IsoBind Blood and Tissue Maxi kit

Cat No. IB-MXBTDNA-100

System: Silica spin columns (manual workflow)

Sample types: Whole blood, buffy coat, body fluids, fresh tissue, frozen tissue

USER MANUAL

Website www.gene-vantage.com

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In vitro diagnostics

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Technical support: info@gene-vantage.com

1. KIT CONTENTS

The Gene Vantage Maxi Blood and Tissue DNA Isolation Kit is designed to provide researchers and diagnostic professionals with a comprehensive tool for high-purity nucleic acid extraction from a variety of sample types. This kit is optimized for maximum efficiency and flexibility, accommodating a wide range of biological samples, including plasma, serum, urine, solid tissues, and challenging samples such as sputum or swabs. It is especially suited for applications requiring high-throughput processing and is compatible with both manual and automated systems. The kit supports critical downstream applications such as RT-PCR, ensuring minimal PCR inhibition and optimal performance. Below is a detailed table of the kit contents, highlighting the components and their specific uses, volumes, and storage recommendations.

Component	Description/ Function	Volume Required per Sample	Short Term Storage	Long Term Storage	Total for 96 Samples	Total for 384 Samples
Lysis Buffer	Lyses cells to release DNA. Specially formulated to ensure complete lysis of cellular material for optimal yield of DNA.	200 µL	Room temperature (15-25°C)	Room temperature (15-25°C)	20 mL	80 mL
Proteinase K	Enhances lysis by breaking down proteins, facilitating more efficient DNA release.	20 uL	Room temperature	-20°C	1.92mL	7.68mL
Binding Buffer *Add Isopropanol(95 %) prior to use	Facilitates binding of DNA to the silica membrane.	200 µL	Room temperature (15-25°C)	4-8°C	20 mL	80 mL
Wash Buffer A * Add EtOH (96-100 %) prior to use	Removes impurities such as cellular debris and proteins without stripping away the bound DNA	400 µL	Room temperature (15-25°C)	Room temperature (15-25°C)	40 mL	155 mL

Wash Buffer B * Add EtOH (96-100 %) prior to use	Removes residual salts without stripping away the bound DNA	400 µL	Room temperature (15-25°C)	Room temperature (15-25°C)	40 mL	155 mL
Elution Buffer	Elutes purified DNA from the spin plate	50 µL	Room temperature (15-25°C)	4-8°C	5 mL	20 mL
Spin Plate	Silica matrix that selectively binds RNA while allowing other compounds to pass through it. Available in 96 well format for the simultaneous processing of multiple samples.	1 plate per 96 samples	Room temperature (15-25°C)	Sealed in ziplock at 4-8°C	1 spin plate	2 spin plates

 Buffers contain skin irritants  Wear gloves

2. IMPORTANT NOTES

Before beginning your work with the Gene Vantage Isobind Blood and Tissue Maxi 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 DNA yields.Tissues should be finely minced using clean blade or homogenized in a bead mill or tissue lyser specific to the sample type (e.g., muscle, liver). For blood samples, ensure thorough mixing to prevent clotting before processing.

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

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

Maximum Capacity: To prevent membrane clogging and ensure efficient DNA purification, do not exceed the recommended sample volume and loading capacity of the spin plates. Overloading can lead to incomplete binding of DNA to the membrane 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 Blood and Tissue Maxi 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 Blood and Tissue Maxi Kit is engineered to efficiently isolate high-quality DNA from blood and tissue samples using a robust process grounded in the solid-phase extraction principle. This process is facilitated by silica-based spin plates specifically designed to maximize DNA yield and purity. This format also allows for simultaneous processing of multiple samples. Below is a detailed explanation of each step involved in the DNA extraction process:

Cell Lysis: The first crucial step involves the breakdown of cell membranes to release DNA into the solution. Effective lysis is key to ensuring that all DNA is accessible for subsequent binding. This kit uses Lysis Buffers, 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 DNA and interfere with the extraction process. This enzymatic treatment further ensures that DNA is 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 DNA must be selectively captured or bound while other cellular debris and impurities are excluded. DNA in the lysate binds to a silica membrane within the spin plate when in the presence of the Binding Buffer. This environment promotes the adherence of DNA to the silica surface due to the formation of hydrogen bonds between the negatively charged phosphate groups of the DNA and the positively charged surface of silica. This step is critical as it determines the yield of DNA.

Washing: Clean DNA is essential for sensitive applications like PCR. The kit contains two sequential wash buffers (Wash A and B), each designed to efficiently remove different types of contaminants. Wash Buffer A primarily removes proteins and other large organic molecules, 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 matrix while the DNA remains bound to the silica membrane. This ensures that only purified DNA remains bound to the matrix.

Elution: The final step is to release the purified DNA from the silica membrane for use in downstream applications. Elution is achieved by applying an Elution Buffer, which disrupts the hydrogen bonds between the DNA and the silica, allowing the DNA to be released into the buffer. The elution buffer is pre-warmed to enhance the efficiency of DNA recovery. The DNA is collected by centrifugation, which forces the eluted DNA into a clean microcentrifuge tube.

Key Features:

Quality of Output: This kit utilises robust silica-based spin plate technology, which selectively binds DNA while efficiently removing contaminants. This results in DNA with high purity, characterised by optimal A260/A280 ratios, indicating minimal protein contamination and readiness for sensitive downstream applications.

Tailored for Higher Throughput: The kit is tailored for the simultaneous processing of up to 96 samples per plate, making it ideal for labs seeking to maintain their turnaround time without compromising on quality or yield

Comprehensive Cell Disruption: The Lysis Buffer and Proteinase K combination effectively disrupts cell types found in blood and tissue samples, ensuring complete release of DNA.

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 optimized for blood and tissue samples, ,including whole blood, buffy coat, body fluids, and various tissue types (both fresh and frozen) the kit's robust lysis and binding conditions are effective in isolating high quality DNA, making it a useful tool in both clinical diagnostic and research settings.

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

Note: Please engage with Gene Vantage technical support (see above: Important notes) should you require 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 DNA 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 DNA. 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 DNA within the solution. This ensures maximum contact between the DNA and the silica binding surface,

increasing the efficiency of DNA 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 DNA extraction protocol.

5.2 Consumables

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

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-free to prevent the degradation of DNA by residual enzymatic activity.

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

column.

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

6. QUICK VIEW PROTOCOL

Step	Procedure	Details
Sample Preparation	Mince 25 mg of tissues manually using a sterile blade or place in a microcentrifuge tube with steel beads and bead beat in tissue lyser until mixture is completely homogenized. For whole blood samples add 200 uL of sample into a deep well plate and proceed to the lysis step.	For solid starting materials (e.g., tissues), crush samples in microcentrifuge tubes using a Tissue Lyser or similar device. This step ensures complete cell lysis.
Lysis	Add 200 uL of Lysis Buffer + 20 uL Proteinase K to the samples > Incubate @ 70 degrees for 1 hour > Spin @ 6000 rpm for 5 mins. > Pipette clear lysate into a new deepwell plate	Check for any precipitate in the buffer; dissolve by heating if necessary. Optionally, add a reducing agent if required. Shake intermittently during the incubation step.
Binding	Add 200 uL of Binding Buffer > Incubate on bench for mins. > Transfer to new spin plate > Spin @ 6000 rpm for 4 minutes > Discard flow through and restack spin plate onto deepwell plate	This step determines your quality and yield of DNA.
Washing	Add 400 ul of Wash buffer > Spin @ 6000 rpm for 4 minutes > Discard flow through and reuse deepwell plate > Repeat steps for Wash Buffer B	Ensure all wash buffers pass through the plate.
Elution	Add 50 uL of preheated Elution Buffer to spin plate > Incubate for 5 mins on the bench > Spin 6000 rpm for 2 mins > discard spin plate and transfer DNA to microcentrifuge tube for storage	Place the spin plate in a clean collection tube. Add 50 µL Elution Buffer, let sit for 1 minute, then centrifuge at 6000 x g for 2 minutes to collect DNA.

7. KIT SPECIFICATIONS

Parameter	Specification
Format	Spin Plate (96 well format)
Sample Material	Blood, tissue
Typical Yield	Up to 30 µg per isolation
Elution Volume	50 µL
Preparation Time	Approx. 45 minutes
Binding Capacity	Up to 100 µg DNA

8. WORKFLOW TIPS

To maximize the effectiveness and reliability of the IsoBind Blood and Tissue Maxi 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 minimize DNA degradation. If immediate processing is not possible, samples must be handled and stored carefully to preserve their integrity.

Blood Samples: Blood samples should ideally be processed within 2 hours of collection to prevent the breakdown of cellular components and to maintain the integrity of the DNA. If immediate processing is not possible, keep the blood at room temperature and proceed to storage guidelines as soon as feasible.

Tissue Samples: Process tissue samples as soon as possible after collection to minimize enzymatic degradation of DNA by endogenous nucleases.If immediate processing is not viable, proceed to freezing as soon as possible, place tissue samples directly in liquid nitrogen to rapidly freeze them, preserving cellular structure and DNA integrity: If liquid nitrogen is not available then freeze according to storage considerations.

Storage Considerations: For short-term storage refrigerate blood samples at 2°C to 8°C immediately after collection. Samples stored under these conditions are stable for up to 7 days for long-term storage; blood samples should be frozen. Freeze samples at -20°C for up to 6 months. For periods longer than 6 months, consider storing at -80°C to better preserve DNA quality.

Handling: Avoid multiple freeze-thaw cycles as they can significantly degrade DNA quality. Thaw frozen samples on ice when ready for processing.

SAMPLE SIZE CONSIDERATIONS

Optimal Sample Volume Blood Samples: For most applications, a blood sample volume of 200 to 500 µL is optimal. This volume is sufficient to achieve a good yield of DNA while ensuring efficient processing.

Column Capacity Blood Samples: Ensure that the volume of the blood sample does not exceed the capacity of the silica spin columns provided in the kit. Overloading the column can result in lower DNA purity and yield because the column may not effectively bind all the DNA from an excessively large volume.

Optimal Sample Volume for Tissue Samples: For tissue samples, up to 25 mg per extraction is recommended. This amount typically provides a sufficient quantity of DNA for downstream applications without overloading the extraction system.Optimal sample size and weight are not only about adhering to technical limits but also about maximizing the efficiency of DNA recovery. Too small a sample might not yield enough DNA for your needs, whereas too large a sample can strain the capacity of the kit components, leading to inefficient extraction and potential loss of material.

Sample Homogeneity for Tissue Samples: To ensure consistent DNA yield and quality, the tissue sample should be homogeneous. This means the tissue should be evenly divided and representative of the whole sample to avoid variability in the results.

Handling Larger Sample Volumes for Tissue Samples: 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.

Concentration and Yield for Tissue Samples: Adjust the volume of Elution Buffer based on the required concentration of DNA; smaller volumes yield more concentrated DNA, which might be crucial for sensitive downstream applications like PCR.

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.

Calibration: Regular calibration of the centrifuge is crucial for achieving the precise speeds necessary for optimal DNA isolation. Inaccuracies in speed can lead to inefficient separation of phases, potentially contaminating the DNA 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: 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 DNA 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. Sample Preparation:

1.1 For blood samples: Transfer 200 µL whole blood in anticoagulant onto a spin plate. Proceed to lysis step.

1.2 For tissue samples: Finely chop up to 25 mg of tissue using a sterile blade. For tougher samples, it is recommended that you use a tissue lyser and bead beating tubes to ensure complete homogenization.

1.3 Transfer the chopped tissue into the spin plate.

2. Lysis:

2.1 Preheat the lysis buffer to 60°C. Add 20 uL ProK and 200 ul of lysis buffer to each sample in a Spin plate.

2.2 Incubate at 70°C for 1 hour, shaking every 10 minutes to ensure the lysis buffer interacts thoroughly with the sample.

2.3 Centrifuge the deep well plate at 6000 rpm for 5 minutes. Carefully pipette the clear upper lysate into a deepwell plate.

3. Binding DNA:

3.1 Add an 200 uL of Binding Buffer to the clear lysate, pipette thoroughly to mix and incubate on the bench for 5 minutes.

3.2 Transfer the mixture to a spin plate stacked on top of a deep well plate. Load the samples using a multichannel pipette, ensuring not to overfill each well (maximum of 750 uL per well).

3.3 Centrifuge in a plate centrifuge at 6 000 rpm for 4 minutes to pass the lysate through the silica membrane. Discard the flow through and stack the spin plate onto the same deep well Plate.

4. Washing:

4.1 Add 400 ul Wash A per well using multichannel pipette. Incubate on the bench for 2 minutes.

4.2 Spin for 4 minutes at 6000 rpm in plate centrifuge. Discard waste liquid from the deep well plate and re-stack for the next wash.

4.3 Add 400 ul Wash B per well using multichannel pipette. Incubate on the bench for 2 minutes.

4.4 Spin for 4 minutes at 6000 rpm in plate centrifuge. Discard waste liquid from the deep well plate and re-stack for the next wash.

4.5 Add 600 ul 80% ethanol well using multichannel pipette. Incubate on the bench for 2 minutes.

4.6 Spin for 4 minutes at 6000 rpm in plate centrifuge. Discard waste liquid from the deep well plate and re-stack.

4.7 Spin for 4 minutes at 6000 rpm in plate centrifuge to dry the membrane. Discard waste liquid from the deep well plate. Transfer spin plate onto an elution plate.

5. Elution of DNA:

5.1 Add 50 uL of pre-warmed Elution Buffer to the center of the spin membrane membrane.

5.2 Incubate on the bench for 5 minutes at room temperature.

5.3 Centrifuge at 6000 rpm for 2 minutes to collect the eluted DNA.

6. Storage of DNA:

6.1 Process immediately or Store the eluted DNA at 4-8°C for short term storage or at -20°C to -80°C for long-term preservation.

11. TROUBLESHOOTING GUIDE

Problem Description	Possible Causes	Suggestions
Low DNA Yield	Incomplete lysis of sample	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.
	DNA degradation	Use fresh samples or store samples properly. Add RNase inhibitors if needed.
Poor DNA purity	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.
DNA Degradation	Improper sample storage	Store samples at 4-8°C for short term storage and at -20 to -80°C for long term storage or use immediately after collection.

	Prolonged exposure to room temperature	Keep samples and lysates on ice during the extraction process.
Inconsistent DNA 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 DNA 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 DNA	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.

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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