

IsoBind Plasmid Isolation Kit

Cat No. IB-PLAS-50

System: Silica spin columns (manual workflow)

Sample types: Bacterial cultures (high-copy plasmid DNA from 1–5 ml of overnight cultures grown in Luria-Bertani (LB) medium or similar bacterial growth media. While the kit is primarily intended for use with bacterial cultures, it may also be adaptable to other sample types that contain plasmid DNA)

USER MANUAL



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1. KIT CONTENTS

The IsoBind Plasmid Isolation Kit by Gene Vantage is meticulously engineered to provide an efficient and reliable method for the isolation of high-quality plasmid DNA from a wide array of sample types. Each component of the kit has been optimised to ensure maximal yield and purity of the isolated Plasmid, which is crucial for downstream applications such as cloning, sequencing, and gene expression analysis. This section outlines the contents of the kit, their functions, and the required volumes per sample, along with storage recommendations to maintain their integrity and performance.

Kit Contents for Gene Vantage IsoBind Plasmid Isolation Kit

Component	Description /Function	Volume Required per Sample	Short Term Storage	Long Term Storage	Total for 50 Samples	Total for 100 Samples
Lysis Buffer	Facilitates the lysis of bacterial cells to release plasmid	200 μL	Room temperature (15-25°C)	Room temperature (15-25°C)	10 mL	20 mL
Binding Buffer *Add Isopropanol (95%) prior to use	Binds plasmid DNA to the silica spin column under high- salt conditions.	250 μL	Room temperature (15-25°C)	Room temperature (15-25°C)	12.5 mL	25 mL
Wash Buffer A * Add EtOH (96-100 %) prior to use	Removes contaminant s and impurities from the bound DNA.	400 μL	Room temperature (15-25°C)	Room temperature (15-25°C)	20 mL	40 mL
Wash Buffer B * Add EtOH (96-100 %) prior to use	Provides an additional wash step to ensure high purity of the eluted plasmid DNA.	400 μL	Room temperature (15-25°C)	Room temperature (15-25°C)	20 mL	40 mL



Elution Buffer	Elutes purified plasmid DNA from the silica spin column in a low-salt buffer.	50 μL	Room temperature (15-25°C)	4-8°C	2.5 mL	5 mL
Silica Spin Columns (blue o- ring)	Allows for the binding, washing, and elution of plasmid DNA.	1 column + collection tube	Room temperature (15-25°C)	Sealed in ziplock at 4-8°C	50 units	100 units



Buffers contain skin irritants



Wear gloves



2. IMPORTANT NOTES

Before beginning your work with the IsoBind Plasmid Isolation Extraction Kit, please take a moment to review these important notes. Adhering to these guidelines will ensure optimal results and efficiency throughout your DNA extraction process.

Sample Preparation: Achieving a homogeneous sample is crucial for consistent DNA yields. Particularly with complex 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 Plasmid Isolation 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 Plasmid Isolation Kit by Gene Vantage is expertly crafted to enable the efficient and reliable extraction of high-quality plasmid DNA from bacterial cultures. This kit incorporates a blend of optimized chemical lysis and advanced purification processes to meet the exacting requirements of molecular biology, genetic engineering, and biotechnology research.

Advanced Purification Technology: The kit employs a proprietary silica spin column-based technology to ensure a streamlined purification process. This technology effectively removes PCR inhibitors and other contaminants, guaranteeing the highest integrity of plasmid DNA for downstream applications such as cloning, PCR, and sequencing.

Dual-Phase Lysis System: The kit is equipped to handle up to 5 mL of bacterial culture, ensuring comprehensive cell disruption for a wide range of bacterial species. This dual-phase lysis system ensures maximum yield and purity of plasmid DNA.

The kit employs a harmonious blend of chemical and mechanical lysis techniques, coupled with an advanced purification process, to ensure the isolation of plasmid DNA with exceptional purity and integrity.

Cell Lysis: The lysis process is initiated by resuspending the bacterial pellet in a specially formulated Lysis Buffer. This buffer contains a balanced mixture of chaotropic agents and detergents that work synergistically to disrupt the bacterial cell wall and membrane, releasing the plasmid DNA into the solution. The lysis step is carefully optimized to ensure complete cell disruption while preserving the integrity of the plasmid DNA.

Plasmid Binding: Following cell lysis, the Binding Buffer is added to the lysate. This buffer creates conditions that favor the selective binding of plasmid DNA to the silica matrix of the spin column. The high salt concentration in the Binding Buffer promotes the interaction between the negatively charged DNA and the positively charged silica surface, enabling efficient capture of the plasmid DNA while allowing other cellular components to pass through.

Washing: After the plasmid DNA is bound to the column, a series of washing steps are performed using Wash Buffers 1 and 2. These wash buffers are meticulously designed to remove contaminants, such as proteins, lipids, and residual salts, from the bound DNA. The wash buffers differ in their composition and stringency to ensure a thorough cleansing of the silica matrix, resulting in plasmid DNA of exceptional purity.

Elution: The final step involves eluting the purified plasmid DNA from the silica column using an Elution Buffer. This buffer has a low ionic strength, which disrupts the interaction between the DNA and the silica, allowing the plasmid DNA to be released into the eluate. The elution process is optimized to maximize the recovery of plasmid DNA while maintaining its quality and suitability for downstream applications.



Key Features:

<u>Quality of Output</u>: 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.

<u>Comprehensive Cell Disruption</u>: The Lysis Buffer and Proteinase K combination effectively disrupts a wide variety of cell types, ensuring complete release of DNA.

<u>Enhanced Recovery</u>: 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.

<u>Time Efficiency</u>: 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.

<u>Ease of Use:</u> 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.

<u>Streamlined Approach</u>: 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.

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

<u>Scalability</u>: 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

The IsoBind Plasmid Isolation Kit provides a streamlined and efficient protocol for isolating plasmid DNA from bacterial cultures. Here is a detailed overview of each step in the procedure:

Sample Collection:

Collect bacterial cultures, ensuring that they are well-grown and contain the desired plasmid. Note details such as strain, growth conditions, and culture volume for accurate record-keeping.

Cell Lysis:

Add Lysis Buffer to the bacterial pellet, ensuring complete resuspension for efficient cell lysis. This step is crucial for releasing the plasmid DNA into the solution.

Plasmid Binding:

Add Binding Buffer to the lysate, promoting the selective adherence of plasmid DNA to the silica matrix of the spin column. This step is essential for capturing the plasmid DNA while allowing other cellular components to pass through.

Washing:

Sequentially add Wash Buffers 1 and 2 to the column, followed by centrifugation to remove contaminants. These washing steps ensure the purity of the isolated plasmid DNA.

Elution:

Add pre-warmed Elution Buffer to the column, incubate, and centrifuge to collect purified plasmid DNA. This final step releases the plasmid DNA from the column, ready for downstream applications



7. KIT SPECIFICATIONS AT A GLANCE

Parameter	Specification
Format	Silica spin columns
Sample Material	Bacterial cultures
Typical Yield	Up to 25 μ g per isolation
Purity Ratios	A260/A280 1.8, indicating high purity
Elution Volume	$50 \mu\text{L}$, can be adjusted for desired concentration
Preparation Time	Approximately 30 minutes, for efficient workflow
Binding Capacity	Up to 25 μ g of plasmid DNA per column

8. WORKFLOW TIPS

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

Keep samples on ice during collection and transport, and process them within a few hours of collection. If this is not possible, aliquot and freeze samples at -80°C as soon as possible.

Solid Tissue Samples: Solid tissues such as plant materials require immediate stabilisation. Flash-freezing in liquid nitrogen is recommended directly after collection to rapidly halt RNAse activity. Store frozen tissues at -80°C in airtight containers with minimal air space to prevent freezer burn, which can degrade tissue integrity and DNA quality.

Storage Considerations: Long-term storage conditions play a crucial role in maintaining DNA integrity. Samples stored at -80°C are generally stable for several years. Avoid frequent temperature fluctuations, as these can lead to ice crystal formation and mechanical breakdown of cellular structures, facilitating DNase activity. For short-term storage (a few days to weeks), refrigerating samples at -20°C may be adequate, but is not recommended for samples sensitive to partial degradation.

SAMPLES SIZE CONSIDERATIONS

The IsoBind Plasmid Isolation Kit is designed to efficiently process 1-5 mL of bacterial culture. This volume range is optimal for achieving high yields of plasmid DNA with excellent purity.



For smaller volumes (less than 1 mL), concentrate the bacterial culture by centrifugation and resuspend in a smaller volume of Lysis Buffer to ensure efficient cell lysis and plasmid recovery.

For larger volumes (greater than 5 mL), divide the culture into multiple aliquots and process each separately. Alternatively, consider using the IsoBind Maxi Kit, which is specifically designed for larger sample volumes and provides a higher binding capacity.

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. Sample Collection

- 1.1 Collect bacterial cultures that have been grown to optimal density in appropriate media, ensuring the presence of the target plasmid.
- 1.2 Document the strain, plasmid type, growth conditions, and volume of each culture for accurate record-keeping and reproducibility.

2. Cell Lysis

- 2.1 Resuspend the bacterial pellet thoroughly in Lysis Buffer. The volume of Lysis Buffer should be sufficient to ensure complete resuspension and efficient cell disruption.
- 2.2 Incubate the lysate for 5 minutes at room temperature, occasionally inverting the tube to promote complete lysis. This step is crucial for releasing plasmid DNA into the solution.

3. Neutralization

- 3.1 Add Neutralization Buffer to the lysate to precipitate cellular debris and proteins. This buffer neutralizes the alkaline conditions of the lysis step, causing the formation of a precipitate containing genomic DNA and cellular proteins.
- 3.2 Centrifuge the mixture to pellet the precipitate, ensuring that the supernatant, which contains the plasmid DNA, is clear.

4. Plasmid Binding

- 4.1 Transfer the supernatant to a spin column containing a silica membrane. The plasmid DNA in the supernatant binds to the silica membrane in the presence of high salt concentrations provided by the Binding Buffer.
- 4.2 Centrifuge the column to remove the flow-through, ensuring that the plasmid DNA remains bound to the membrane.



5. Washing

- 5.1 Add Wash Buffer 1 to the spin column to remove residual proteins and other contaminants. This buffer contains a lower salt concentration, which maintains the binding of plasmid DNA to the membrane while allowing impurities to be washed away.
- 5.2 Centrifuge the column and discard the flow-through. Repeat this step with Wash Buffer 2, which further cleans the bound plasmid DNA and removes any remaining salts.

6. Elution

- 6.1 Add pre-warmed Elution Buffer to the center of the silica membrane in the spin column. The low salt concentration and slightly alkaline pH of the Elution Buffer allow the plasmid DNA to be released from the membrane.
- 6.2 Centrifuge the column to collect the eluted plasmid DNA in a clean microcentrifuge tube. The eluted plasmid DNA is now ready for downstream applications.



11. TROUBLESHOOTING

The following troubleshooting guide addresses common issues encountered during the sample preparation and extraction process using Gene Vantage's Plasmid Extraction Kit:

Problem Description	Possible Causes	Suggestions
Low plasmid yield	Incomplete cell lysis	Ensure complete resuspension of the bacterial pellet in Lysis Buffer. Increase lysis time and/or incubation temperature.
	Overloading of the spin column	Process smaller volumes of bacterial culture or split the lysate into multiple aliquots before loading onto the column.
	Inefficient binding of plasmid to the column	Check the pH and salt concentration of the Binding Buffer. Ensure thorough mixing with the lysate.
Plasmid DNA degradation	Prolonged exposure to room temperature	Minimize the time between lysis and neutralization. Keep lysate on ice if there are delays.
	Mechanical shearing during handling	Avoid vigorous vortexing or pipetting. Use widebore tips for transferring lysate.
Contamination with genomic DNA or proteins	Incomplete removal of cellular debris	Centrifuge the lysate thoroughly after lysis. Ensure the supernatant is clear before proceeding to the binding step.
	Inadequate washing of the spin column	Perform all washing steps as described, ensuring complete removal of wash buffers. Extend wash incubation times if needed.
Poor elution efficiency	Suboptimal elution conditions	Pre-warm the Elution Buffer to 50-60°C. Ensure it is added directly to the center of the spin column membrane.
	Insufficient incubation time during elution	Increase the incubation time with the Elution Buffer to 2-5 minutes to enhance plasmid release.
Buffer precipitation or degradation	Improper storage conditions	Store buffers at the recommended temperatures. Check for precipitation or discoloration before use.
	Old or expired reagents	Replace any reagents that are beyond their shelf life or show signs of degradation.
Equipment malfunction	Centrifuge not reaching required speed	Verify the centrifuge speed with a tachometer. Calibrate and service the centrifuge regularly.
	Inconsistent vortexing or mixing	Ensure the vortex mixer is functioning correctly. Use a consistent and thorough mixing technique.
Incomplete removal of ethanol from the column	Inadequate drying of the spin column	Extend the drying time by centrifuging the empty column for an additional 2-5 minutes.



High salt content in eluted plasmid DNA	Residual wash buffer remaining in the column	Ensure complete removal of Wash Buffer 2 by performing an additional centrifugation step before elution.
Low plasmid purity (A260/ A280 ratio below 1.8)	Contamination with proteins or phenol	Optimize washing steps. Increase the number of washes or the volume of Wash Buffer 2 if necessary.
Inconsistent plasmid yield between replicates	Variation in bacterial culture density or growth conditions	Standardize culture growth conditions. Ensure consistent starting material for each extraction.
Presence of genomic DNA in plasmid preparations	Incomplete neutralization of the lysate	Ensure thorough mixing of the Neutralization Buffer with the lysate. Centrifuge until the debris is completely pelleted.

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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