions on Page 5.
    - If constant vortexing for 5 minutes is not possible, vortex for 30 seconds every 2 minutes for 5 minutes.

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

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

27. Remove the 96-well processing plate or tube containing the Mag-Bind® Particles CH from the magnetic separation device.

28. Add 400 µL 80% ethanol. Vortex for 2 minutes.

29. Place the 96-well processing plate or tube 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. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

31. Repeat Steps 27-30 for a second 80% ethanol wash step.

32. Leave the 96-well processing plate or tube 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.

33. Remove the 96-well processing plate or tube containing the Mag-Bind® Particles CH from the magnetic separation device.

34. Add 50-100 µL Nuclease-free Water.

35. Vortex for 5 minutes to mix.

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

36. Place the 96-well processing plate or tube 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.

37. Transfer the cleared supernatant containing purified RNA to a clean 96-well plate (not provided) or a new microcentrifuge tube.

38. Store RNA at -80°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Low DNA Yields	Incomplete resuspension of magnetic particles	Resuspend the magnetic particles by vortexing before use.
	RMP Buffer was not prepared correctly	Prepare RMP Buffer according to the instructions on Page 5.
	Loss of magnetic particles during operation	Increase the particle collection/ magnetization time.
	Proteinase K Solution not added or added at the wrong step	Proteinase K Solution must be added after the second 90°C incubation step. The plate must be removed from the incubator and incubated at room temperature after addition.
Problem	**Cause**	**Solution**
Low RNA Yields	Incomplete resuspension of magnetic particles	Resuspend the magnetic particles by vortexing before use.
	PHM Buffer was not prepared correctly	Prepare PHM Buffer according to the instructions on Page 5.
	Loss of magnetic particles during operation	Increase the particle collection/ magnetization time.
Problem	**Cause**	**Solution**
Problem with downstream application	DNA is excessively cross-linked due to over-fixation	Extend incubation time at 90°C to 90 minutes.
		The incubator must reach 90°C before starting incubation.
		The solution within the plate must reach 90°C at both 90°C incubation steps, Step 6 and Step 10, in both the Sequential Protocol and the RNA Only Protocol.

Problem	Cause	Solution
DNA contamination	Incomplete digestion of DNA during DNase Digestion Step	After Step 10 of the RNA Purification Procedure (Page 14) or after Step 21 of RNA Only Protocol (Page 23), perform the following: 1. Add 100 µL Nuclease-free Water to each sample and vortex for 5 minutes. 2. Add 73.5 µL DNase Digestion Buffer and 1.5 µL Mag-Bind DNase I. 3. Let sit at room temperature for 15 minutes. 4. Add 525 µL PHM Buffer and vortex for 5 minutes. 5. Continue with Step 14 of the RNA Purification Protocol (Page 15) or Step 25 of the RNA only Protocol (Page 24). This will require additional PHM Buffer that is not provided with this kit. Please contact your Omega Bio-tek representative at **1-800-832-8896** for ordering information.
Poor $A_{260/230}$ purity	Contamination carry-over in plasticware	When performing the extraction manually, follow the steps outlined below to reduce contamination. *DNA Purification Procedure:* • After adding RMP Buffer (Page 12, Step 17), transfer the mixed contents to a new 1.5 mL microcentrifuge tube then proceed with the procedure. • Repeat this process for 80% ethanol wash step (Page 12, Step 21). *RNA Purification Procedure:* • After adding 80% ethanol (Page 15, Step 17), transfer the mixed contents to a new 1.5 mL microcentrifuge tube then proceed with the procedure. *DNA Only Protocol:* • After adding RMP Buffer (Page 19, Step 17), transfer the mixed contents to a new 1.5 mL microcentrifuge tube then proceed with the procedure. • Repeat this process for 80% ethanol wash step (Page 19, Step 21). *RNA Only Protocol:* • After adding 80% ethanol (Page 24, Step 28), transfer the mixed contents to a new 1.5 mL microcentrifuge tube then proceed with the procedure.

Problem	Cause	Solution
Carryover of the magnetic particles in the elution	Carryover of the magnetic particles in the eluted DNA or RNA will not effect downstream applications.	To remove the carryover magnetic particles from the eluted DNA or RNA, simply magnetize the magnetic particles and carefully transfer the DNA or RNA eluate to a new tube or plate.
PCR inhibition	Contaminants in eluate	Increase the elution volume to at least 100 µL or bring the eluate to at least 100 µL using the appropirate solution, Elution Buffer (DNA) or Nuclease-free Water (RNA).
Small RNAs causing low DV_{200}	Incorrect alcohol percentage at RNA bind	At Step 1 (RNA Purification Procedure) or Step 13 (RNA Only Protocol), use 70% alcohol instead of 100% alcohol during RNA purification for small RNAs to be excluded. If 70% alcohol is used, small RNAs will not be purified in the total RNA.

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
v5.0, October 2025	The protocol has been updated to reflect new sample processing procedure.
v3.3, January 2024	Warnings and Safety Information section has been added to the manual.
v3.2, May 2022	An important statement has been included to clarify that the actual number of preparations is dependent on various factors and may be lower than the number of preparations specified with the kit.
v3.1, June 2021	Notices & Disclaimer section has been added to the manual.
v3.0, January 2020	A new protocol for the extraction of RNA only from FFPE has been added to the kit.
v2.0, July 2019	An addtional dry step has been added to the DNase Digestion process in the RNA Only protocol.
v1.0, June 2019	Initial release.

Notices & Disclaimers

For European Union Use.

MB4 Buffer contains Triton X-100, 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol (CAS 9002-93-1), a substance included in the European Authorisation list (Annex XIV) of REACH Regulation (EC) No 1907/2006. Substances and mixtures used for the purpose of Scientific Research and Development (SR&D) are exempt from authorization requirements if used below 1 tonne per year in volume.

Scientific Research and Development includes experimental research or analytical activities at a laboratory scale such as synthesis and testing of applications of chemicals, release tests, etc. as well as the use of the substance in monitoring and routine quality control or in vitro diagnostics.

Trademarks and Licenses

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

innovations in nucleic acid isolation

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