IsoBind Universal Pathogen Kit

Catalog No. IB-UPAT-100

Catalog No. IB-UPAT-250

System: Silica spin columns (manual workflow)

Sample types: biological fluids including whole blood, nucleated blood, buffy coat, saliva, body fluids, swabs, hair and feathers, FPPE, storage buffers and FTA cards, cultured bacterial and fungal tissues, solid tissues (muscle, liver, kidney, lung, heart etc.)

USER MANUAL

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In Vitro Diagnostic Device

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Technical support: info@gene-vantage.com

1. KIT CONTENTS

The IsoBind Universal Pathogen Kit by Gene Vantage is meticulously designed for the efficient and reliable isolation of pathogenic nucleic acids, ensuring high-quality results for various applications. The kit components are optimized for ease of use and compatibility with a wide range of biological sample types, including, biological fluids swabs, hair and feathers, FPPE, storage buffers and FTA cards, cultured bacterial and fungal tissues as well as solid tissues. 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	Function / Description	Volume Per Sample	Short term Storage	Long Term Storage	Total for 100 Samples	Total for 250 Samples
Lysis Buffer A - DNA	Lyses cells to release DNA. Specially formulated to ensure complete lysis of cellular material for optimal yield of DNA.	200 µL	Room temperature	Room Temperature	20 mL	45 mL
Lysis Buffer B - RNA	Lyses cells to release RNA. Specially formulated to ensure complete lysis of cellular material for optimal yield of RNA.	200 uL	Room temperature	Room temperature	20 mL	45 mL
Proteinase K	Enhances lysis by breaking down proteins, facilitating more efficient nucleic acid release.	10 µL	Room Temperature	-20°C	1 mL	2.5 mL
Binding Buffer *Add Isopropanol (95%) prior to use	Facilitates binding of nucleic acid to silica membrane	150 uL	Room temperature	4-8°C	15 mL	35 mL

Wash Buffer A * Add EtOH (96-100 %) prior to use	Removes impurities such as cellular debris and proteins without stripping away the bound nucleic acid	700 µL	Room temperature	Room temperature	70 mL	175 mL
Wash Buffer B * Add EtOH (96-100 %, molecular grade) prior to use	Removes residual salts without stripping away the bound RNA	500 µL	Room temperature	Room temperature	50 mL	125 mL
Booster Compound	Increases selectivity of the silica membrane to bind nucleic acids	6 uL	Room temperature	-20°C	1 mL	2.5 mL
Elution Buffer	Elutes purified nucleic acids from the column	100 µL	Room temperature	4-8°C	10 mL	25 mL
Silica Spin Columns	Silica matrix that selectively binds nucleic acids while allowing other compounds to pass through	1 column + collection tube	Room temperature / 4-8°C	Sealed ziplock bag at 4-8°C	100 units	250 units

 Buffers contain skin irritants  Wear gloves

2. IMPORTANT NOTES

Before beginning your work with the Gene Vantage Isobind Universal Pathogen 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 nucleic acid yields. Particularly with complex tissues, thorough mechanical breakdown is necessary to ensure all cells are lysed and nucleic acid is accessible. Use a bead mill or tissue homogeniser for solid tissues such as tissue and organ samples and ensure complete mixing with the lysis buffer.

Handling of Samples: Biological samples should be handled with care to prevent degradation of nucleic acids. Keep samples on ice when possible during preparation and process them promptly after collection to minimize nucleic acid 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 37Â°C, stirring until the solids have dissolved.

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

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

Correct Protocol: This kit is useful for both DNA and RNA Extractions from a variety of pathogen and human samples. Please follow the correct instructions and protocol for your specific sample type and the type of extraction you are doing (DNA or RNA). This ensures that you isolate the correct type of nucleic acid and get as good quality yield as possible.

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 Universal Pathogen 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 Universal Pathogen Kit is engineered to efficiently isolate high-quality nucleic acids from a variety of biological samples using a robust process grounded in the solid-phase extraction principle. This process is facilitated by silica-based spin columns specifically designed to maximize nucleic acid yield and purity. Below is a detailed explanation of each step involved in the extraction process:

Cell Lysis: The first crucial step involves the breakdown of cell membranes to release nucleic acid into the solution. Effective lysis is key to ensuring that all nucleic acid is accessible for subsequent binding. This kit uses 2 different lysis buffers: Lysis Buffer A for DNA extraction and Lysis Buffer B for RNA extraction. The buffers contain a combination of surfactants and buffering agents that disrupt cellular and nuclear membranes. The addition of Proteinase K, an enzyme, aids in digesting proteins that could otherwise bind nucleic acids and interfere with the extraction process. This enzymatic treatment further ensures that nucleic acids are fully liberated from the cellular membrane. The lysis reaction is enhanced by incubating the sample mixture at 56Â°C which optimizes the activity of Proteinase K and ensures complete lysis.

DNA Binding: Following lysis, the free nucleic acids must be selectively captured or bound while other cellular debris and impurities are excluded. Nucleic acid in the lysate binds to a silica membrane within the spin column when in the presence of the Binding Buffer. This buffer promotes the adherence of nucleic acid to the silica surface due to the formation of hydrogen bonds between the negatively charged phosphate groups of the nucleic acids and the positively charged surface of silica. The addition of the Booster Compound further increases the selectivity of the membrane to ensure that only nucleic acids bind to the silica matrix. This step is critical as it determines the yield of nucleic acid.

Washing: Clean nucleic acid is essential for sensitive applications like PCR. The kit contains two sequential wash buffers (Wash Buffer A and Wash Buffer B), each designed to efficiently remove different types of contaminants. Wash Buffer A primarily removes proteins and other large organic molecules while Wash Buffer B is designed to eliminate smaller molecules and salts. Each wash involves adding a specific volume of buffer, followed by centrifugation to pull the liquid through the column while the nucleic acid 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 purified nucleic from the silica membrane for use in downstream applications. Elution is achieved by applying an Elution Buffer, which disrupts the hydrogen bonds between the nucleic acid and the silica, allowing the nucleic to be released into the buffer. The elution buffer is pre-warmed to enhance the efficiency of nucleic acid recovery. The nucleic acid is collected by centrifugation, which forces the eluted nucleic acid into a clean microcentrifuge tube.

Key Features:

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

Comprehensive Cell Disruption: The Lysis Buffer and Proteinase K combination effectively disrupts a wide variety of cell types, ensuring complete release of RNA. Bead beating can be added as an additional step for another level of cell disruption.

Time Efficiency: The entire 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.

Versatility: The kit is suitable for both RNA and DNA extraction from a variety of pathogen and human samples. The kits robust lysis and binding conditions are effective in isolating high quality nucleic acid.

Compatibility with Downstream Applications: The high-quality nucleic acid 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 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 nucleic acid. A temperature control feature to protect 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 nucleic within the solution. This ensures maximum contact between the RNA and the silica binding surface, increasing the efficiency of nucleic acid 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 extraction protocol.

5.2 Consumables

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 DNase and RNase-free to prevent the degradation of nucleic acids 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 nucleic acid to the column.

B-ME or DTT: Used as a reducing agent to remove tannins and polyphenols and denature proteins by reducing disulfide bonds. Aids in cell lysis.

6. QUICK VIEW PROTOCOL

Step	Procedure	Notes
Sample Preparation	Prepare and homogenize samples according to type. See complete protocol for details.	Ensure samples are collected and stored under conditions that preserve RNA/DNA integrity.
Lysis	Add 200 uL Lysis Buffer to samples Vortex > Add 20 uL Proteinase K > Incubate at 56°C for 1 hour	In addition, bead beating can be included for additional mechanical lysis for tissue samples
Binding	Transfer lysate to new tube > Add 1 volume of Binding Buffer + 6 uL Booster > Vortex > Transfer to spin column > Spin @ 8000 rpm for 1 min. > Discard flow through and reuse collection tube	Binding conditions must be precise - follow volume ratios accurately for effective binding.
Washing	Add 700 uL of Wash Buffer A > Spin @ 8000 rpm for 1 minute > Discard flow through and reuse the collection tube > Repeat with 500 uL of Wash Buffer B	ENsure all wash buffer flows through the column.
Dry Centrifuge	Place column back into collection tube > spin @ 8000 rpm for 2 minutes to dry the column	This step is crucial in ensuring no residual wash buffer remains on the column prior to elution
Elution	Add 50 uL of Elution Buffer > Incubate on the bench for 2 mins> Spin @ 8000 rpm for 1 minute to elute the DNA/RNA.	Use pre-warmed Elution Buffer if higher DNA/RNA concentration is needed. Elution Buffer volume can be adjusted depending on the desired concentration.
Storage	Store the eluted nucleic acids appropriately or proceed to downstream applications.	Immediate use is ideal; otherwise, store at 4-8°C for short term storage or at -20 to -80°C for long-term preservation.

7. KIT SPECIFICATIONS

Parameter	Specification
Format	Spin column
Sample Material	Blood, serum, plasma, tissue, swabs, body fluids
Typical Yield	Variable, depends on sample type and pathogen load
Purity Ratios (A260/A280)	1.8-2.0
Elution Volume	30-100 µL
Preparation Time	Approx. 60 min/20 preps
Binding Capacity	Up to 25 µg of RNA

8. WORKFLOW TIPS

To maximize the effectiveness and reliability of the IsoBind Universal Pathogen Kit, it is crucial to consider additional aspects of the extraction process that impact both the quality of the nucleic acid 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: If processing cannot be done immediately after collection, store the sample in its transport media at 4°C to minimise enzymatic activities and microbial growth that can degrade nucleic acid. This is particularly important for ensuring that the nucleic acid remains stable and intact for extraction.

Long-Term Storage: If samples cannot be processed right away, they should be stored at 4°C in their original collection vials or pouches for processing. However, it's essential that this temperature is strictly maintained to prevent any fluctuations that could affect sample stability. Avoid long-term storage as the preservatives in the transport medium may begin to degrade or alter the nucleic acid over time, storing samples in their original collection vials at 4°C is typically safe for a few days, but this can vary based on the specific preservatives used in the transport media.

Minimizing Contamination: Use sterile pipettes and filter tips for sample handling. Cross-contamination can introduce extraneous DNA or degrade the sample quality.

Documentation and labelling: Carefully label all samples with the date of collection, sample type, and specific storage requirements. Maintain detailed records of storage duration and conditions to help correlate sample quality with experimental outcomes.

SAMPLE SIZE CONSIDERATIONS

Scaling Buffer Volumes: If additional sample volume is required to increase the size of the pellet, adjust the volumes of Lysis Buffer A, Lysis Buffer B and Binding Buffer proportionally. For instance, doubling the sample volume should also double the volume of Lysis Buffer S from 200 ul to 400 ul. It is essential that the volume of each buffer matches the sample size to ensure complete lysis, optimal binding, and effective washing.

Handling Larger Sample Volumes: For samples exceeding the standard 1 ml size, it may be necessary to process the material in multiple batches or adjust the protocol to accommodate larger volumes, which includes scaling up the volumes of all reagents and possibly using multiple spin columns.

Sample Homogeneity: Ensure that cell pellets are fully resuspended in lysis buffer and in the case of teeth and bone they are crushed to a fine, even powder. This is crucial for consistent lysis across samples and significantly impacts the yield and purity of the extracted nucleic acid.

Considerations for High Metabolite Content: Transport media components like ethanol or methanol can interfere with nucleic acid extraction. Adequately removing these through centrifugation and discarding the transport medium before adding the lysis buffer is vital.

Concentration and Yield: Ensure that the elution buffer is pre-warmed to help release the nucleic acid more effectively from the silica matrix. Adjust the volume of the elution buffer based on the required concentration of nucleic acid; smaller volumes yield more concentrated nucleic acid, which might be crucial for sensitive downstream applications like PCR.

Special Considerations for Low Biomass Samples: In cases where the sample biomass is low, take extra precautions to minimize loss during transfers and spins. Use low-retention pipette tips and ensure all centrifugation steps are precisely timed to avoid losing precious nucleic acid material.

9. PREPARING BUFFERS AND EQUIPMENT

Before Starting:

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.

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 nucleic acid 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.

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
Functionality Check: 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
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 nucleic acid 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 DNase-free environment. Use DNase decontamination solutions and maintain clean bench practices throughout the procedure.

10. COMPLETE PROTOCOL

1. Biological Fluids

1.1. For total DNA isolation from <= 200 ul of whole blood, nucleated blood, buffy coat, saliva, sputum, milk, etc.

1.2. STORAGE BUFFERS: For urine, serum and other low concentration body fluids stored in storage buffers (Zymo DNA/RNA Shield or Invitrogen RNA Later), add 20 ul of Proteinase K to 400 ul of the sample mixture prepared according to the manufacturers specifications. Proceed to step 4.

1.3. Mix thoroughly or vortex 10-15 seconds and then incubate the tube at room temperature for 20 minutes. Proceed to step 4.

2. Mammalian/Insect Cell Cultures

2.1. For total DNA isolation from <= 5 x 106 cells such as HeLa cells, HEK-293 cells, Drosophila cell lines, etc.

2.2. Media should be removed before processing by pelleting cells (at approximately 8,000 x g for 2 minutes depending on volume and cell type) and removing the supernatant. Proceed to step 4.

Note: For mammalian cell samples, it is possible to reduce Proteinase K digestion time to 5 minutes at 55°C.

3. Solid Tissues

3.1. For total DNA isolation from <= 25 mg tail snips, ear punches, organ biopsies (brain, liver, heart, kidney, muscle, stomach, bladder, intestine, etc.).

3.2. For tissues stored in storage buffers such as DNA/RNA shield or RNAlater, spin down the sample tube at 10,000 g for 5 minutes and discard supernatant. For each 25 mg of tissue use the buffer volumes stipulated below. i.e. if you have 50 mg of tissue, double the lysis and binding volumes.

Note: Wash buffers can remain as is, although we recommend
- a double 80 % ethanol wash
- Overnight Proteinase K digestion at 55° C can be used to increase yields
- For hair and feather samples see Appendix B for a protocol.

3.3. For tissues stored without storage buffers, load <= 25 mg wet tissue into a bead beating tube, add 300 ul Elution Buffer. Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for > 5 minutes.

Note: Required processing time will vary depending on the device and application and therefore should be evaluated on a case by case basis. For example, processing times may be as little as 3 minutes when using high-speed cell disruptors (e.g., the portable TerraLyzerTM Sample Processor, FastPrepÂ® -24, or similar) or as long as 20 minutes when using lower speeds (e.g., Disruptor GenieTM, or standard benchtop vortexes). See manufacturer's literature for operating information.

3.4. Centrifuge at 10,000 x g for 2 minutes. Transfer 180 ul supernatant to a new microcentrifuge tube. Proceed to step 4.

4. Lysis

4.1. Add 180 ul Lysis A, mix, incubate (55°C / 1-12 hr) to digest proteins.

4.2.. Add 180 ul Lysis B, mix, incubate (55°C / 5 min). Centrifuge at 10,000 x g for 2 minutes.

4.3.Transfer clear supernatant to new tube, without disturbing the lower phase. Do not transfer any particulate matter.

5. Binding

5.1. Add 0.7 volumes Binding Buffer, mix, incubate 2 minutes at room temperature to allow nucleic acids to precipitate.

5.2. Transfer to spin column and centrifuge at 10,000 x g for 1 minute. Discard the flow through waste, return column to collection tube.

6. Washing

6.1. Add 280 ul Wash A to the spin column, incubate for 1 minute at room temperature.

6.2. Centrifuge at 10,000 x g for 1 minute. Discard the flow through waste, return column to collection tube.

6.3. Add 400 ul Wash B to the spin column and centrifuge at 10,000 x g for 1 minute. Discard the flow through waste, return column to collection tube.

6.4. Add 700 ul 80 % ethanol to the spin column directly on the matrix. Centrifuge at 10,000 x g for 1 minute. Discard the flow through waste, return column to collection tube.

7. Dry Centrifuge

7.1. Centrifuge at 10,000 x g for 1 minute to dry the silica column. Discard collection tube.

8. Elution

8.1. Transfer the spin column to a clean microcentrifuge tube.

8.2. Add 50 ul of preheated Elution Buffer directly on the matrix. Incubate for 5 minutes at room temperature, then centrifuge at 10,000 g for 1 min. Discard the spin column.

8.3. The eluted DNA can be used immediately for molecular based applications or stored either short term at 4-8°C or long term at -20 to -80°C.

9. Bacterial and fungal cell cultures

9.1. For total DNA isolation (e.g. genomic, plasmid, etc.) from <= 5 x 106 E. coli cells.

9.2. If culture is stored in transport media, the sample should be spun at max speed for 10 minutes and the supernatant discarded.

9.3. If culture is being retrieved from plates/flasks, add 50 - 100 mg (wet weight) fungal or bacterial cells that have been resuspended in up to 300 ul of Elution buffer to a Bead beating tube.

9.3.1 Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for > 5 minutes.

Note: Required processing time will vary depending on the device and application and therefore should be evaluated on a case by case basis. For example, processing times may be as little as 3 minutes when using high-speed cell disrupters (e.g., the portable TerraLyzerTM Sample Processor, FastPrepÂ® -24, or similar) or as long as 20 minutes when using lower speeds (e.g., Disruptor GenieTM, or standard benchtop vortexes). See manufacturer's literature for operating information.

9.4. Add 20 uL of beta-mercaptoethanol OR 8 uL of DTT. Add 400 ul of Lysis A vortex, incubate (55°C / 15 min).

9.5. Add 200 ul of Lysis B vortex, incubate (55°C / 5 min), centrifuge at 10,000 x g for 2 minutes.

9.6 Transfer clear supernatant to new 2 ml microcentrifuge tube. When transferring, ensure that the lower phases created during centrifugation are not disturbed/transferred, or contaminants will be carried forward.

9.7. Add 1 volume Bind + 5 ul Booster, vortex and incubate for 2 minutes on the bench.

9.8. Transfer 700 ul of the mixture from Step 5 to a IsoBind spin column and centrifuge at 10,000 x g for 1 minute. Discard the flow through waste, return column to collection tube.

9.9. Repeat step 6 until the full volume of the sample is used.

9.10. Add 280 ul Wash A to the spin column, incubate for 1 minute at room temperature.

9.11.Centrifuge at 10,000 x g for 1 minute. Discard the flow through waste, return column to collection tube.

9.12. Add 400 ul Wash B to the spin column and centrifuge at 10,000 x g for 1 minute. Discard the flow through waste, return column to collection tube.

9.13. Add 700 ul 80 % ethanol to the spin column directly on the matrix. Centrifuge at 10,000 x g for 1 minute. Discard the flow through waste, return column to collection tube.

9.14. Centrifuge at 10,000 x g for 1 minute to dry the silica column. Discard collection tube.

9.15. Transfer the spin column to a clean microcentrifuge tube. Add 50 ul Elution Buffer directly on the matrix. Incubate for 5 minutes at room temperature, then centrifuge at 10,000 g for 1 min.

9.16.The eluted DNA can be used immediately for molecular based applications or stored either short term at 4-8°C or long term at -20 to -80°C.

10. Blood, buffy coat, body fluids, saliva, swabs

10.1. BUFFY COAT: Centrifuge 10 mL of whole blood (in EDTA or other anticoagulant) at 3200 rpm for 15 minutes at room temperature (or use a personal protocol). Combine 200 ul of buffy coat + 200 uL of Lysis A + 20 uL of Proteinase K + 20 uL of beta-mercaptoethanol OR 8 uL of DTT.

10.2. BLOOD: use liquid sample as is. Combine 200 uL of liquid sample + 200 uL of Lysis A + 20 uL of Proteinase K + 20 uL of beta-mercaptoethanol OR 8 uL of DTT. Proceed to step 1 below.

10.3. BODY FLUIDS, SALIVA: Combine 200 uL of liquid sample + 200 uL of Lysis B + 20 uL of Proteinase K + 20 uL of beta-mercaptoethanol OR 8 uL of DTT. Incubate the sample shaker for 5 minutes with shaking at +- 600 rpm at room temperature. Proceed to step 2 below.

10.4. BUCCAL SWABS: Thoroughly rinse mouth with water before isolating cells. Brush the inside of the cheek with a buccal swab for 15 seconds (approximately 20 brushes), making sure to cover the entire area of the inner cheek. Rinse the brush into a microcentrifuge tube using a mixture of 300 ul of Lysis A and 200 ul of Elution Buffer or another isotonic solution. Add 20 ul of Proteinase K. Proceed to step 1 below.

10.5. OTHER SWABS: For each swab, add 200 uL of the transport medium (saline, elution buffer, do not use PBS) and 200 uL of Lysis B. Vortex thoroughly. Incubate the sample in a thermomixer for 15 minutes with shaking at +- 600 rpm at 56°C. Proceed to step 2 below.

10.6. Pipette to mix until the sample and lysis buffer are homogenous. Incubate the sample in a thermomixer for 30 minutes with shaking at +- 600 rpm at 56°C.

10.7. Centrifuge at 10,000 x g for 2 minutes. Transfer clear supernatant to new tube, without disturbing the lower phase. Do not transfer any particulate matter.

10.8. Add 1 volume Bind + 5 ul Booster and pipette at least 5 times to mix. Immediately transfer to spin column and centrifuge at 10,000 x g for 1 minute. Discard the flow through waste, return column to collection tube.

10.9. Repeat step 3 until the full volume of sample is used.

10.10. Add 280 ul Wash A to the spin column, incubate for 1 minute at room temperature.

10.11. Centrifuge at 10,000 x g for 1 minute. Discard the flow through waste, return column to collection tube.

10.12. Add 400 ul Wash B to the spin column and centrifuge at 10,000 x g for 1 minute. Discard the flow through waste, return column to collection tube.

10.13. Add 700 ul 80 % ethanol to the spin column directly on the matrix. Centrifuge at 10,000 x g for 1 minute. Discard the flow through waste, return column to collection tube.

10.14. Centrifuge at 10,000 x g for 1 minute to dry the silica column. Discard collection tube.

10.15. Transfer the spin column to a clean microcentrifuge tube. Add 50 ul Elution Buffer directly on the matrix. Incubate for 5 minutes at room temperature, then centrifuge at 10,000 g for 1 min.

10.16. The eluted DNA can be used immediately for molecular based applications or stored <= -20°C for future use. The total yield can be improved by eluting the DNA with 60-70°C Elution Buffer.

10.17. The eluted DNA can be used immediately for molecular based applications or stored either short term at 4-8°C or long term at -20 to -80°C.

11. TROUBLESHOOTING GUIDE

Problem Description	Possible Causes	Suggestions
Low RNA/DNA yield	Insufficient lysis of sample	Increase lysis time or use mechanical disruption methods like bead beating for tough tissues.
	Incomplete homogenization	Ensure thorough homogenization of the sample, especially for fibrous tissues.
	Low amount of starting material	Increase the amount of starting material, keeping the buffer volumes consistent.
RNA degradation	RNase contamination	Use RNase-free consumables and reagents. Handle samples on ice and minimize exposure to air.
	Prolonged sample storage	Process fresh samples or store properly at -80°C for long-term storage.
Poor RNA/DNA purity (low A260/ A280 ratio)	Contamination with proteins or phenol	Ensure complete removal of contaminants during washing steps. Increase wash buffer volumes.
	Residual wash buffer in the sample	Perform an additional centrifugation step to remove residual wash buffer before elution.
Incomplete elution of RNA/DNA	Insufficient elution buffer volume	Increase the volume of elution buffer or perform a second elution step.
	Low elution temperature	Preheat the elution buffer to 60°C to enhance RNA/DNA elution.
Clogged spin column	Excessive cell debris or particulate matter	Centrifuge the lysate before applying to the column to remove debris.
	Overloading of the column	Reduce the amount of starting material or split the lysate into multiple columns.
Inconsistent RNA / DNA yields	Variability in sample quality	Standardize sample collection and storage methods to reduce variability.
	Inconsistent pipetting	Calibrate pipettes regularly and use consistent pipetting techniques.

Equipment malfunction	Centrifuge or thermomixer issues	Check equipment for proper function before starting the procedure. Perform maintenance checks.
	Incorrect centrifuge speed or temperature settings	Verify that the centrifuge and thermomixer are set to the correct parameters.
Contamination in RNA/DNA samples	Cross-contamination between samples	Use separate pipette tips for each sample and reagent. Clean work area before and after use.
	Contaminated reagents or consumables	Use fresh reagents and RNase-free consumables. Store reagents properly to prevent degradation.

12. PRODUCT USE RESTRICTION / WARRANTY

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