IsoBind Plant DNA Extraction Kit

Cat No. IB-PDNA-100

System: Silica spin columns (manual workflow)

Sample types: Leafy tissues, woody and fibrous tissue, herbaceous tissue, seed grains, and fruits

USER MANUAL

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1. KIT CONTENTS

The IsoBind Plant DNA Extraction Kit by Gene Vantage is meticulously designed to provide researchers with all the necessary components for efficient and high-quality extraction of DNA from plant samples. This comprehensive kit includes an array of reagents and consumables, each serving a specific function in the DNA extraction process. From the initial lysis of plant tissues to the final elution of purified DNA, the kit ensures a streamlined workflow with optimised buffers and spin columns. In this section, we provide a detailed overview of the kit contents, including descriptions of each component, their volumes for different kit sizes, storage conditions, and essential notes on handling and safety. By understanding the role and requirements of each component, users can achieve consistent and reproducible results in their plant DNA extraction experiments.

Component	Description/ Function	Volume per Sample	Short Term Storage	Long Term Storage	Total for 50 Samples	Total for 100 Samples
Plant DNA Spin Columns	Silica matrix that selectively binds DNA while allowing other compounds to pass through it	1 column + collection tube	Room temperature (15-25°C)	Sealed in ziplock at 4-8°C	50 units	100 units
Lysis Buffer PL	Lyses cells to release DNA. Specially formulated to ensure complete lysis of cellular material for optimal yield.	600 µL	Room temperature (15-25°C)	Room temperature (15-25°C)	30 mL	60 mL
Binding Buffer PB *Add Isopropanol (95%) prior to use	Facilitates DNA binding to the silica spin column.	Equal volume to lysate	Room temperature (15-25°C)	Room temperature (15-25°C)	Variable	Variable
Wash Buffer A * Add EtOH (96-100 %) prior to use	Removes impurities such as cellular debris and proteins without stripping away the bound DNA	500 µL	Room temperature (15-25°C)	Room temperature (15-25°C)	25 mL	50 mL
Wash Buffer B * Add EtOH (96-100 %) prior to use	Removes residual salts without stripping away the bound DNA	500 µL	Room temperature (15-25°C)	Room temperature (15-25°C)	25 mL	50 mL

Wash Buffer C * Add EtOH (96-100 %) prior to use	Final wash to remove remaining traces of chaotrophic agents.	500 µL	Room temperature (15-25°C)	Room temperature (15-25°C)	25 mL	50 mL
Elution Buffer PE	For eluting purified DNA from the silica spin column.	50 µL	Room temperature (15-25°C)	4-8°C	2.5 mL	5 mL
RNase A (Lyophilized)	For the removal of RNA from DNA preparations.	4 µL	4-8°C	-20°C	200 µL	400 µL

 Buffers contain skin irritants  Wear gloves

2. IMPORTANT NOTES

Before beginning your work with the Gene Vantage Isobind Plant DNA 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 DNA yields. Particularly with complex plant materials, thorough mechanical breakdown is necessary to ensure all cells are lysed and DNA 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.

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 DNA.

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 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 column clogging and ensure efficient DNA purification, do not exceed the recommended sample volume and loading capacity of the spin columns. Overloading can lead to incomplete binding of DNA 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 Plant DNA 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

Basic Principle

The IsoBind Plant DNA Extraction Kit is based on a solid-phase extraction method that efficiently isolates high-quality genomic DNA from various plant samples. The process involves:

Cell Lysis: The initial step involves the mechanical and chemical disruption of plant cells to release cellular contents. The Lysis Buffer PL is formulated with detergents and chaotropic agents that break down cell membranes and denature proteins, ensuring the efficient release of genomic DNA into the solution. For tough plant samples, mechanical homogenization using a bead mill or mortar and pestle is recommended to ensure complete lysis.

DNA Binding: Once the cell lysate is cleared of debris by centrifugation, the supernatant is mixed with Binding Buffer PB. This buffer contains chaotropic salts that create conditions favorable for the binding of DNA to the silica membrane of the Plant DNA Spin Columns. The chaotropic salts disrupt hydrogen bonding between water molecules and the DNA, allowing the DNA to interact with the silica surface.

Washing: After binding, the spin columns are subjected to a series of wash steps using Wash Buffers A, B, and C. These wash buffers are designed to remove contaminants such as proteins, polysaccharides, and salts while retaining the bound DNA on the silica membrane. The sequential washing ensures the removal of different types of impurities, resulting in highly pure DNA.

Elution: The final step involves the elution of purified DNA from the silica membrane. Elution Buffer PE, with a low ionic strength, is used to elute the DNA. The low salt concentration disrupts the interaction between the DNA and the silica, allowing the DNA to be released into the elution buffer. The eluted DNA is now ready for downstream applications such as PCR, sequencing, and genotyping.

Key Features:

Quality of Output: Utilises advanced silica-based spin column technology, which selectively binds DNA while efficiently removing contaminants. This results in DNA 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 and Proteinase K combination effectively disrupts a wide variety of cell types, ensuring complete release of DNA.

Enhanced Recovery: Tailored for samples that are difficult to lyse, the system ensures that even tightly bound nucleic acids are made available for extraction, which is critical for achieving consistent results across different sample types.

Time Efficiency: The entire DNA 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 plant RDNA from a variety of agricultural and human samples, the kits robust lysis and binding conditions are effective in isolating high quality DNA.

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.

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 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 binding buffer to improve the yield and quality of DNA by ensuring more efficient binding of DNA to the column.

RNase:

Ribonuclease (RNase) is used to degrade RNA that may be present in the sample, ensuring that the extracted nucleic acid is predominantly DNA.

6. QUICK VIEW PROTOCOL

Step	Procedure	Details
1. Sample Collection and Storage	Collect plant samples and store them > Note details	How to prepare samples stored in different ways for DNA extraction
2. Sample Homogenization and Lysis	Grind tissue > add lysis buffer PL >incubate > spin.	Chloroform, DNase, RNase and Proteinase K can be optionally added to modify the workflow
3. DNA Binding	Add the binding buffer >. Transfer to silica spin column > centrifuge > discard flow-through.	Add 0.6 volumes of binding buffer e.g. 400 ul sample + 240 ul binding buffer and Add 6 ul Booster. Do not exceed 700 ul per column. Vortex thoroughly before spin step. Adjust depending on the amount of starting material.
8. Wash Steps	Add Wash A, B, C sequentially, centrifuge, discard flow through.	Add 300 ul of Wash A, 600 ul of Wash B, and 600 ul of Wash C, respectively.
9. Dry Centrifuge Step	Place silica spin column back in the collection tube and spin	Done to ensure there are no remnants of previous buffers remaining which could affect the elution step.
10. Elution Step	Heat elution buffer > add to column > incubate > spin and elute DNA.	100 ul heated Elution Buffer. Adjust the amount of Elution buffer needed depending on desired yield and amount of starting material.

7. QUICK SPECIFICATIONS

Parameter	Specification
Format	Spin column
Sample Material	Plant tissue (leaves, stems, roots)
Typical Yield	Up to 30 µg (depending on sample type)
Purity (A260/A280)	1.8 - 2.0
Elution Volume	50 µL
Preparation Time	Approx. 60 minutes
Binding Capacity	Up to 50 µg DNA per column

8. WORKFLOW TIPS

To maximize the effectiveness and reliability of the IsoBind Plant DNA 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.

Freezing: Freeze samples in liquid nitrogen immediately after collection to preserve nucleic acids. This step is crucial for samples that cannot be processed right away.

Long-Term Storage: Store frozen samples at temperatures between -80°C to -65°C. Ensure that the storage facility maintains consistent temperatures to avoid freeze-thaw cycles that can degrade DNA.

Dried or Lyophilized Samples

Drying: Ensure that plant samples are completely dried within 24 hours of collection. Use a lyophilizer or air-dry the samples in a clean, dry environment.

Storage: Store dried samples at room temperature in airtight containers. Protect the samples from moisture and humidity, which can lead to fungal growth and DNA degradation.

Sample Containers

Cryovials: For frozen samples, use cryovials that are designed to withstand low temperatures without cracking.

Airtight Containers: For dried samples, use airtight containers with desiccants to absorb any residual moisture.

Proper storage conditions are vital for maintaining sample quality until extraction. Follow these guidelines to ensure the best possible results with the IsoBind Plant DNA Extraction Kit.

Sample Size Considerations

The IsoBind Plant DNA Kit is designed to accommodate the typical sample volumes required for effective plant DNA extraction. Adhering to the recommended sample volume relative to the weight of the starting material is crucial for optimizing the extraction process. Proper sample size ensures efficient DNA recovery and prevents overloading, which can adversely affect both yields and purity.

When preparing samples, consider the following

Optimal Sample Volume: Determine the ideal sample volume based on the specific requirements of the IsoBind Plant DNA Kit. This volume is critical for achieving efficient lysis and DNA binding.

Weight of Starting Material: Ensure that the weight of the starting material is proportional to the recommended sample volume. Overloading with excessive material can hinder the extraction process.

Sample Homogeneity: For heterogeneous samples, ensure that the portion used for extraction is representative of the whole sample to maintain consistency in DNA yield and quality.

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

Calibration: Calibrate pipettes regularly according to the manufacturer’s guidelines to ensure they dispense volumes accurately, which is critical for the precise preparation of buffers and reagents.

Maintenance: Clean pipettes frequently to prevent cross-contamination between samples. Check the pipette tips for any residual sample before each use, and replace pipette tips between samples to maintain sample integrity.

Vortex Mixer

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 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. Tissue Homogenization

1.1 Fresh or Frozen Tissue: Grind up to 10 - 20 mg of plant tissue in liquid nitrogen using a mortar and pestle to a fine powder.

1.2 Dried Tissue: Rehydrate by adding a small volume of RNase-free water and grind to a fine powder.

1.3 Place tissue into SafeLock tube, add 5mm steel bead and crush to fine powder in Tissue Lyser.

2. Cell Lysis

2.1 Addition of Lysis Buffer PL: Add 600 ul lysis buffer PL to each sample & pipette to mix.

Optional: Add a reducing agent of your choosing (BME or DTT) as per your CTAB protocol. Also, add Proteinase K to prevent contamination of your DNA by proteins if necessary.

2.2 Incubate in a heat bath or oven at 70 C for 30 minutes.

2.3 Heat Elution Buffer: Elute in heat bath/oven as lysis step is taking place

Optional: Cool samples to ambient temp, add 300 ¼l chloroform, vortex thoroughly (until milky).

2.4 Incubate at room temperature for 5 minutes.
2.5 Centrifugation: Spin for 4 min @ 8000 rpm

3. DNA Binding

3.1 Transfer supernatant to a new safe lock tube.

3.2 Add 0.6 volumes of binding buffer PB e.g. 400 ul sample + 240 ul binding buffer PB.

3.3 Add 6 ul Booster.

3.4 Vortex thoroughly and load onto the silica spin column with a collection tube.

3.5 Incubate for 2 minutes at room temp.

3.6 Spin for 1 minute at 8000 rpm and discard flow through

Optional: Change collection tubes between this step and the wash steps to reduce chances of DNA contamination.

4. Washing

4.1 Wash A: Add 300 ul Wash A per sample to the silica spin column. Incubate for 1 minute.

4.2 Spin for 1 minute at 8000 rpm and discard flow through. Reuse collection tube.

4.3 Wash B: Add 600 ul Wash B per sample to the silica spin column. Incubate for 30 seconds.
4.4 Spin for 1 minute at 8000 rpm and discard flow through. Reuse collection tube

4.5 Wash C: Add 600 ul Wash C per sample to the silica spin column. Incubate for 30 seconds.

4.6 Spin for 2 minutes at 8000 rpm and discard flow through.

4.7 Dry Centrifugation Step: Transfer silica spin column back to collection tube and centrifuge at 8000 rpm for 2 minutes to ensure all buffers have been removed from the column matrix. Discard the collection tube.

5. Elution

5.1 Transfer the silica spin column to a new, sterile DNase free microcentrifuge tube.

5.2 Add 100 ul of pre-heated elution buffer directly to the silica spin column matrix.

5.3 Incubate for 5 minutes at room temperature.

5.4 Spin for 2 minutes at max speed to retrieve DNA. Discard the silica spin column and keep the centrifuge tube with eluted DNA.

11. TROUBLESHOOTING GUIDE

Problem Description	Possible Causes	Suggestions
Low DNA Yield	Incomplete cell lysis	Ensure complete homogenization of the sample. For tough samples, increase vortexing time or use a bead beater.
	Insufficient sample quantity	Increase the amount of starting material, keeping within the recommended range for the kit.
	Loss of DNA during purification steps	Be careful during the transfer of supernatant to avoid loss. Ensure the spin column does not dry out completely before elution.
DNA Purity Issues (Low A260/A280 Ratio)	Contamination with proteins or other organic compounds	Repeat the extraction with an increased volume of wash buffers. Ensure all buffers are prepared correctly.
	Residual ethanol in the DNA sample	Extend the final drying step of the spin column before elution. Ensure complete evaporation of ethanol and ensure that the tip of the silica spin column does not touch the flow through.

	Contamination with RNA	Perform RNase treatment according to manufacturer's instructions.
High DNA Degradation	Harsh mechanical lysis	Reduce vortexing or bead beating time. Avoid high-speed centrifugation that might shear the DNA.
	Improper storage of isolated DNA	Store the eluted DNA at -20Â°C for long-term storage or 4Â°C for short-term storage. Avoid repeated freeze-thaw cycles.
Inconsistent DNA Quality Across Samples	Variation in sample types or sizes	Standardize sample type and size. Pre-test samples to adjust the lysis buffer volume and incubation time as needed.
	Cross-contamination	Use new pipette tips for each sample and reagent. Clean work area and equipment between samples.
PCR Inhibition	Residual contaminants or inhibitors	Increase the volume of the wash buffer or perform an additional wash step. Test the DNA dilution effect on PCR efficiency.
Equipment Malfunction	Centrifuge not reaching required speed	Verify centrifuge performance and calibration. Use an alternative centrifuge if available.
	Vortexer not providing sufficient mixing	Ensure the vortexer is set to the highest speed. If the problem persists, check the equipment for faults.
Buffer Precipitation	Cold storage of buffers that should be at room temperature	Warm the buffers to dissolve precipitates before use. Store buffers according to the manufacturer's instructions.
	Incorrect preparation of buffers	Re-check buffer preparation instructions to ensure correct dilution ratios and components.
Difficulty in Eluting DNA	Spin column membrane dried out	Do not over-dry the spin column before elution. If the membrane is dry, add elution buffer and wait a few minutes before centrifugation.
	Incomplete elution	Perform a second elution step (note: this will cause the sample to become more diluted)
	Elution buffer not adequately heated	Heat the elution buffer to the recommended temperature to improve DNA recovery.

Clogged Column	Too much sample	Reduce the amount of sample added. Make sure to not exceed the required buffer to sample ratio and do not overload columns.

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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