IsoBind PCR Kit

Catalog No. IB-PCR-50

Catalog No. IB-PCR-100

System: Silica spin columns (manual workflow)

Sample types: PCR products

USER MANUAL

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Table of Contents

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1. KIT CONTENTS

The IsoBind PCR mini clean-up kit is designed for the ultra-quick purification of PCR products using spin columns. This kit provides all necessary components for the purification of single or double-stranded DNA from PCR or other enzymatic reactions. Ensure all components are present before starting.

Component	Description/ Function	Volume Required per Sample	Short Term Storage	Long Term Storage	Total for 50 Samples	Total for 100 Samples
Buffer GHI	Binding buffer for DNA purification	115 µL per 25 µL PCR rxn	Room temperature (15-25°C)	Room temperature (15-25°C)	30 mL	60 mL
Wash C ***add EOH (90-100%) prior to use**	Wash buffer to remove impurities	700 µL	Room temperature (15-25°C)	Room temperature (15-25°C)	35 mL	70 mL
Elution Buffer	Buffer for eluting purified DNA	40 µL	Room temperature (15-25°C)	4-8°C	5 mL	10 mL
Spin Columns (blue o-ring)	Silica spin columns for DNA binding and purification	1 column per sample + 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 PCR 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: Ensure samples are homogenized thoroughly before processing. This is crucial for achieving consistent DNA yields. For samples with high humic acid content, additional steps may be necessary to remove these substances effectively.

Reagent Preparation: Some reagents may require preparation or dilution before use. Verify the conditions and preparation instructions for each reagent, especially the lysis and wash buffers. In case of buffer precipitation, gently warm the solution to dissolve any solids and allow it to cool to room temperature before use.

Centrifugation Protocols: The protocols involve centrifugation at specific speeds. Using the correct speed is vital for the efficient separation of supernatant and pelleted material. Ensure your centrifuge is calibrated and capable of achieving the required speeds.

Column Handling: Do not exceed the maximum loading volume for spin columns. Overloading can lead to incomplete DNA purification and reduced yield. If your sample volume exceeds this limit, process the sample in multiple aliquots.

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:

Access to Assistance: 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 PCR 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 PCR mini clean-up kit is a meticulously engineered solution for the purification of PCR products, leveraging the efficiency of silica spin column technology to deliver high-quality DNA suitable for a range of downstream applications.

The kit operates on a solid-phase extraction principle, utilizing the selective affinity of DNA for silica in the presence of chaotropic salts.

Cell Lysis: The process begins with the addition of Buffer GHI to the PCR sample. Buffer GHI contains chaotropic salts, which disrupt the hydrogen bonding network in water, leading to the denaturation of proteins and the release of nucleic acids into solution. This step ensures that the DNA is freed from the complex matrix of the PCR reaction components.

DNA Binding: The chaotropic salts in Buffer GHI also facilitate the binding of DNA to the silica membrane within the spin column. In the presence of these salts, the silica surface becomes positively charged, attracting the negatively charged phosphate backbone of the DNA. This interaction is selective for DNA, allowing other components of the PCR reaction to be washed away in subsequent steps.

Washing: After the DNA has bound to the silica membrane, Wash C buffer is used to remove any remaining contaminants, including proteins, salts, and unincorporated nucleotides. The washing process is critical for the purity of the final DNA product, as it ensures that only DNA remains bound to the silica membrane.

Elution: The final step involves the elution of purified DNA from the silica membrane. This is achieved by adding a low-salt Elution Buffer to the column, which disrupts the interactions between the DNA and the silica surface. The eluted DNA is then collected in a clean tube, ready for downstream applications. The efficiency of the elution process is enhanced by preheating the Elution Buffer, which helps to solubilize the DNA and facilitate its release from the membrane.

Key Features:

Versatility**:** The IsoBind PCR mini clean-up kit is designed to purify PCR products ranging from 150 bp to 5 kb in length, making it suitable for a wide array of molecular biology applications. This versatility ensures that researchers can use the kit for various PCR assays, including those requiring precise fragment size selection.

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.

Enhanced Recovery: Tailored for samples that are difficult , 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.

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	Description	Volume/ Condition	Key Notes
Lysis	1. Add 4.6 volumes of Buffer GHI to 1 volume of the PCR sample. 2. Mix thoroughly by pipetting.	115 µL Buffer GHI to 25 µL sample	Ensures complete lysis of the PCR components, releasing the DNA into solution.
Binding	1. Transfer the lysate to the spin column. 2. Centrifuge at 13,000 rpm for 30 seconds.	Discard flow-through	DNA binds to the silica membrane in the presence of chaotropic salts, ensuring selective purification.
Washing	1. Add 700 µL of Wash C buffer to the column. 2. Centrifuge at 13,000 rpm for 30 seconds. 3. Perform an additional centrifugation step without adding more wash buffer to dry the silica membrane.	700 µL Wash C	Removes impurities effectively. The final spin without additional buffer ensures complete drying of the membrane.
Elution	1. Preheat the Elution Buffer to 60-70°C. 2. Add 40 µL of the preheated Elution Buffer to the spin column. 3. Incubate at room temperature for 1 minute. 4. Centrifuge at 13,000 rpm for 30 seconds.	40 µL Elution Buffer	The heated Elution Buffer enhances DNA recovery by promoting efficient release from the silica membrane.

7. KIT SPECIFICATIONS

Parameter	Specification
Format	Silica spin column
Sample Material	PCR products
Typical Yield	Up to 95% recovery of PCR products
Purity Ratios	A260/A280 > 1.8
Elution Volume	40 µL (adjustable based on desired concentration)
Preparation Time	Less than 15 minutes from start to finish
Binding Capacity	Up to 10 µg of DNA per column
Sample Volume	Up to 25 µL of PCR reaction
Fragment Size Range	150 bp - 5 kb
Storage Conditions	Room temperature for all components
Shelf Life	12 months at room temperature
Applications	Sequencing, cloning, PCR, restriction digestion, etc.

8. WORKFLOW TIPS

COLLECTION AND STORAGE OF STARTING MATERIAL

Proper collection and storage of PCR samples are critical for maintaining the integrity and quality of DNA, which can significantly impact the effectiveness of the IsoBind PCR mini clean-up kit. Here are detailed guidelines:

Immediate Processing: Use sterile, nuclease-free tubes for sample collection to prevent contamination. Contaminants can inhibit PCR reactions and affect the purification process.
If possible, process samples immediately after collection to prevent degradation. If immediate processing is not feasible, stabilize the samples as described below.

Short-term Storage: For short-term storage (a few days), store samples at 4°C. This temperature is sufficient to prevent significant DNA degradation for short periods.

Long-term Storage: For long-term storage, freeze samples at -20°C or -80°C. Freezing at -20°C is suitable for most PCR samples for several months, but for storage longer than six months or for very sensitive applications, -80°C is recommended to ensure DNA integrity**.**

Avoid Repeated Freeze-Thaw Cycles: Repeated freezing and thawing can fragment the DNA and reduce its quality. To avoid this, aliquot samples into usable volumes before freezing so that each aliquot is thawed only once.

Documentation: Clearly label each sample with relevant information (e.g., date of collection, sample type, concentration) to ensure proper identification and traceability. Maintain a detailed log of sample storage conditions and duration. This documentation can be critical for troubleshooting issues related to sample quality affecting the PCR results.

SAMPLE SIZE CONSIDERATIONS

Proper sample size is crucial for the effectiveness of the IsoBind PCR mini clean-up kit. This section provides detailed guidelines on how to adjust sample and buffer volumes based on the size and concentration of your PCR samples.

Optimal Sample Size: The kit is optimized for PCR samples typically ranging from 25 µL to 100 µL. This range ensures the best interaction with the kit's buffers and the silica membrane for efficient DNA binding and purification.

Buffer Volume Scaling: For PCR reactions larger than 100 µL, it is recommended to proportionally increase the amount of Buffer GHI used during the lysis stage to maintain the correct chemistry for DNA binding. However, do not process more than 100 µL in a single column to avoid overloading. Instead, split larger volumes into multiple columns.

Buffer Volume Adjustments: Buffer GHI: Always use 4.6 volumes of Buffer GHI relative to your sample volume. For example, for a 50 µL sample, you would need 230 µL of Buffer GHI.

Wash Buffer: Regardless of the sample volume, use a full 700 µL of Wash C buffer per washing step to ensure all contaminants are effectively removed from the DNA bound to the silica membrane.

Sample Concentration: If dealing with highly concentrated DNA samples, consider diluting the sample to fall within the optimal range for the kit (not exceeding 10 µg total DNA per column) to avoid saturation of the silica membrane and potential loss of yield.

For low-concentration samples, ensure the total volume does not exceed 100 µL. It may be beneficial to concentrate the sample before processing if the volume is too great.

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

The success of DNA purification using the IsoBind PCR mini clean-up kit depends on careful sample preparation and extraction. Follow these detailed guidelines to ensure optimal results:

1. Sample Collection:

1.1 Collect your PCR samples in sterile, nuclease-free tubes. This minimizes the risk of contamination that could affect the purity and yield of the extracted DNA.

Homogenisation (if applicable):
1.2 For PCR samples that require homogenization, such as those containing particulate matter or complex mixtures, ensure complete disruption of the sample. This can be achieved through methods like vortexing, bead beating, or sonication, depending on the sample type.

2. Lysis:

2.1 Add 4.6 volumes of Buffer GHI to 1 volume of PCR sample. For example, mix 115 µL of Buffer GHI with 25 µL of PCR sample.

2.2 Mix thoroughly by pipetting up and down several times to ensure complete lysis. The chaotropic salts in Buffer GHI will denature proteins and release the DNA into solution.

2.3 Ensure complete mixing during the lysis step to maximize DNA yield.

3. Binding:

3.1 Transfer the lysate to the spin column provided in the kit. The silica membrane in the spin column selectively binds DNA in the presence of chaotropic salts.

3.2 Centrifuge the column at 13,000 rpm for 30 seconds. The DNA will bind to the membrane while the rest of the lysate passes through as flow-through, which should be discarded.

4. Washing:

4.1 Add 700 µL of Wash C buffer to the spin column. This buffer removes contaminants while keeping the DNA bound to the membrane.

4.2 Centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.

4.3 Repeat this washing step to ensure thorough removal of contaminants.

4.4 Perform an additional centrifugation step without adding more wash buffer to dry the silica membrane completely.

4.5 Be thorough in the washing steps to remove all contaminants, which can affect downstream applications.

4.6 Make sure the spin column is completely dry after the final wash step to prevent ethanol carryover, which can inhibit downstream reactions.

5. Elution:

5.1 Preheat the Elution Buffer to 60-70°C. The elevated temperature helps to increase the efficiency of DNA elution from the silica membrane.

5.2 Transfer the spin column to a clean 1.5 mL microcentrifuge tube.

5.3 Add 40 µL of preheated Elution Buffer to the center of the silica membrane in the spin column. 5.4 Incubate at room temperature for 1 minute to allow the DNA to dissolve into the buffer.

5.5 Centrifuge at 13,000 rpm for 30 seconds to collect the eluted DNA in the tube. The eluted DNA is now ready for use in downstream applications.
If the DNA concentration is too low, consider reducing the elution volume or performing a second elution step with a fresh Elution Buffer.

11. TROUBLESHOOTING GUIDE

Problem Description	Possible Causes	Suggestions
Low DNA Yield	Incomplete cell lysis	Ensure thorough mixing of Buffer GHI with the PCR sample. Incubate for an additional 1-2 minutes to enhance lysis.
	Inefficient DNA binding	Check the pH of Buffer GHI. Adjust if necessary to ensure optimal binding conditions. Verify the integrity of the spin column membrane.
	Sample overload	Reduce the sample volume or split the sample into multiple columns to prevent overloading.
Impure DNA (Low A260/A280 Ratio)	Residual ethanol from Wash C	Extend the drying time after the final wash step to ensure complete removal of ethanol. Perform an additional centrifugation step if needed.
	Contaminants in eluate	Increase the number of wash steps. Ensure thorough removal of Wash C buffer before elution.
Inconsistent DNA Yields	Variation in sample concentration	Standardize the concentration of PCR samples. Consider quantifying DNA before processing.
	Incomplete lysis	Verify that each sample is fully lysed by checking for clarity in the lysate. Adjust lysis time if necessary.
Equipment Malfunction (Centrifuge)	Incorrect centrifuge speed	Confirm that the centrifuge is set to the correct speed (13,000 rpm) and is properly calibrated.

	Mechanical issues	Inspect the centrifuge for any signs of wear or damage. Perform maintenance or repairs as needed.
Contamination in DNA Samples	Cross-contamination during handling	Use sterile, nuclease-free consumables. Practice good pipetting techniques to avoid cross-contamination.
	Contaminated reagents or buffers	Prepare fresh buffers and reagents. Store them under appropriate conditions to prevent contamination.
Difficulty in Eluting DNA	Incomplete incubation with Elution Buffer	Ensure the Elution Buffer is preheated and incubated with the column for at least 1 minute. Gently pipette the buffer onto the membrane to enhance elution.
	Low elution volume	Increase the volume of Elution Buffer if a higher DNA yield is desired. Consider a second elution step to maximize recovery.
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.
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 -20°C for long-term storage or 4°C for short-term storage. Avoid repeated freeze-thaw cycles.

12. PRODUCT USE RESTRICTION / WARRANTY

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