IsoBind Viral RNA Kit

Catalog No. IB-VRNA-100

System: Silica spin columns (manual workflow)

Sample types: buccal swabs, buffy coat, whole blood, FTA cards, tissue, cultured cells, cartilage, body fluids, plant tissue

USER MANUAL

Website www.gene-vantage.com

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In Vitro Diagnostic Device

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1. KIT CONTENTS

The IsoBind Viral RNA Extraction Kit by Gene Vantage is meticulously designed for the efficient and reliable isolation of viral RNA, ensuring high-quality results for various applications. The kit components are optimised for ease of use and compatibility with a wide range of sample types, from buccal swabs to whole blood & FTA cards. 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 temp.	Room temp.	60 mL
Binding Buffer *Add Isopropanol (95%) prior to use	Facilitates binding of RNA to the silica membrane.	600 µL	Room temp.	4-8°C	60 mL
Wash Buffer A * Add EtOH (96-100 %) prior to use	Removes impurities such as cellular debris and proteins without stripping away the bound RNA	700 µL	Room temp.	Room temp.	70 mL
Wash Buffer B * Add EtOH (96-100 %) prior to use	Removes residual salts without stripping away the bound RNA	700 µL	Room temp.	Room temp.	70 mL
Wash Buffer C * Add EtOH (96-100 %) prior to use	Final wash to remove remaining traces of chaotrophic agents	700 µL	Room temp.	Room temp.	70 mL
Elution Buffer	Elutes purified RNA from the column	50 µL	Room temp.	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 u
Proteinase K	Enhances lysis by breaking down proteins, facilitating more efficient RNA release.	20 µL	Room temperature	-20°C	2mL

 Buffers contain skin irritants  Wear gloves

2. IMPORTANT NOTES

Before beginning your work with the Gene Vantage Isobind Viral RNA Kits, 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 or plant materials, 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 leaf and soil samples and ensure complete mixing with the lysis buffer.

- **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**: Buffer Inspection and Treatment: 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:** Optimal Speed and Time: 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 Viral RNA kit.

Personal Protective Equipment (PPE): Always wear appropriate personal protective equipment (PPE), including a lab coat, disposable gloves, and protective goggles, when handling chemicals or performing procedures with this kit.

Chemical Handling:

- Reagents containing guanidine salts must be handled with caution as they can form highly reactive and potentially hazardous compounds if combined with bleach.
- In the event of a spill involving these buffers, immediately clean the area with a suitable laboratory detergent and water.
- For spills involving potentially infectious agents, first clean the area with laboratory detergent and water, followed by disinfection with a 1% (v/v) sodium hypochlorite solution.

Ventilation and Awareness: All reagents included in the kit should be handled in a well-ventilated workspace to avoid inhalation of fumes or aerosols. Familiarise yourself with the Safety Data Sheets (SDS) for all chemical components to understand the potential hazards and obtain detailed first aid measures in case of accidental exposure.

Pathogen Safety: When working with pathogenic or potentially infectious samples (e.g., human blood), always treat all specimens as potentially infectious material. Adhere to universal precautions for handling biological samples to minimise risks to both the individual and the laboratory environment.

Waste Disposal: Dispose of all waste materials, including used reagents, consumables, and biological waste, in accordance with your institution's safety guidelines and relevant local regulations. Proper waste disposal protocols are critical to mitigating environmental and laboratory hazards.

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

4. KIT PRINCIPLES

The IsoBind Viral RNA Extraction Kit is crafted for the efficient extraction of viral RNA employing silica spin column technology. This extraction kit is specifically engineered for the isolation of viral RNA from a diverse array of sample types, including serum, plasma, cell-free body fluids, and swab samples. Clients have also successfully used this kit for the extraction of viral RNA from samples such as leaf tissue and municipal water. This kit ensures the extraction of high-quality viral RNA suitable for a variety of downstream applications.

- **Lysis:** The kit employs a denaturing lysis buffer that disrupts cells and viral particles, releasing nucleic acids and simultaneously inactivating RNases to protect RNA from degradation.
- **Binding:** RNA binds selectively to a silica membrane within the spin columns, facilitated by the buffer conditions optimised for RNA affinity. This specificity is crucial for eliminating most proteins and other contaminants.
- **Washing**: Multiple ethanol-based wash steps follow binding to remove residual impurities, ensuring the RNA's purity on the membrane, crucial for avoiding downstream analytical complications.
- **Elution**: Purified RNA is then eluted using a low-ionic strength buffer, which releases the RNA from the membrane for collection. This step is critical for maintaining the integrity and stability of the isolated RNA.

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 effectively disrupts a wide variety of cell types, ensuring complete release of RNA.
- **Enhanced Recovery:** Tailored for samples that are difficult to lyse, the system ensures that even tightly bound nucleic acids are made available for capture, which is critical for achieving consistent results across different sample types.
- **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 grape vine RNA, the kits robust lysis and binding conditions are effective in isolating high quality RNA for this complex tissue.
- **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 impact the purity and yield of the extracted RNA. A temperature control feature is beneficial, as it protects sensitive samples from heat degradation during extended spin cycles.

- **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 kit reagents, increasing the efficiency of RNA recovery, as well as its purity

- **Thermomixer/ heating block/ oven:** Required for the incubation of samples at controlled temperatures during the lysis and elution steps.

5.2 Consumables

- **Microcentrifuge Tubes (1.5 mL RNAse free):** 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 wash away impurities without stripping the RNA from the column.

- **Isopropanol (95%, molecular grade):** Added to the binding buffer to improve the yield and quality of RNA.

- **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).

6. QUICK VIEW PROTOCOL

Step	Procedure	Details
Sample Preparation (if applicable)	For solid starting materials, crush samples in round bottom safe lock tubes with a steel bead using a Tissue Lyser or any other applicable sample crusher.	For samples such as soil and plant tissue this step is crucial in ensuring complete cell lysis.
Lysis	Combine 200 uL sample with 150 ul lysis Lysis Buffer > Vortex	Check Lysis Buffer for any precipitate and dissolve if necessary by heating. Add a reducing agent at this step, if required
Binding	Add 0.6 volumes of Binding Buffer + 20 uL booster > Vortex > load onto column	Volume of lysate to binding buffer should be 1:1 i.e 500 uL: 500 mL
Washing	Add 700 uL Wash A > Spin @ 14 000 g > discard flow through. Repeat for Wash Buffers B and C	Ensure all wash buffer passes through column
Dry Centrifuge	Spin @ max speed for 1 minute to dry column	This step is crucial to ensure there is no residual buffer bound to your column
Elution	Place column in new tube > Add 50 uL Elution Buffer > spin at 14 000 x g for 1 minute	
Storage	Eluted RNA is suitable for immediate use or for storage	Store eluted RNA at -20 degrees for short term storage and -80 degrees for long term storage

7. KIT SPECIFICATIONS

Parameter	IsoBind Viral RNA Extraction Kit
Format	Spin columns
Sample Material	Serum, plasma, cell-free body fluids, swab samples, plants, FTA cards
Typical Yield	Depends on sample type and viral load
Elution Volume	30-100 µL
Preparation Time	Approximately 60 min/20 preps
Binding Capacity	Up to 25 µg of RNA

8. WORKFLOW TIPS

To maximize the effectiveness and reliability of the IsoBind Viral RNA Kit, it is crucial to consider additional aspects of the extraction process that impact both the quality of the DNA 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 plant materials 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.

- **Plant Tissues**: Plant tissues are particularly susceptible to enzymatic degradation because of their high RNase content. To ensure the integrity of RNA, clean and segment the plant material quickly after harvesting. Freeze plant segments in liquid nitrogen immediately after cleaning and cutting. Store the frozen samples at -80°C in RNase-free containers until ready for RNA extraction. Avoid thawing during transfer to preserve RNA quality.

- **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

Centrifuges

- Performance Check: 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.
- 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

- 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.

Workspace and consumable 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

A. Biological Fluids:

- For the isolation of viral RNA from < 200 ul of whole blood, nucleated blood, buffy coat, saliva, sputum, milk, etc.

- For urine, serum, and other low-concentration body fluids stored in storage buffers (e.g., Zymo DNA/RNA Shield or Invitrogen RNAlater), add 20 ul of Proteinase K to 400 ul of the sample mixture prepared. Mix thoroughly or vortex for 10-15 seconds and then incubate the tube at room temperature for 20 minutes.

Note: we do not recommend the use of RNAlater for storage of absorbent samples as it impacts the chemistry of the extraction process which will impact your yield and quality.

B. Swab Samples:

- For the isolation of viral RNA from swab samples, including buccal swabs and other types of swabs:

- Buccal Swabs: Thoroughly rinse the mouth with water before isolating cells. Brush the inside of the cheek with a buccal swab for 15 seconds, ensuring thorough coverage of the inner cheek area. Transfer the swab contents into a microcentrifuge tube containing a mixture of 300 ul of Lysis Buffer. Add 20 ul of Proteinase K and proceed with the lysis step as outlined in the protocol.
- Other Swabs: For each swab, mix 200 ul of the transport medium (saline or elution buffer; not PBS) with 200 ul of Lysis Buffer. Vortex thoroughly. Incubate the sample in a thermomixer for 15 minutes with shaking at approximately 600 rpm at 56°C. Proceed with the subsequent steps of the extraction protocol.

C. Serum and Plasma Samples:

- For the isolation of viral RNA from serum and plasma samples:

- Centrifuge the blood sample at 3,200 rpm for 15 minutes at room temperature to separate the serum or plasma. Carefully transfer 200 ul of the supernatant to a new tube. Add 200 ul of Lysis Buffer and 20 ul of Proteinase K. Mix well and incubate the sample at 56°C for 10 minutes with occasional mixing. Proceed with the binding step of the protocol.

Note: The detailed steps for the lysis, binding, washing, and elution phases are outlined in the main protocol section of the user manual. It is crucial to adhere to the recommended volumes and incubation times to ensure the efficient recovery of viral RNA. For specific sample types not mentioned here, please contact technical support for guidance.

Main Protocol - Purification of Viral RNA from Various Sample Types

1. Sample Preparation

- For serum, plasma, and cell-free body fluids: Transfer 140-200 µl of the sample to a microcentrifuge tube.
- For swab samples: Add 400 µl of Lysis Buffer to the swab and vortex vigorously to release the viral RNA into the buffer.
- For solid tissues: Transfer 20-25 mg of tissue to a bead beating tube and add 600 µl of Lysis Buffer.

2. Adjust Lysis Conditions

- Add an appropriate volume of Proteinase K to the sample (refer to the kit manual for specific volumes) and mix well.
- Incubate the mixture at room temperature for 5 minutes, with occasional mixing to ensure complete lysis.

3. Sample Lysis

- For solid tissues and swab samples, proceed with mechanical disruption using a bead beater or a vortex adapter for 5-10 minutes.
- For liquid samples, continue with gentle mixing or inversion to ensure complete lysis.

4. Clarify Lysate

- Centrifuge the lysed samples at 12,000 x g for 5 minutes to pellet debris and obtain a clear supernatant.
- Carefully transfer the supernatant to a new microcentrifuge tube, avoiding any transfer of the pellet.

5. Bind RNA

- Add an equal volume of Binding Buffer to the supernatant and mix well by pipetting.
- Add 20 ul booster
- Transfer the mixture to an IsoBind Viral RNA Spin Column placed in a collection tube and centrifuge for 1 minute at 8,000 x g
- Discard the flow-through.

6. Wash RNA

- Add 500 µl of Wash Buffer A to the column and centrifuge for 1 minute at 8,000 x g. Discard the flow-through.
- Add 500 µl of Wash Buffer B to the column and centrifuge for 1 minute at 8,000 x g. Discard the flow-through.
- Add 500 µl of Wash Buffer C to the column and centrifuge for 1 minute at 8,000 x g. Discard the flow-through.

7. Dry Column

- Centrifuge the empty column for 1-2 minutes at maximum speed to remove any residual wash buffer.

8. Elute RNA

- Place the column in a new microcentrifuge tube and add 30-50 µl of preheated (60-70°C) Elution Buffer directly to the center of the membrane.
- Incubate for 1 minute at room temperature, then centrifuge for 1 minute at 8,000 x g to collect the eluted RNA.
- Store the eluted RNA at -80°C for long-term storage or proceed to downstream applications.

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 incubation time	Extend the incubation time during the lysis step.
	RNA degradation	Use fresh samples or store samples properly. Add RNase inhibitors if needed.
Poor RNA purity (low A260/A280 ratio)	Contamination with proteins or phenolic compounds	Increase the number of wash steps. Ensure complete removal of Wash Buffers.
	Incomplete removal of wash buffers	Perform additional dry centrifugation steps to remove residual wash buffers.
RNA degradation	Improper sample storage	Store samples at -80°C or use immediately after collection.
	Prolonged exposure to room temperature	Keep samples and lysates on ice during the extraction process.
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

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