omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

Mag-Bind® Total RNA 96 Kit

M6731-00	1 x 96
M6731-01	4 x 96

Manual Date: November 2024
Revision Number: v5.2

For Research Use Only

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Mag-Bind® Total RNA 96 Kit

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Introduction

Mag-Bind® Total RNA 96 Kit allows for rapid and reliable isolation of high-quality total cellular RNA from a wide variety of tissue and cultured cells. Total RNA can be purified from 5-10 mg tissue or 1 x 10^6 cultured cells. Purified RNA is suitable for all major downstream applications such as RT-PCR, restriction digestion, and hybridization applications.

If using the Mag-Bind® Total RNA 96 Kit for the first time, please read this booklet in its entirety to become familiar with the procedures. Samples are lysed in OTRK Lysis Buffer. Proteins are removed with a proteinase K digestion step. DNA and RNA are then bound to the paramagnetic beads. Genomic DNA is removed with a DNase I digestion step. After two quick wash steps with 80% ethanol, RNA is eluted.

Since the Mag-Bind® Total RNA 96 Kit uses paramagnetic beads, it can also be adapted to liquid handlers including Beckman Coutler's Biomek FX, Tecan Genesis, Thermo Kingfisher Flex, and Hamilton Star instruments.

New in this Edition:

November 2024:
- Removal of 100% isopropanol from Materials to be Supplied by User.
- M6731-02 pack size has been discontinued and is no longer available to purchase.

April 2024:
- Addition of Warnings and Safety Information.

May 2018:
- A mastermix of OTRK Lysis Buffer and Proteinase K Solution has been added to the protocol.
- The DNase Digestion protocol has been modified and a new buffer has been added to the kit.

March 2018:
- The storage temperature for DNase Digestion Buffer has changed. DNase Digestion Buffer should now be stored at -20°C along with the Mag-Bind® DNase I.
- Magnetic separation device Cat# MSD-01B) has been discontinued and is no longer available for purchase.
- Multichannel reservoirs (Cat# AC1331-01) has been discontinued and is no longer available for purchase.

Kit Contents

Product Number	M6731-00	M6731-01
Purifications	1 x 96 preps	4 x 96 preps
Mag-Bind® Particles CNR	2.2 mL	8.8 mL
OTRK Lysis Buffer	50 mL	200 mL
VHB Buffer	22 mL	88 mL
RNA Wash Buffer II	50 mL	3 x 50 mL
Proteinase K Solution	3 mL	12 mL
Mag-Bind® DNase I	220 µL	4 x 220 µL
DNase I Digestion Buffer	25 mL	25 mL
PHM Buffer	20 mL	65 mL
RNA Elution Buffer	25 mL	100 mL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® Total RNA 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles CNR must be stored at 2-8°C. Mag-Bind® DNase I, DNase Digestion Buffer, and PHM Buffer must be stored at -20°C. 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 in some buffers. Dissolve such deposits by warming the solution at 37°C with gently shaking.

Warnings

This kit is for research use only.

Please read all instructions carefully before using the kit.

Decontaminate and dispose of all potentially infectious materials in accordance with applicable local, state, and national regulations. Please refer to safety data sheets (SDSs) for information on disposal of different components included in this kit.

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.

Preparing Reagents

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

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

Kit	100% Ethanol to be Added
M6731-00	28 mL
M6731-01	112 mL

2. Dilute RNA Wash Buffer II with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M6731-00	200 mL
M6731-01	200 mL per bottle

Tissue Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge capable of 4,000*g* with swing-bucket rotor for 96-well plates
- Adapter for 96-well deep-well plates
- Magnetic separation device
- Equipment for disrupting tissue (MM300 Mixer Mill or Geno/Grinder 2000/2010) or mortar and pestle
- Vortexer
- 100% ethanol
- Multi-channel pipettor and nuclease-free pipette tips
- Sealing film
- Multi-channel reservoirs
- 96-well microplates with a minimum capacity of 1 mL (Recommend Cat# SSI-Bio 775A-S0-62100 or Thermo 96-well deep V microplate for Kingfisher Flex Part No. 95040450)

Before Starting:

- Prepare buffers according to instructions on Page 5.
- Vortex the Mag-Bind® Particles CNR thoroughly before use.

1. Prepare a mastermix of OTRK Lysis Buffer and Proteinase K Solution according to the table below.

Component	Amount per Prep	Total Amount per 96-well Plate
OTRK Lysis Buffer	450 µL	47.5 mL*
Proteinase K Solution	20 µL	2.1 mL*

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

2. Choose one of the two following methods for homogenization of samples:

A. Manual Sample Preparation

To prepare samples, collect fresh tissue sample in a 30 mL mortar and freeze by dipping in liquid nitrogen using tweezers or tongs to fill the tube. Grind the tissue using a clean pestle. Transfer the grounded powder and liquid nitrogen into 96-well deep-well plate and allow the liquid nitrogen to evaporate. Add 470 µL OTRK Lysis Buffer/Proteinase K Solution mastermix.

B. Mechanical Tissue Disruption:
Place sample into a stainless steel grinding plate with appropriate steel beads. Add 470 µL OTRK Lysis Buffer/Proteinase K Solution mastermix to the samples. Grind sample at 30 Hz for 1-2 minutes according to manufacturer's instructions. For GenoGrinder 2000/2010, grind at 1500 RPM for 2 minutes. Remove the plate from the homogenizer and remove the caps. It may be necessary to centrifuge the plate briefly to remove debris from the caps.

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

4. Transfer 400 µL lysate to a 96-well plate. Do not disturb the debris pellet.

   Note: The 96-well plate must have minimum volume of 1.0 mL and be compatible with the magnetic stand used.

5. Add 300 µL 100% ethanol and 20 µL Mag-Bind® Particles CNR to each sample.

   Note: The Mag-Bind® Particles CNR will settle and clump together at the bottom of the bottle during storage. Vortex the Mag-Bind® Particles CNR thoroughly before use. Mag-Bind® Particles CNR and ethanol can be made as a mastermix.

6. Vortex for 5 minutes at room temperature.

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

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

9. Remove the plate from the magnetic separation device.

10. Add 400 µL VHB Buffer to each sample.

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

11. Resuspend the Mag-Bind® Particles CNR by vortexing for 3 minutes or pipetting up and down 20 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CNR.

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

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

14. Remove the plate from the magnetic separation device.

15. Add 400 µL RNA Wash Buffer II to each sample.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

16. Resuspend the Mag-Bind® Particles CNR by vortexing for 2 minutes or pipetting up and down 20 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CNR.

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

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

    Note: All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait one minute and remove any residual liquid from the well.

19. Let sit at room temperature for 5 minutes to dry the samples. Remove residual liquid with a pipettor.

20. Remove the plate from the magnetic separation device.

21. Add 100 µL RNA Elution Buffer to each sample.

22. Vortex at room temperature for 5 minutes. While waiting, prepare the Mag-Bind® DNase I mix according to the table below.

Component	Amount per Prep	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.

23. Add 52 µL Mag-Bind® DNase I mix to each sample. Mix by pipetting up and down to fully resuspend the magnetic beads.

24. Let sit at room temperature for 10 minutes.

25. Add 150 µL PHM Buffer to each sample. Mix by vortexing for 1 minute.

26. Add 300 µL RNA Wash Buffer II to each sample.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

27. Vortex the samples for 10 minutes.

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

29. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

30. Remove the plate from the magnetic separation device.

31. Add 400 µL RNA Wash Buffer II to each sample.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

32. Resuspend the Mag-Bind® Particles CNR by vortexing for 2 minutes or pipetting up and down 20 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CNR.

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

34. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

35. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

    Note: All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait one minute and remove any residual liquid from the well.

36. Remove the plate from the magnetic separation device.

37. Add 50-100 µL RNA Elution Buffer to each sample.

38. Resuspend the Mag-Bind® Particles CNR by vortexing for 5 minutes.

    Note: Complete resuspension is required for efficient elution.

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

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

41. Store RNA at -80°C.

Cultured Cells Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge capable of 4,000*g* with swinging-bucket rotor for 96 well plates
- Adapter for 96-well deep-well plates
- Magnetic separation device
- Vortexer
- 100% ethanol
- Pre-chilled PBS if using cells grown in a monolayer
- Trypsin if using cells grown in a monolayer
- Multi-channel pipettor and nuclease-free pipette tips
- Sealing film
- Multi-channel reservoirs
- 96-well microplates with a minimum capacity of 1 mL (Recommend Cat# SSI-Bio 775A-S0-62100 or Thermo 96-well deep V microplate for Kingfisher Flex Part No. 95040450)

Before Starting:

- Prepare buffers according to instructions on Page 5.
- Vortex the Mag-Bind® Particles CNR thoroughly before use.

1. Prepare a master mix of OTRKLysis Buffer and Proteinase K Solution according to the table below.

Component	Amount per Prep	Total Amount per 96-well Plate
OTRK Lysis Buffer	450 µL	47.5 mL*
Proteinase K Solution	20 µL	2.1 mL*

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

2. Harvest cells by choosing one of the following methods (A or B).

   A) For cells grown in suspension:
   1. Determine the number of cells. Do not use more than 1 x 10^6 cells.
   2. Pellet the appropriate number of cells by centrifuging at 500*g* for 5 minutes.
   3. Add 470 µL OTRK Lysis Buffer/Proteinase K Solution mastermix to each sample.
   4. Pipet up and down several time to mix the samples.

B) For cells grown in a monolayer:

These cells can either be lysed directly in the cell culture dish or trypsinized and collected as a cell pellet prior to lysis. Cells grown in cell culture flasks should always be trypsinized.

Direct cell lysis:

1. Determine the number of cells.
2. Aspirate the cell culture medium completely.
3. Add 470 µL OTRK Lysis Buffer/Proteinase K Solution mastermix to each sample.
4. Pipet up and down several time to mix the samples.

Trypsinization of cells:

1. Determine the number of cells.
2. Aspirate the cell culture medium completely.
3. Wash cells with 4°C PBS.
4. Aspirate the PBS.
5. Wash cells with 4°C PBS containing 0.1-0.25% trypsin.
6. Check cells for detachment. Make sure cells are detached before proceeding.
7. Add cell culture medium containing serum to inactivate the trypsin.
8. Transfer cells to an RNase-free microplate.
9. Centrifuge at 500*g* for 5 minutes.
10. Aspirate the supernatant completely.
11. Add 470 µL OTRK Lysis Buffer/Proteinase K Solution mastermix to each sample.
12. Pipet up and down several time to mix the samples.

Note: Not removing cell-culture medium completely will inhibit lysis and dilute the lysate. This will affect the conditions for binding of RNA to the Mag-Bind® Particles CNR.

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

4. Transfer 400 µL lysate to a 96-well plate. Do not disturb the debris pellet.

Note: The 96-well plate must have a minimum volume of 1.0 mL and be compatible with the magnetic separation device used.

5. Add 300 µL 100% ethanol and 20 µL Mag-Bind® Particles CNR to each sample.

   Note: The Mag-Bind® Particles CNR will settle and clump together at the bottom of the bottle during storage. Vortex the Mag-Bind® Particles CNR thoroughly before use. Mag-Bind® Particles CNR and ethanol can be made as a mastermix.

6. Vortex for 5 minutes at room temperature.

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

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

9. Remove the plate from the magnetic separation device.

10. Add 400 µL VHB Buffer to each sample.

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

11. Resuspend the Mag-Bind® Particles CNR by vortexing for 2 minutes or pipetting up and down 20 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CNR.

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

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

14. Remove the plate from the magnetic separation device.

15. Add 400 µL RNA Wash Buffer II to each sample.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

16. Resuspend the Mag-Bind® Particles CNR by vortexing for 1 minute or pipetting up and down 20 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CNR.

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

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

    Note: All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait one minute and remove any residual liquid from the well.

19. Let sit at room temperature for 5 minutes to dry the samples. Remove any residual liquid with a pipettor.

20. Remove the plate from the magnetic separation device.

21. Add 100 µL RNA Elution Buffer to each sample.

22. Vortex at room temperature for 5 minutes. While waiting, prepare the Mag-Bind DNase I mix according to the table below.

Component	Amount per Prep	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.

23. Add 52 µL Mag-Bind® DNase I mix to each sample. Mix by pipetting up and down to fully resuspend the magnetic beads.

24. Let sit at room temperature for 10 minutes.

25. Add 150 µL PHM Buffer to each sample. Mix by vortexing for 1 minute.

26. Add 300 µL RNA Wash Buffer II to each sample.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

27. Vortex the samples for 10 minutes.

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

29. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

30. Remove the plate from the magnetic separation device.

31. Add 400 µL RNA Wash Buffer II to each sample.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

32. Resuspend the Mag-Bind® Particles CNR by vortexing for 1 minute or pipetting up and down 20 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CNR.

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

34. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

35. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

    Note: All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait one minute and remove any residual liquid from the well.

36. Remove the plate from the magnetic separation device.

37. Add 50-100 µL RNA Elution Buffer to each sample.

38. Resuspend the Mag-Bind® Particles CNR by vortexing for 5 minutes.

    Note: Complete resuspension is required for efficient elution.

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

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

41. Store RNA at -80°C.

Troubleshooting Guide

Please use this guide to solve any problems that may arise. We hope that it will aid in clearing up any questions for you. If for any reason you need further assistance, please contact our technical support staff at our **Toll Free Number 1-800-832-8896.**

Possible Problems and Suggestions

Problem	Likely Cause	Suggestions
Low RNA yields	Incomplete resuspension of Mag-Bind® Particles CNR	Resuspend the Mag-Bind® Particles CNR by vortexing before use.
	RNA degraded during sample storage	Make sure the sample is properly stored and make sure the samples are processed immediately after collection or removal from storage.
	Loss of Mag-Bind® Particles CNR during procedure	Be careful not to remove the Mag-Bind® Particles CNR during the procedure.
	Ethanol was not added to RNA Wash Buffer II	Add 100% ethanol to RNA Wash Buffer II as instructed on Page 5.
	Mag-Bind® Particles CNR not resuspended during binding	Vortex vigorously for 2 minutes after addition of ethanol and Mag-Bind® Particles CNR.
Problem with downstream application	Insufficient RNA was used	RNA in the sample already degraded. Do not freeze/thaw the sample more than once. Do not store at room temperature.
Carryover of the Mag-Bind® Particles CNR in the elution	Carryover of the Mag-Bind® Particles CNR in the eluted RNA will not effect downstream applications.	To remove the carryover Mag-Bind® Particles CNR from the eluted RNA, simply place the plate on the magnetic separation device and wait until the eluate has cleared. Carefully transfer the RNA eluate to a new 96-well plate.

Notes:

Notes:

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

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

omega BIO-TEK

innovations in nucleic acid isolation

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