IsoBind Marine RNA Kit

Catalog No. IB-FRNA-100

System: Silica spin columns (manual workflow)

Sample types: fish tissue, brain and gills. However, this kit can be adapted to accommodate a variety of other marine tissue types

USER MANUAL

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1. KIT CONTENTS

The IsoBind Marine RNA Kit by Gene Vantage is meticulously designed for the efficient and reliable isolation of fish tissues, ensuring high-quality results for various applications. The kit components are optimised for ease of use and compatibility with marine sample types. This section details each component included in the kit, its function, and the volume provided, tailored for both small-scale and semi-automated applications.

Component	Description/ Function	Volume per Sample	Short Term Storage	Long Term Storage	Total for 100 Samples
Lysis Buffer	Lyses cells to release RNA. Specially formulated to ensure complete lysis of cellular material for optimal yield.	600 µL	Room temperature	Room temperature	60 mL
Proteinase K	Enhances lysis by breaking down proteins, facilitating more efficient RNA release.	50 µL	Room temperature	-20°C	2mL
Binding Buffer *Add Isopropanol (95%) prior to use	Facilitates binding of RNA to the silica membrane.	0.7x volume of lysate	Room temperature	4-8°C	Variable
Wash Buffer A * Add EtOH (90-100 %) prior to use	Removes impurities such as cellular debris and proteins without stripping away the bound RNA	800 µL	Room temperature	Room temperature	80 mL
Wash Buffer B * Add EtOH (96-100 %) prior to use	Removes residual salts without stripping away the bound RNA	800 µL	Room temperature	Room temperature	80 mL
Wash Buffer C * Add EtOH (96-100 %) prior to use	Final wash to remove remaining traces of chaotrophic agents	800 µL	Room temperature	Room temperature	70 mL

Elution Buffer	Elutes purified RNA from the column	100 µL	Room temperature	4-8°C	5 mL
Silica Spin Columns	Silica matrix that selectively binds RNA while allowing other compounds to pass through it	1 column + collection tube	Room temperature	Sealed in ziplock at 4-8°C	100 units

 Buffers contain skin irritants  Wear gloves

2. IMPORTANT NOTES

Before beginning your work with the Gene Vantage Isobind Marine RNA Kit, please take a moment to review these important notes. Adhering to these guidelines will ensure optimal results and efficiency throughout your extraction process.

Sample Preparation: Achieving a homogeneous sample is crucial for consistent RNA yields. Particularly with complex tissues, thorough mechanical breakdown is necessary to ensure all cells are lysed and RNA is accessible. Use a bead mill or tissue homogeniser for solid tissues such as gills and brains and ensure complete mixing with the lysis buffer.

RNAlater Removal: We do not recommend storage of tissues in RNAlater. However, if this is unavoidable please ensure you the RNAlater is fully removed using RNAlater removal columns prior to proceeding with the extraction process. If resampling is possible, resample and process immediately or store collected samples in Lysis Buffer for a few days prior to processing.

Handling of Samples: Biological samples should be handled with care to prevent degradation of RNA. Keep samples on ice when possible during preparation and process them promptly after collection to minimise RNA breakdown.

Buffer Preparation: Prior to use, inspect all buffers for precipitation which can occur due to cold storage or prolonged shelf life. If precipitates are observed, gently warm the buffers to 37°C, stirring until the solids have dissolved. Cool the buffers to room temperature before application to prevent thermal degradation of RNA.

Centrifugation Parameters: Follow the kit's specified centrifugation speeds and times rigorously. These parameters are optimised to ensure maximum recovery of RNA while effectively separating it from proteins, lipids, and other cellular debris. Deviations might lead to lower yields or contamination of the eluted RNA.

Maximum Capacity: To prevent column clogging and ensure efficient RNA purification, do not exceed the recommended sample volume and loading capacity of the spin columns. Overloading can lead to incomplete binding of RNA to the column or carryover of impurities.

Component Stability: Proper storage of kit components is critical for maintaining their efficacy. Store enzymes and sensitive reagents at temperatures specified in the kit documentation to preserve their activity and shelf life. Most reagents in this kit are stable at room temperature, but always check the label for specific storage instructions.

Concentration and Yield: The elution volume can be adjusted based on the desired concentration. A smaller volume results in higher concentration but may reduce overall yield. It's important to balance these factors based on the requirements of subsequent applications.

Optimal Recovery: For optimal recovery, ensure that the elution buffer is in direct contact with the entire surface of the silica membrane by allowing it to incubate on the bench for 2 minutes before centrifuging during the elution step.

Technical Support: Gene Vantage offers comprehensive technical support. If you encounter any issues or have questions about the kit's usage, do not hesitate to contact our technical support team. We are here to help you achieve the best possible results with our products.

3. SAFETY PRECAUTIONS

Ensure the safety of all laboratory personnel by adhering to standard laboratory practices when using the Isobind Marine RNA kit.

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. Guanidine salts can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Many of the reagents included in the kit are chemical in nature and should be handled in a well-ventilated area. Users should be familiar with the safety data sheets (SDS) for each chemical component for information on potential hazards and first aid measures in case of accidental exposure.

Treat all samples as potentially infectious material. Following the universal precautions for handling biological materials will help protect not only the individual conducting the experiment but also the wider laboratory environment.

Dispose of all waste materials according to your institution's safety guidelines and regulations. This includes the proper disposal of used reagents, consumables, and biological waste to mitigate any potential hazards.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

4. KIT PRINCIPLES

The IsoBind Marine RNA Extraction Kit is crafted for the efficient extraction of RNA employing silica spin column technology. This extraction kit is specifically engineered for the isolation of RNA from fish sample types, including brain, gills and tissue. This kit ensures the extraction of high-quality viral RNA suitable for a variety of downstream applications.

Lysis: The fist crucial step involves the breakdown of cell membranes to release RNA into the solution. Effective lysis is key to ensuring that RNA is accessible for subsequent binding. This kit uses a Lysis Buffer, which contains a combination of surfactants and a buffering agent that disrupts cellular and nuclear membranes. The addition of Proteinase K, an enzyme, aids in digesting proteins that could otherwise bind RNA and interfere with the extraction process. This enzymatic treatment further ensures that RNA is fully liberated from the cellular membrane. This lysis reaction is enhanced by incubating the mixture at 56 degree Celsius which optimises the activity of Proteinase K and ensure complete lysis. An additional lysis step of bead beating can be added to ensure complete mechanical lysis of the tissues. An optional, separation step with chloroform and Viral RNA Lysis Buffer can be performed to separate RNA from dissolved lipids and proteins, as well.

Binding: Following lysis, the free RNA must be selectively captured or bound while other cellular debris and impurities are excluded. RNA in the lysate binds to a silica membrane within the spin column when in the presence of the Binding Buffer. This promotes the adherence of RNA to the silica membrane due to the formation of of hydrogen binds between the negatively charge phosphate groups of the RNA and the positively charged surface of the the silica. This step is crucial as it determines the yield of RNA.

Washing: Clean RNA is essential for sensitive applications like PCR. The kit contains three sequential wash buffers (Wash Buffers A, B and C), each designed to efficiently remove different molecules. Wash Buffer A primarily removes proteins and other large molecules, Wash Buffer B is designed to eliminate smaller molecules and salts and Wash. Buffer C ensures that no residual wash buffer remains on the spin column matrix. Each wash involves adding a specific volume of buffer, followed by centrifugation to pull the liquid through the column while the RNA remains bound to the silica membrane. This ensures that only purified nucleic acid remains on the column.

Elution: The final step is to release the puffed RNA from the silica membrane for use in downstream applications. Elution is achieved by applying an Elution offer, which disrupts the hydrogen bond between the RNA and the silica, allowing the silica to be released into the buffer. The elution buffer is pre-heated to enhance the efficiency of the RNA recovery. The RNA is collected by centrifugation, which forces the eluted RNA into a clean micro centrifuge tube.

Key Features:

Quality of Output: Utilises advanced silica-based spin column technology, which selectively binds RNA while efficiently removing contaminants. This results in RNA with high purity, characterised by optimal A260/A280 ratios typically ranging between 1.8 and 2.0, indicating minimal protein contamination and readiness for sensitive downstream applications.

Comprehensive Cell Disruption: The Lysis Buffer, Proteinase K and optional bead beating combination effectively disrupts a wide variety of cell types, ensuring complete release of RNA.

Time Efficiency: The entire RNA extraction process can be completed in approximately 45 minutes for 24 samples, which is ideal for labs seeking to maintain their turn around times without compromising on the quality of results.

Ease of Use: The protocol is designed to be straightforward with clear step-by-step instructions, reducing the potential for operator error and the need for extensive training.

Streamlined Approach: Specifically optimised for the extraction of RNA from a variety of marine and fish samples, the kits robust lysis and binding conditions are effective in isolating high quality RNA.

Compatibility with Downstream Applications: The high-quality RNA extracted is suitable for a variety of molecular biology techniques, including PCR, qPCR and next-generation sequencing, ensuring broad applicability.

Scalability: The kit is suitable for both low and high-volume sample processing, with options for manual (individual spin column) and semi-automated (96 well spin plates) workflows. This flexibility allows laboratories of all sizes to integrate this kit into their existing workflows efficiently.

Note: Please engage with **Gene Vantage** technical support (see above: Important Notes) should you require a higher throughput.

5. HARDWARE AND CONSUMABLES (SUPPLIED BY THE USER)

5.1 Hardware

Centrifuge: A high-speed centrifuge capable of achieving at least 13,000 x g is essential for the effective sedimentation of cellular debris and the precise separation of supernatants during the RNA extraction process.

The centrifuge must be reliable and capable of maintaining consistent speeds to avoid variations that could affect the purity and yield of the extracted RNA. A temperature control feature to protect sensitive samples from heat degradation during extended spin cycles.

* Tissue Lyser: Used during the bead beating step to ensure mechanical disruption of the tissue samples. Crucial for ensuring complete mechanical lysis of the sample tissue.

Vortex Mixer: A vortex mixer is required to thoroughly mix samples with lysis and binding buffers, which is crucial for the complete lysis of cells and the homogeneous suspension of RNA within the solution. This ensures maximum contact between the RNA and the silica binding surface, increasing the efficiency of RNA recovery.

Thermomixer/ heating block/ oven: Required for the incubation of samples at controlled temperatures during the lysis and elution steps. The ability to set precise temperatures is essential, as optimal lysis conditions can vary depending on the sample type and the specific requirements of the RNA extraction protocol.

5.2 Consumables

* RNAlater Removal Columns: If storing tissues in RNAlater, it is imperative that you remove the RNAlater prior to proceeding with the RNA extraction protocol as RNAlater has compounds that may interfere with the extraction process which can lead to a decreased yield and/or lower quality of purified RNA. Please see the **Section 6: Quick View Protocol** or S**ection 10: Complete Protocol** for information on how to remove the RNAlater.

Microcentrifuge Tubes (1.5 or 2 mL): Used for sample preparation and for collecting the eluted RNA.

Pipettes and Aerosol-Barrier Pipette Tips: Precision pipettes and aerosol-barrier tips are crucial for the accurate measurement and transfer of fluids, which is vital for maintaining the correct

buffer ratios and avoiding cross-contamination between samples. This is particularly important when working with infectious agents or when performing multiple extractions to ensure reproducible and reliable results.

The pipettes should be regularly calibrated to ensure accuracy, and the tips should be certified RNase-free to prevent the degradation of RNA by residual enzymatic activity.

Ethanol (96-100%, molecular grade): Added to wash buffers to help in washing away impurities without stripping the RNA from the column.

Isopropanol (95%, molecular grade): Added to binding buffer to improve the yield and quality of RNA by ensuring more efficient binding of RNA to the column.

* DNase: Deoxyribonuclease (DNase) is used to remove DNA contamination from RNA samples, ensuring that the extracted RNA is pure and suitable for downstream applications. This is an optional step that can be included at the users discretion should the downstream assay require it (e.g cDNA synthesis)

* Chloroform: Used as part of an additional lysis step in conjunction with the Viral RNA Lysis buffer (supplied as part of the kit). Denatures and disrupts cellular and nuclear membranes allowing the RNA to be released from the cell and available for the extraction process.

* indicates an optional step in the RNA extraction process

6. QUICK VIEW PROTOCOL

Step	Procedure	Details
Sample Collection and Storage	Collect marine samples and process immediately or store them in lysis buffer for a few days > Proceed to tissue homogenisation step. For samples stored in RNAlater, decant sample into RNAlater removal columns > Spin for 30 seconds @ 2000 rpm > Discard flow through > retrieve tissue with an inoculation loop and place into provided bead beating tubes	If possible, process samples immediately after collection. If this is not an option, store the samples in the Lysis Buffer (**We do not recommend storing samples in RNAlater)** until they are able to be processed. Samples will be preserved for a few days at room temp. in the lysis buffer. If you have used RNAlater, remove the RNAlater before proceeding to the lysis step.
Tissue Homogenisation	Place tissue into bead beating tubes > Add steel beads + 150 ul of Crushing reagent + 50 ul BME > Crush in Tissue Lyser	Mechanical lysis via bead beating ensures proper tissue homogenization and release of RNA
Lysis	Transfer crushed tissue to new 1.5mL tube > Add 600 uL of Lysis Buffer + 50 uL Proteinase K > Vortex > Incubate at 37 C for 8 minutes > Spin @ 12 000 g for 5 mins.	Check Lysis Buffer for any precipitate and dissolve if necessary by heating. Optional second cell lysis step using chloroform can be performed: Add 400 ul chloroform + 200 ul Viral RNA lysis buffer to sample > incubate for 2 min. on the bench and then spin @ 12000 rpm for 5 min
Binding	Transfer supernatant to new microcentrifuge tube > Add 0.7 volume of Binding buffer + 5 ul of Booster > Vortex > Load sample onto spin column placed in a collection tube > Spin @ 12000 rpm for 2 minutes > Discard flow through > Reuse collection tube	Volume of lysate to binding buffer should be 0.7:1 e.g. 400 µl sample + 280 µl Binding Buffer + 5 ul Booster. An optional on-column DNAse treatment can be performed here to remove trace DNA molecules

Washing	Add 800 uL Wash A > Spin @ 12 000 g > Discard flow through > Repeat for Wash Buffers B and C	Ensure all wash buffer passes through column
Dry Centrifuge	Spin @ max speed for 2 minutes to dry column	This step is crucial to ensure there is no residual buffer bound to your column
Elution	Place spin column in new tube > Add 100 uL Elution Buffer > spin at 12 000 x g for 1 minute > process eluted RNA immediately or store	Store RNA at -20°C for short term storage or -80°C for long term storage

7. KIT SPECIFICATIONS

Parameter	Specification
Format	Silica spin column
Sample Material	Fish tissue (muscle, gill, brain)
Typical Yield	Varies depending on sample type
Purity Ratios	A260/A28 >= 2.0
Elution Volume	30-100 μL
Preparation Time	Approximately 30 minutes
Binding Capacity	Up to 25 μg RNA per column

8. WORKFLOW TIPS

To maximize the effectiveness and reliability of the IsoBind Marine RNA Kit, it is crucial to consider additional aspects of the extraction process that impact both the quality of the RNA obtained and the user's experience. These additional suggestions provide guidance on sample quality and preparation, elution efficiency, and quality control measures:

COLLECTION AND STORAGE OF STARTING MATERIAL

Immediate Processing: Ideally, samples should be processed immediately after collection to minimise RNA degradation. If immediate processing is not possible, samples must be handled and stored carefully to preserve their integrity.

Solid Tissue Samples: Solid tissues such as gills and brain require immediate stabilisation. Flash-freezing in liquid nitrogen is recommended directly after collection to rapidly halt RNAse activity. Store frozen tissues at -80°C in airtight containers with minimal air space to prevent freezer burn, which can degrade tissue integrity and RNA quality. You can also store it in lysis buffer for a few days prior to processing.
Storage Considerations: Long-term storage conditions play a crucial role in maintaining RNA integrity. Samples stored at -80°C are generally stable for several years. Avoid frequent temperature fluctuations, as these can lead to ice crystal formation and mechanical breakdown of cellular structures, facilitating RNase activity. For short-term storage (a few days to weeks), refrigerating samples at -20°C may be adequate, but is not recommended for samples sensitive to partial degradation.

SAMPLES SIZE CONSIDERATIONS

Adjustments Based on Material: The amount of starting material can greatly influence the efficiency of RNA extraction. For optimal results, adjust buffer volumes and processing methods proportionally:

For tissue samples, use 20-25 mg per extraction.
Increase buffer volumes accordingly if starting with larger sample sizes to ensure complete lysis and effective RNA binding.

9. PREPARING BUFFERS AND EQUIPMENT

Before Starting:

Centrifuges: Before beginning any procedures, ensure that the centrifuge is functioning correctly. Perform a test run to check for any unusual noises or vibrations that could indicate a maintenance issue. Ensure that the rotor is securely fastened and that the lid closes properly.

Pipette Calibration: Regular calibration of the centrifuge is crucial for achieving the precise speeds necessary for optimal RNA isolation. Inaccuracies in speed can lead to inefficient separation of phases, potentially contaminating the RNA sample or resulting in lower yields.

Cleaning: Clean the centrifuge and rotor regularly to prevent the buildup of dust and biological material, which could interfere with operations or contaminate samples. Use appropriate disinfectants to wipe down the interior and rotor, especially after handling potentially infectious samples.

Pipettes: Accuracy Verification: Verify the accuracy of all pipettes before use. This can be done by pipetting distilled water onto a precision scale to check if the dispensed volumes are within the manufacturer's specified tolerance.

Calibration: Calibrate pipettes regularly according to the manufacturer's guidelines to ensure they dispense volumes accurately, which is critical for the precise preparation of buffers and reagents. Maintenance: Clean pipettes frequently to prevent cross-contamination between samples. Check the pipette tips for any residual sample before each use, and replace pipette tips between samples to maintain sample integrity.

Vortex Mixer: Ensure that the vortex mixer is operating correctly. Test the mixer by running it at different speeds to ensure it can provide the vigorous agitation needed for thorough mixing of lysis buffers with samples.

Stability: Check the stability of the vortex mixer on the bench to prevent any movement during operation, which could affect the homogeneity of sample mixing.

Balance Calibration and Accuracy: Regularly check and calibrate balances used to weigh samples or reagents to ensure precision. Incorrect measurements can alter the concentration of reagents, affecting the efficiency of the RNA extraction.

Cleanliness: Keep the balance area clean and free from vibrations and drafts, which could affect the accuracy of measurements.

Preparation: Prepare all consumables in advance by arranging them in an orderly manner on the workstation. This organisation helps prevent confusion and potential contamination during the extraction process. Ensure that all reagents are within their expiration dates and have been stored under the correct conditions. Any reagent that appears cloudy or precipitated should be warmed gently, if permissible, and mixed thoroughly to redissolve any solids.

Workspace Preparation: Disinfect the workspace thoroughly before starting the extraction to create an RNase-free environment. Use RNase decontamination solutions and maintain clean bench practices throughout the procedure.

10. COMPLETE PROTOCOL

1. Sample Storage

1.1. **Tissues for immediate processing:** Proceed to tissue homogenization step

1.2. **Tissues stored in lysis buffer**: Decant lysis buffer and proceed to tissue homogenization step

1.3. **Samples stored in RNAlater**: Decant the sample into RNAlater removal columns. Centrifuge for **30 seconds @ 2000 rpm** or until the visible RNAlater buffer has moved into the collection tube & can be discarded.

1.4. Retrieve the tissue with an inoculation loop and load into bead beating tubes for crushing.

2. Tissue Homogenization

2.1. Load **20 ug** of sample into a microcentrifuge tube

2.2. Add s**teel beads + 150 ul of Crushing Reagent + 50 ul BME** to the sample tube

2.3. Crush in Tissue Lyser until the sample has be completely homogenized

3. Lysis

3.1. Add **600 µl Lysis Buffer + 50 ul Proteinase K** to the sample. Vortex to mix thoroughly.

3.2. Incubate samples **@ 37 °C for 8 min**.

* Optional: Cool samples to ambient temperature and add **400 ul chloroform + 200 ul Viral RNA lysis buffer**, vortex thoroughly. Incubate at room temperature for 2 minutes and proceed to the centrifugation step.

3.3 . Centrifuge samples at **12 000 x g for 5 minutes.**

4. Binding

4.1. Transfer supernatant to a new 1.5 mL microcentrifuge tube.

4.2. Add **0.7 volume of Binding Buffer** eg. 400 µl sample + 280 µl Binding Buffer.

4.3. Add **5 ul of Booster Compound** and vortex thoroughly to mix.

4.4. Load onto a silica spin column placed in a collection tube.

4.5. Incubate for **2 minutes** at room temperature.

4.6. Centrifuge at **12 000 x g for 2 minutes** and discard flow through. Reuse the collection tube

* Optional: Add an on-column DNAse treatment here as per the manufacturer's instruction to degrade any trace DNA that might be bound to the column.

5. Washing

5.1. Add **800 ul Wash A** onto the silica spin column and incubate on bench for 2 minutes

5.2. Centrifuge at **12 000 x g for 2 minutes** and discard flow through. Reuse collection tube.

5.3. Add **800 ul Wash B** onto the silica spin column and incubate on bench for 2 minutes

5.4. Centrifuge at **12 000 x g for 2 minutes** and discard flow through. Reuse collection tube.

5.5. Add **800 ul Wash C** onto the silica spin column and incubate on bench for 2 minutes

5.6. Centrifuge at **12 000 x g for 2 minutes** and discard flow through. Reuse collection tube.

5.7. Transfer silica spin column back to collection tube and centrifuge at **12 000 x g for 2 minutes** to ensure all buffers have been removed from the column matrix. Discard the collection tube.

6. Elution

6.1. Transfer the silica spin column to a new, sterile microcentrifuge tube. Add **100 ul of pre-heated Elution buffer** directly to the silica spin column matrix.

6.2. Incubate for 2 minutes at room temperature.

6.3. Centrifuge for 2 **minutes at 12 000 x g** to retrieve RNA. Discard the silica spin column and keep the microcentrifuge tube with eluted RNA.

7. **Storage**

7.1. RNA is now ready for downstream applications. Store the extracted RNA at -20°C for short-term storage or -80°C for long-term storage.

11. TROUBLESHOOTING GUIDE

Problem Description	Possible Causes	Suggestions
Low RNA yield	Incomplete lysis of sample tissue	Ensure thorough homogenization of the sample. Increase the amount of Lysis Buffer if necessary.
	Insufficient sample quantity	Increase the amount of starting material, keeping within the recommended range for the kit
Poor RNA purity (low A260/A280 ratio)	Contamination with proteins or phenolic compounds	Do a second wash with Wash Buffer C
	Incomplete removal of ethanol	Extend dry centrifuge step to ensure all wash buffer has been removed
	Incomplete removal of RNAlater	Ensure that all RNAlater is removed using RNAlater removal columns before proceeding with the rest of the protocol. Extend the centrifugation step during the removal step or add in a dry centrifuge step to ensure all RNAlater is removed. If possible, resample and do not store samples in RNAlater, store in the lysis buffer provided as part of the kit.
RNA degradation	Harsh mechanical lysis	Reduce bead beating time. Avoid high speed centrifugation that might shear the RNA
	Improper storage of RNA	Store the eluted RNA at -20 degrees Celsius to -80- degrees Celsius for long term storage. Avoid repeated freeze thaw cycles
Inconsistent RNA yield	Variation in sample size or type	Standardize the amount and type of starting material.
	Inconsistent execution of the protocol	Follow the protocol steps precisely and consistently.
Clogged spin column	Excessive sample material	Reduce the amount of starting material or increase the volume of Lysis Buffer.
	Insufficient centrifugation	Increase the centrifugation speed or duration.

Contamination in RNA samples	Cross-contamination between samples	Use sterile equipment and consumables. Practice good laboratory hygiene.
	Contamination of reagents or equipment	Use fresh reagents and clean equipment before use.
Equipment malfunction	Centrifuge not reaching required speed	Check the centrifuge settings and performance. Calibrate or repair the centrifuge if necessary.
	Pipettes delivering inaccurate volumes	Calibrate pipettes regularly. Use pipettes with the correct volume range for the protocol.
Buffer Precipitation	Cold storage of buffers that should be at room temperature	Ensure all buffers are stored according to the specifications provided in the manual. Label storage containers clearly with the appropriate storage temperatures and routinely check storage conditions.
	Incorrect preparation of buffers	Adhere strictly to the buffer preparation instructions provided in the manual. Measure all components accurately and mix thoroughly
Difficulty in Eluting RNA	Spin Column membrane dried out	Pre wet the column before adding the elution buffer with a few micro litres of RNase free water. Ensure the spin column does not sit out for too long after the final wash step.
	Elution buffer not adequately heated.	Heat the elution buffer to the specified temperature. Let it incubate in the column before centrifugation. If yield is still low, perform a second elution

12. PRODUCT USE RESTRICTION / WARRANTY

GENE VANTAGE kit components are intended, developed, designed, and sold for research purposes only. All kit components are for general laboratory use only and should only be used by qualified personnel wearing the appropriate protective clothing. GENE VANTAGE does not assume any responsibility for damages due to improper application of our products in other fields of application. Any user, whether by direct or resale of the product, is liable for any and all damages resulting from any application outside of research.

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