Amplimax KingPlex Viral RNA

Cat No. AM-VRNA-KP-200

System: Magnetic Beads, suitable for manual or automated workflows

Sample types: serum, plasma, nasopharyngeal swabs, bronchoalveolar lavage (BAL), urine, stool, sputum, whole blood, cervical swabs, urethral swabs

USER MANUAL

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In Vitro Diagnostics

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1. KIT CONTENTS

The AmpliMax KingPlex Viral RNA Kit is designed to provide an efficient and reliable method for the extraction of high-quality viral RNA from various sample types. Utilizing advanced magnetic bead technology, the kit ensures the isolation of RNA suitable for downstream applications such as RT-PCR.

Component	Description/ Function	Volume Required per Sample	Short Term Storage	Long Term Storage	Total for 50 Samples	Total for 100 Samples	Total for 1000 Samples
Lysis Buffer	Lyses cells to release RNA. Specially formulated to ensure complete lysis of cellular material for optimal yield.	500 µL	Room temperature	Room temperature	25 mL	50 mL	500mL
Binding Buffer *Add Isopropanol (95%) prior to use	Facilitates binding of RNA to magnetic beads	115 µL	Room temperature	4-8°C	5.75 mL	11.5 mL	115mL
Wash Buffer A * Add EtOH (96-100 %) prior to use	Removes impurities such as cellular debris and proteins without stripping away the bound RNA	400 µL	Room temperature	Room temperature	20 mL	40 mL	400mL
Wash Buffer B * Add EtOH (96-100 %) prior to use	Removes residual salts without stripping away the bound RNA	400 µL	Room temperature	Room temperature	20 mL	40 mL	400mL

Wash Buffer C * Add EtOH (96-100 %) prior to use	Final wash to remove remaining traces of chaotrophic agents	400 µL	Room temperature	Room temperature	20 mL	40 mL	400mL
Elution Buffer	Elutes purified RNA from the magnetic beads	100 µL	Room temperature	4-8°C	5 mL	10 mL	100mL
Magnetic Beads	Binds RNA in the presence of binding buffer	20 µL	Room Temperature	4-8°C	1 mL	2 mL	20mL
Booster	Enhances binding efficiency of RNA to magnetic beads	6 µL	4-8°C	-20°C	300 µL	600 µL	6mL
Proteinase K	Enhances lysis by breaking down proteins, facilitating more efficient RNA release.	20 µL	Room temperature	-20°C	1mL	2mL	20mL

 Buffers contain skin irritants

 Wear gloves

2. IMPORTANT NOTES

Before beginning your work with the Gene Vantage Amplimax KingPlex Viral 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: It is crucial that samples like tissues or cells are thoroughly homogenized before processing.For samples with a high fat content or fibrous material, additional mechanical or chemical disruption methods may be required.Bead beating using a Tissue Lyser can be included to ensure complete cellular disruption and RNA release. Proper homogenization is essential for consistent RNA yields.

Handling of Samples: This kit is designed to accommodate a wide range of sample types, including those with high levels of secondary metabolites, which are challenging for RNA extraction. Special attention should be given to these samples to ensure that all components interact effectively, achieving optimal lysis and binding. All samples should also be handled with care to prevent degradation of RNA.

Mag Beads: These are the cornerstone for the RNA purification process, ensuring high selectivity and binding capacity. Prior to use, ensure the beads are fully resuspended to achieve uniform consistency. This is crucial for reproducibility and efficiency in RNA recovery across samples. Do no centrifuge or freeze the beads, or they will have to be discarded.

Proteinase K: This enzyme is vital for digesting cellular proteins and releasing nucleic acids. It is sensitive to degradation by repeated freeze-thaw cycles; hence, aliquot and store for long-term use. The enzyme can be shipped at ambient. Short term storage is best at 2-8°C while long term storage (> 6 months) should be at -20°C.

Booster: This component is designed to augment the yield of RNA, especially when working with samples that have low nucleic acid concentrations. It must be added with precision as per the recommended volumes, considering the variation in nucleic acid content among different sample types.

Component Stability: All kit components are sensitive to extreme temperatures. Store them according to the specified conditions to prevent degradation. Ensure they are brought to the appropriate temperature before use, as many enzymatic and binding reactions are temperature dependent.

Cross-contamination Prevention: Use fresh pipette tips for each reagent and sample to prevent cross-contamination.

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.

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 Amplimax KingPlex Viral 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 AmpliMax KingPlex Viral RNA Kit is based on magnetic bead technology, which provides a highly efficient and reliable method for isolating viral RNA from a variety of sample types. The kit is designed to deliver high-quality RNA suitable for a range of downstream applications, including RT-PCR, qPCR, and sequencing. The basic principle behind the kit involves four main steps: cell lysis, RNA binding, washing, and elution.

Lysis: The kit utilizes a lysis buffer that efficiently disrupts cell membranes, releasing RNA into the solution. This is achieved through a combination of chemical and mechanical methods, ensuring the complete breakdown of cellular structures.

RNA Binding: The released RNA binds selectively to the magnetic beads in the presence of the binding buffer. The binding conditions are optimized to ensure high specificity and yield.The magnetic beads are coated with a material that selectively binds RNA, minimizing the capture of unwanted biomolecules.

Washing: The bound RNA is washed multiple times with wash buffers to remove impurities and contaminants. Each wash buffer is formulated to ensure the removal of specific types of contaminants. Wash Buffer A removes proteins and other cellular debris,Wash Buffer B targets residual salts and other soluble impurities and Wash Buffer C provides a final purification step to ensure the highest RNA purity.

Elution: The purified RNA is eluted from the magnetic beads using an elution buffer. The elution conditions are optimized to release the RNA while maintaining its integrity. The elution buffer is formulated to efficiently dissociate the RNA from the beads, ensuring maximum recovery.

Key Features:

Automation Compatibility: The kit is compatible with the Kingfisher Flex, which allows for hands-free operation and increases throughput, reducing the potential for human error and variability between runs.

Versatility: The kit is compatible with a diverse range of sample types, including plant tissues, cultured cells, and biological fluids. This versatility allows researchers to use the same kit for different experimental needs, streamlining the workflow and reducing the need for multiple specialized kits.

Quality of Output: The kit is engineered to maximize RNA recovery and purity. Typical yields are high, with an A260/A280 purity ratio consistently near 1.8, indicative of low protein contamination, which is ideal for high-sensitivity applications such as qPCR and sequencing.

Time Efficiency: The kit reduces the time from sample to analysis by streamlining the RNA extraction process, minimizing the steps involved, and thus significantly cutting down on the total time required to run the workflow.

Ease of Use: The kit provides a simplified workflow with minimal hands-on time, making it accessible to researchers of varying skill levels.

Technical Support: For any technical inquiries or issues with kit components, please contact Gene Vantage customer support. Our experts are ready to assist you in ensuring successful DNA extractions.

5. HARDWARE AND CONSUMABLES (SUPPLIED BY THE USER)

5.1 Hardware

Vortex Mixer: A vortex mixer is used to thoroughly mix samples and reagents at various stages of the protocol, ensuring homogenous mixtures and efficient binding of RNA to the magnetic beads.

Thermomixer or Heating Block: A thermomixer or heating block capable of maintaining precise temperatures is necessary for incubating samples during the lysis and elution steps. Consistent temperature control is crucial for effective cell lysis and efficient elution of RNA from the magnetic beads.

Magnetic Stand or Separator: A magnetic stand or separator is required for the separation of magnetic beads from the liquid phase. This equipment allows for the easy and rapid collection of beads, ensuring that the RNA remains bound to the beads while contaminants are washed away.

Tissue Lyser: Effective RNA extraction begins with thorough tissue disruption. A TissueLyser or similar mechanical disruptor is essential for breaking down tough plant cell walls, which is particularly critical for fibrous or woody samples. This equipment uses high-speed shaking with beads or steel balls to pulverize the plant material, ensuring that the cellular contents are fully accessible for subsequent chemical lysis.

5.2 Consumables

Filter Tips: Filter tips are necessary to prevent cross-contamination between samples and ensure the accuracy of pipetting. The use of filter tips is crucial for maintaining the integrity of the DNA/RNA samples and the reliability of the extraction process.

Microcentrifuge Tubes: Microcentrifuge tubes are used for the final collection and storage of the eluted DNA/RNA.

Disposable Pipette (25 mL): A disposable pipette (Cat. No. 4489, Corning) with a capacity of 25 mL is required for the transfer of larger volumes of reagents. This pipette is used during the setup of the extraction run to load reagents into the deep well plates or reagent troughs.

Ethanol (96-100%) and Isopropanol (95%), Molecular grade: These solvents are used in the washing steps to remove contaminants and impurities from the RNA/DNA. Ensure that they are of molecular biology grade for optimal results.

6. QUICK VIEW PROTOCOL

Step	Procedure	Details
Sample Preparation	For plant tissues, use a bead beater or mortar and pestle with liquid nitrogen to achieve fine homogenization. For cultured cells, resuspend pelleted cells in the lysis buffer, ensuring complete resuspension for optimal lysis.	Homogenize the sample thoroughly using these appropriate mechanical or chemical methods to ensure complete lysis.
Lysis	Add 500 µL of lysis buffer to the sample > incubate at room temperature for 10 mins.	Vortex intermittently during incubation step
Binding	Centrifuge for 1 min to clear Lysate> Transfer Lysate to a clear microcentrifuge tube > Add 115 µL of binding buffer + 6 µL of Booster Compound > Shake> Place on magnetic stand > Discard supernatant.	Pipette buffer off slowly so that you do not aspirate the magnetic beads

Washing	Add 400 µL of Wash Buffer A to magnetic beads > Incubate for 2min on the bench> Place the tube back on the magnetic stand> Discard supernatant once clear> Repeat steps for Wash Buffer B and then for Wash Buffer C.	Pipette buffer off slowly so that you do not aspirate the magnetic beads Vortex during incubation to ensure that there will be no clumping of beads
Elution	Add 100 µL of elution buffer> incubate for 2min>Place the tube on the magnetic stand> pipette supernatant and transfer the eluted RNA to a new microcentrifuge tube>Discard the magnetic beads.	

7. KIT SPECIFICATIONS

Parameter	Amplimax KingPlex Viral RNA Kit
Format	Magnetic Bead Based, Automated (Kingfisher Flex)
Sample Material	Viral samples from various biological fluids and Plant tissue.
Typical Yield	Up to 100 µg (depending on the sample and viral load)
Purity Ratio (A260/A280)	1.8 - 2.0
Elution Volume	50 - 100 µL
Preparation Time	Approx. 45 minutes
Binding Capacity	Up to 100 µg RNA per well

8. WORKFLOW TIPS

To maximize the effectiveness and reliability of the Amplimax KingPlex Viral 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

Plant Tissues: Fresh plant tissues should be flash-frozen in liquid nitrogen immediately after collection to prevent RNA degradation and stored at -80°C until ready for use. Avoid thawing the samples before processing to minimize RNA degradation. Process the samples directly from the frozen state.

Cultured Cells: For cultured cells, harvest the cells by centrifugation and remove the supernatant. Flash-freeze the cell pellet in liquid nitrogen and store at -80°C. Ensure that the cells are not exposed to RNases during collection and handling. Use RNase-free reagents and consumables.

Buccal Swabs and FTA Cards: Buccal swabs and FTA cards should be allowed to dry completely at room temperature in a sterile environment before storage.Store dried swabs and cards at room temperature in a sealed container with a desiccant to prevent moisture accumulation.

Blood and Serum Samples: Collect blood samples in anticoagulant-treated tubes (e.g EDTA) and store at 4°C for short-term storage or aliquot and freeze at -80°C for long-term storage. Serum samples should be separated by centrifugation, aliquoted, and stored at -80°C to prevent degradation.

SAMPLES SIZE CONSIDERATIONS

Plant Tissues: For fresh plant tissues, such as leaves or roots, use 50-100 mg of material. If the tissue is particularly rich in RNA, such as young leaves, the lower end of this range may be sufficient.

For tougher or woody tissues, such as stems or bark, it may be necessary to increase the amount of starting material to ensure adequate lysis and RNA release.

Cultured Cells: For cultured cells, use up to 1 x 10^6 cells per extraction. For cell types with low RNA content, you may need to increase the number of cells to obtain sufficient RNA yield. Ensure that the cells are evenly resuspended in the lysis buffer to maximize the efficiency of cell lysis and RNA release.

Adjusting Buffer Volumes: When increasing the amount of starting material, it is important to proportionally increase the volumes of lysis and binding buffers used in the protocol to maintain optimal buffer-to-sample ratios. For example, if you double the amount of starting material, you

should also double the volume of lysis and binding buffers to ensure complete lysis and efficient RNA binding.

Processing Methods: For larger sample sizes, consider processing the sample in multiple aliquots to avoid overloading the extraction columns or magnetic beads. Divide the sample into smaller portions, process each aliquot separately, and then pool the eluted RNA at the end of the procedure.

9. PREPARING BUFFERS AND EQUIPMENT

Before Starting

KingFisher System by Thermo Fisher: The AmpliMax KingPLex Viral RNA Kit is designed for compatibility with the KingFisher system, an automated platform that facilitates high-throughput magnetic bead-based nucleic acid purification.

Ensure that the KingFisher system is properly calibrated and has undergone routine maintenance checks according to the manufacturer's instructions. This will ensure consistent and reliable performance during the RNA isolation process.

Familiarize yourself with the KingFisher software and set up the appropriate program for the Amplimax KingPlex Viral RNA Kit protocol. The software allows for customization of parameters such as mixing speed, incubation times, and magnetic separation durations to optimize the extraction process.

Load the KingFisher system with the necessary consumables, including tip combs and deep-well plates, ensuring that they are correctly positioned and free from contamination.

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.

Centrifuge 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: 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.

Pipette 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.

Balances: 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 organization 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.

Magnetic Bead Suspension: Ensure that the magnetic beads are well-suspended before use. Vortex the beads and verify homogeneity to guarantee efficient RNA binding.

10. COMPLETE PROTOCOL

1. Setup and Preparation

1.1 Power on the KingFisher Flex system and ensure it is equipped with the correct magnetic head and block for your sample volume (e.g., 96 or 24 deep-well).

1.2 Place the deep-well plates in the designated positions outside the system for binding, washing, and elution steps.

1.3 Prepare 80% ethanol by mixing absolute ethanol with nuclease-free water.

2. Reagent Preparation

2.1 Vortex the Total Nucleic Acid Binding Beads to ensure they are well mixed.

2.2 Prepare the Binding Bead Mix by adding Binding Solution and Total Nucleic Acid Binding Beads into a new deep-well plate following the required volumes per sample.

3. Sample Preparation

3.1 Add Proteinase K to each sample well according to the sample volume.

3.2 Add the sample to the wells containing Proteinase K.

3.3 Gently mix the Binding Bead Mix again and add to each sample well.

4. Lysis

4.1 Add 500 uL lysis buffer to the sample.

4.2 Incubate the sample at room temperature for 10 minutes and vortex intermittently during the incubation step.

4.3 Transfer 500 uL of lysate to a clean microcentrifuge tube.

5. Binding

5.1 To the lysate, add 115 uL of Binding Buffer and 6 uL of Booster Compound.

5.2 Vortex the mixture thoroughly.

5.3 Incubate or shake using a thermomixer at room temperature for 5 minutes.

5.4 Place the tubes onto the magnetic stand for 2 minutes. Allow the magnetic beads to pellet at the bottom of the tube. Remove the tube from the magnetic stand.

5.5 Discard the lysate by slowly pipetting the lysate off the magnetic beads without aspirating the beads.

6. Washing

6.1 Add 400 uL of Wash Buffer A while the tube is off the magnetic stand.

6.2 Vortex the tube and incubate on the bench for 2 minutes.

6.3 Place the tube back onto the magnetic stand for 2 minutes. Allow the magnetic beads to pellet at the bottom of the tube. Remove the tube from the magnetic stand.

6.4 Discard the supernatant

6.5 Add 400 uL of Wash Buffer B while the tube is off the magnetic stand.

6.6 Vortex the tube and incubate on the bench for 2 minutes.

6.7 Place the tube back onto the magnetic stand for 2 minutes. Allow the magnetic beads to pellet at the bottom of the tube. Remove the tube from the magnetic stand.

6.8 Discard the supernatant

6.9 Add 400 uL of Wash Buffer C while the tube is on the magnetic stand.

6.10 Let the tube sit for 2 minutes to allow the magnetic beads to pellet at the bottom of the tube. Remove the tube from the magnetic stand.

6.11 Discard the supernatant

7. Elution

7.1 Add 100 uL of Elution while the tube is off the magnetic stand.

7.2 Vortex the tube and incubate on the bench for 2 minutes.

7.3 Place the tube back onto the magnetic stand for 2 minutes. Allow the magnetic beads to pellet at the bottom of the tube. Remove the tube from the magnetic stand.

7.4 Pipette the supernatant into a clean, sterile microcentrifuge tube. This contains your eluted RNA which is ready for downstream processing and/or storage. Discard the magnetic beads.

8. Run the Extraction

8.1 Load the instrument with the prepared plates.

8.2 Start the extraction program specifically designed for viral RNA extraction.

8.3 Once the run is complete, carefully remove the elution plate.

9. Storage

9.1 Transfer the eluted RNA into a clean tube or plate.

9.2 Store the RNA at -80°C for long-term storage or at -20°C if it will be used soon.

11. TROUBLESHOOTING GUIDE

Problem Description	Possible Causes	Suggestions
Low RNA yield	Inadequate sample homogenization	Ensure thorough homogenization of the sample. Increase the amount of starting material if necessary.
	Insufficient lysis	Increase the incubation time with Lysis Buffer. Ensure complete mixing of the sample with the buffer.
	Incomplete binding of RNA to magnetic beads	Verify that the magnetic beads are properly mixed with the lysate. Check the binding buffer ratio.
Poor RNA purity (low A260/A280 ratio)	Residual contaminants in the RNA sample	Increase the number of washing steps. Ensure complete removal of wash buffers during the washing steps.
	Contamination with phenol or other organic solvents	Avoid using phenol-based reagents. Use only RNase-free reagents and consumables.
RNA degradation	RNase contamination	Use RNase-free reagents and consumables. Handle samples and reagents with care to avoid contamination.

	Improper storage of RNA	Store RNA at -80°C immediately after extraction. Avoid repeated freeze-thaw cycles.
Inconsistent results between replicates	Variability in sample processing	Standardize the sample processing steps. Use the same amount of starting material for each replicate.
	Equipment calibration issues	Ensure that the KingFisher Flex system and other equipment are properly calibrated and maintained.
Equipment malfunction (KingFisher Flex system)	Mechanical or software issues	Refer to the KingFisher Flex system user manual for troubleshooting specific to the instrument. Contact Thermo Fisher Scientific support if the issue persists.
	Error messages or alerts on the KingFisher Flex system	Follow the on-screen instructions to resolve the issue. Check for any blocked or misaligned components.

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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