



IsoBind Gel Kit

Catalog No. IB-GEL-100

System: Silica spin columns (manual workflow)

Sample types: Standard Agarose Gels, Low-Melt Agarose Gels,
Various Gel Concentrations

USER MANUAL

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1. KIT CONTENTS

The IsoBind Gel Melt Kit is designed for the rapid and efficient purification of agarose gel slices, ensuring the isolation of high-quality DNA fragments for downstream applications such as PCR, ligation, and restriction enzyme digests. Before beginning your experiment, ensure that all components are present and properly stored.

Component	Description/ Function	Volume Required per Sample	Short Term Storage	Long Term Storage	Total for 50 Samples	Total for 100 Samples
Gel Melt Buffer	Buffer for melting agarose gel slices and washing the column.	~650 μ L (melt & wash)	Room temperature (15-25°C)	Room temperature (15-25°C)	15 mL	30 mL
Wash C Buffer *Add EOH (90-100%) prior to use	Wash buffer to remove impurities and contaminants.	700 μ L	Room temperature (15-25°C)	Room temperature (15-25°C)	35 mL	70 mL
Elution Buffer	Buffer for eluting purified DNA from the spin column.	50 μ L	Room temperature (15-25°C)	4-8°C	5 mL	10 mL
Blue O-ring Silica Spin Columns	For DNA binding, washing, and elution.	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 Gene Vantage Isobind Gel Extraction 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: The kit is optimized for ~1% agarose gel slices. For higher percentage gels, adjust the volume of Gel Melt Buffer accordingly. For example, if using a 2% gel, double the volume of Gel Melt Buffer to ensure complete dissolution of the agarose.

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 minimise DNA 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 37°C, stirring until the solids have dissolved. Cool the buffers to room temperature before application to prevent thermal degradation of DNA.

Centrifugation Parameters: 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 Gel 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 Gel Extraction Kit is engineered to efficiently clean up and recover high-quality DNA from samples that have gone through gel electrophoresis. This process is facilitated by silica-based spin columns specifically designed to maximize DNA yield and purity. Below is a detailed explanation of each step involved in the DNA extraction process:

Gel Melting: The Gel Melt Buffer melts the agarose gel slice, releasing the DNA fragments into the solution.

DNA Binding: 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 column 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 a wash buffer that is designed to efficiently remove different types of contaminants. The Wash Buffer involves adding a specific volume of buffer, followed by centrifugation to pull the liquid through the column while the DNA remains bound to the silica membrane. This ensures that only purified DNA remains on the column.

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 nucleic acid into a clean microcentrifuge tube.

Key Features:

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

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

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 the purification of DNA fragments ranging from 150 bp to 5 kb in length, making it a useful tool in both clinical diagnostic and research settings.

Time efficiency: The entire DNA extraction process can be completed in approximately 45 minutes for 24-48 samples, which is ideal for labs seeking to maintain their turnaround times without compromising on the quality of results.

Compatibility with Downstream Applications: It is optimized for standard or low-melt agarose gels in TAE or TBE buffer. 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 volume sample processing, with options for manual (single spin column) and semi-automated (96 well spin plate) workflows.

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 buffers, which is crucial for 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 melting and elution steps. The ability to set precise temperatures is essential, to ensure controlled heating for gel melting. Must be able to reach and maintain temperatures of 60°C - 70°C.

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 a 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 (if applicable)	Warm the Elution Buffer to 60-70°C in a heating block or oven.	
Gel Slice Treatment	Cut agarose gel slice containing the DNA > transfer to a clean microtube > Add Gel Melt Buffer to gel slice > incubate @ 55°C until dissolved.	2.5 volumes of buffer per 1 volume of gel
Binding	Add isopropanol to gel mixture > apply mixture to spin column > Centrifuge for 30 secs to bind DNA to the column.	(100 µL per 100 mg of gel)
Washing	Wash column with 400 µL of Gel Melt Buffer > Add 700 µL of Wash C, Centrifuge for 30 secs.	Ensure the wash buffer passes through column
Dry Centrifuge	Spin @ max speed for 1 minute to dry column	This step is crucial to ensure there is no residual buffer bound to your column
Elution	Add 50 µL of Elution Buffer to the column > incubate for 1 min > centrifuge to collect purified DNA.	
Storage	Eluted DNA is suitable for immediate use or for storage	Store eluted DNA at -20 degrees for short term storage and -80 degrees for long term storage

7. KIT SPECIFICATIONS

Parameter	IsoBind Gel Extraction Kit
Format	Spin columns
Sample Material	Standard and low-melt agarose gels, various gel concentrations
Typical Yield	Depends on the amount and purity of DNA in the gel slice
Elution Volume	50 µL
Preparation Time	Approximately 30 min for typical protocol
Binding Capacity	Up to 10 µg of DNA

8. WORKFLOW TIPS

To maximize the effectiveness and reliability of the IsoBind Gel Extraction 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.

Short-Term Storage: Store agarose gel slices containing the DNA fragments of interest at 4°C in a sealed container or a gel storage buffer to prevent drying out. This is suitable for storage up to a week.

Long-Term Storage: For longer storage, place the gel slices at -20°C. Ensure the slices are well protected in airtight containers or wrapped in plastic wrap to prevent dehydration.

Light Exposure: Minimize exposure to light, especially UV light, as it can cause DNA damage. Wrap the container or use an amber-colored container for storage.

Handling: Handle the gel slices with clean, nuclease-free tools to avoid contamination. Use disposable gloves and change them frequently.

SAMPLES SIZE CONSIDERATIONS

Optimal Gel Slice Size: Determine the ideal size of the agarose gel slice based on the specific requirements of the IsoBind Gel Extraction Kit. This is critical for achieving efficient gel dissolution, DNA binding, and recovery.

Avoiding Overloading: Ensure that the weight of the agarose gel slice is proportional to the capacity of the kit. Overloading with excessive gel material can hinder the extraction process. Typically, the kit can accommodate up to 10 µg of DNA.

Scaling Buffer Volumes: Consider the percentage of the agarose gel. Higher percentage gels (e.g., 2%) require more Gel Melt Buffer to ensure complete dissolution. Adjust the volume of Gel Melt Buffer accordingly.

Fragment Size Range: Ensure that the DNA fragments within the gel slice fall within the optimal size range of 150 bp to 5 kb. This range is ideal for efficient binding and elution from the spin column.

Clean Excision: When excising the DNA band from the gel, aim to cut as close to the band as possible to minimize contamination with other DNA fragments and ensure the purity of the extracted DNA.

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.

Centrifuge 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 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 a DNase-free environment. Use DNase decontamination solutions and maintain clean bench practices throughout the procedure.

10. COMPLETE PROTOCOL

1. Sample collection

- 1.1 Run your previously eluted DNA on agarose gel.
- 1.2 Identify your fragment of interest and cut it out of the agarose using a scalpel. Minimize the volume of agarose by trimming excess from the fragment.
- 1.3 Transfer the slice to a clean microtube.

2. Gel Melting

- 2.1 Add 2.5 volumes of Gel Melt Buffer to 1 volume of agarose gel. e.g. 250 ul Gel melt buffer + 100 mg 1 % gel. Increase Gel Melt buffer to 500 ul if using 2 % gel, i.e. double the volume of the buffer if the agarose gel percent is doubled.
- 2.2 Incubate at 55°C for 10 min or until the gel slice fully dissolved. Mix as needed.
- 2.3 Add 100 ul of 95% isopropanol per 100 mg of gel. Pipette to mix.
- 2.4 Transfer sample to a silica spin column with a collection tube.
- 2.5 Centrifuge for 30 seconds at 10 000 rpm and discard flow-through. Reuse collection tube.
NB Ensure all samples have passed through the column!
- 2.6 Add 400 ul Gel Melt Buffer to the column.
- 2.7 Centrifuge for 30 seconds at 10 000 rpm and discard flow through. Reuse collection tube.

3. Washing

- 3.1 Add 700 ul Wash C to the silica spin column.
- 3.2 Centrifuge for 30 seconds at 10 000 rpm and discard flow through. Replace collection tube

4. Dry Centrifuge Step

- 4.1 Centrifuge at 10 000 rpm for 30 seconds to dry the column.

5. Elution:

- 5.1 Transfer silica spin column to clean, sterile microcentrifuge tube for elution.
- 5.2 Add 50 ul of heated Elution Buffer to the silica spin column.
- 5.3 Incubate for 1 minute on the bench.
- 5.4 Centrifuge for 30 seconds to retrieve samples. Continue to downstream assay.

11. TROUBLESHOOTING GUIDE

Problem Description	Possible Causes	Suggestions
Low DNA Yield	Insufficient sample quantity	Increase the amount of starting material, keeping within the recommended range for the kit.
	Insufficient incubation time	Extend the incubation time during the melting step
	DNA degradation	Use fresh samples or store samples properly. Add DNase inhibitors if needed.
Poor DNA purity (low A260/A280 ratio)	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 -80°C or use immediately after collection.
	Prolonged exposure to room temperature	Keep samples on ice during the extraction process.
Inconsistent DNA Yield	Variation in sample types or sizes	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 Buffers
	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.
	Elution buffer not adequately heated	Heat the elution buffer to the specified temperature. Let it incubate in the column before centrifugation. If yield is still low, perform a second elution

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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