



# Amplimax Plant DNA kit

Cat No. AM-PDNA-100

**System:** Magnetic Beads, suitable for manual or automated workflows

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

## USER MANUAL

**Website** [www.gene-vantage.com](http://www.gene-vantage.com)

**Technical support** [info@gene-vantage.com](mailto:info@gene-vantage.com)

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Technical support: [info@gene-vantage.com](mailto:info@gene-vantage.com)

## 1. KIT CONTENTS

Gene Vantage's AmpliMax Plant DNA Extraction Kit is designed to provide researchers with an efficient solution for extracting high-quality DNA from a wide range of plant materials. Optimised for compatibility with automated systems, the kit features a streamlined protocol that ensures high purity and yield, making it ideal for various downstream applications such as RT-PCR. This kit is crafted to handle diverse biological samples, accommodating the unique challenges presented by plant tissues, including those rich in polyphenols and polysaccharides. This kit supports critical applications in genomics, agrigenomics, and molecular diagnostics, with specific optimization for RT-PCR and other sensitive downstream assays

| Component                                                                      | Description/Function                                                                                             | Volume Required per Sample | Short Term Storage | Long Term Storage | Total for 100 Samples | Total for 250 Samples |
|--------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|----------------------------|--------------------|-------------------|-----------------------|-----------------------|
| <b>Lysis Buffer</b>                                                            | Lyses cells to release DNA. Specially formulated to ensure complete lysis of cellular material for optimal yield | 400 $\mu$ L                | Room temperature   | Room temperature  | 40 mL                 | 100 mL                |
| <b>Binding Buffer</b><br>* Add Isopropanol (95%, molecular grade) prior to use | Facilitates binding of DNA to the silica membrane.                                                               | 300 $\mu$ L                | Room temperature   | 4-8°C             | 30 mL                 | 75 mL                 |
| <b>Booster Compound</b>                                                        | Increases selectivity of the magnetic beads and creates optimal conditions for DNA binding                       | 20 $\mu$ L                 | Room temperature   | 4-8°C             | 2 mL                  | 5 mL                  |
| <b>Wash Buffer A</b><br>* Add EtOH (96-100%, molecular grade) prior to use     | Removes impurities such as cellular debris and proteins without stripping away the bound DNA                     | 400 $\mu$ L                | Room temperature   | Room temperature  | 40 mL                 | 100 mL                |

|                                                                            |                                                                                                                                                         |        |                     |                  |        |         |
|----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|--------|---------------------|------------------|--------|---------|
| <b>Wash Buffer B</b><br>* Add EtOH (96-100%, molecular grade) prior to use | Removes residual salts without stripping away the bound DNA                                                                                             | 400 µL | Room temperature    | Room temperature | 40 mL  | 100 mL  |
| <b>Wash Buffer C</b><br>*Add EtOH (96-100%, molecular grade) prior to use  | Final wash to remove traces of chaotropic agents                                                                                                        | 400 µL | Room temperature    | Room temperature | 40 mL  | 100 mL  |
| <b>Elution Buffer</b>                                                      | Elutes purified DNA from the beads                                                                                                                      | 100 µL | Room temperature    | 4-8°C            | 10 mL  | 25 mL   |
| <b>Magnetic Beads</b>                                                      | Superparamagnetic particles that selectively bind the nucleic acid, creating a complex which is easily separated from the aqueous phase using a magnet. | 25 µL  | At room temperature | 4-8°C            | 2.5 mL | 6.25 mL |



Buffers contain skin irritants



Wear gloves

## 2. IMPORTANT NOTES

To ensure optimal performance and safety when using the AmpliMax Plant DNA Extraction Kit, please adhere to the following detailed guidelines and precautions:

**Sample Preparation:** It is crucial that plant samples are thoroughly homogenized before processing. This involves physical disruption, which can be achieved by grinding the tissue in liquid nitrogen or using a high-speed blender. Proper homogenization is essential for consistent DNA yields, especially from tough or fibrous plant tissues.

**Handling of Diverse Plant Materials:** This kit is designed to accommodate a wide range of plant species, including those with high levels of secondary metabolites like polyphenols and polysaccharides, which are challenging for DNA extraction. Special attention should be given to these samples to ensure that all components interact effectively, achieving optimal lysis and binding.

**Reagent Preparation:** Before starting your extraction, ensure all reagents are brought to room temperature (15-25°C). This is particularly important for enzymes and buffers, as temperature can significantly impact their effectiveness.

**Buffer Resuspension:** If any precipitate is observed in buffers, particularly lysis buffer which may contain SDS that precipitates at lower temperatures, gently warm the buffer to 37°C while stirring until the solution is clear. Allow the buffer to cool to room temperature before use to maintain reagent integrity.

**Column Capacity:** It is critical not to exceed the maximum loading volume recommended for the silica spin columns. Overloading may result in incomplete purification and can compromise both the yield and quality of the extracted DNA. If the sample volume exceeds this limit, it should be processed in multiple aliquots.

**Elution Efficiency:** The volume of elution buffer can be adjusted based on the required DNA concentration for downstream applications. While decreasing the volume increases the concentration, it may reduce the overall yield. It is important to balance between yield and concentration based on the specific needs of subsequent analytical procedures.

**Storage and Stability:** To maintain the stability and performance of the kit components, store all reagents according to the guidelines provided. Most reagents are stable at room temperature, but some, particularly enzymes and certain buffers, should be stored at 4-8°C or even -20°C for long-term storage.

**Magnetic Beads:** These are the cornerstone for the DNA purification process, ensuring high selectivity and binding capacity. Prior to use, ensure the beads are fully resuspended to achieve uniform consistency. This is crucial for reproducibility and efficiency in DNA recovery across samples. Do not centrifuge or freeze the beads, or they will have to be discarded.

**Booster:** This component is designed to augment the yield of DNA, especially when working with samples that have low nucleic acid concentrations. It must be added with precision as per the recommended volumes, considering the variation in nucleic acid content among different sample types.

**Technical Support:** Should you encounter any difficulties or have questions regarding the use of the kit, our technical support team is readily available to provide assistance. We are committed to ensuring that you achieve the best possible results with our products and are here to support you through every step of your scientific journey.

### 3. SAFETY PRECAUTIONS

Ensure the safety of all laboratory personnel by adhering to standard laboratory practices when using the Amplimax 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

The AmpliMax Plant DNA Extraction Kit is designed to efficiently isolate high-quality DNA from a variety of plant tissues using a solid-phase extraction method based on silica spin column technology. This section details the scientific principles and mechanisms underlying each step of the DNA extraction process, providing a comprehensive understanding of how the kit functions at a molecular level.

**Cell Lysis:** The initial step in the DNA extraction process is cell lysis, which involves breaking open the plant cells to release their nucleic acids and other cellular components into solution. This is critical as the integrity and purity of DNA extracted depend significantly on effective lysis. The kit uses a combination of physical and chemical lysis methods -

Physical Lysis: Mechanical disruption is achieved through grinding plant material in liquid nitrogen or using a bead beater. This method is particularly effective for tough, fibrous tissues which are resistant to chemical lysis alone.

Chemical Lysis: A specially formulated lysis buffer containing detergents such as SDS (Sodium Dodecyl Sulfate) disrupts the lipid bilayer of cell membranes and organelles. This detergent-based method helps solubilize proteins and other macromolecules, facilitating the release of DNA into the solution.

Enzymatic Treatment: Depending on the tissue type, an enzyme such as Proteinase K may be added to digest proteins and help in liberating tightly bound DNA from chromatin, enhancing yield and purity.

**DNA Binding:** After lysis, the released DNA must be separated from other cellular debris. In this kit, DNA binding to the silica membrane of the spin columns is facilitated by the presence of chaotropic salts in the binding buffer. The chaotropic salts disrupt the hydration layer around nucleic acids and proteins, making the DNA more hydrophobic and thus promoting its adherence to the silica membrane in the spin column. This selective binding under high-salt conditions allows other contaminants to remain in solution or be washed away.

**Washing:** The objective of the washing steps is to remove any remaining impurities such as proteins, polysaccharides, phenolic compounds, and salts, which can interfere with downstream applications.

**Wash Buffer A:** Contains a moderate concentration of ethanol, which helps in eliminating proteins and other organic compounds while retaining the DNA bound to the column.

**Wash Buffer B:** Often contains a higher concentration of ethanol and sometimes other additives to ensure the removal of more stubborn contaminants such as polysaccharides and pigments, common in plant extracts.

**Elution:** The final step in the DNA extraction process is to elute the purified DNA from the silica membrane, recovering it in a buffer suitable for storage and use in downstream applications. Typically, a low ionic strength buffer or water, which disrupts the interactions between the DNA and the silica membrane, allowing the DNA to rehydrate and elute into the solution.

### Key Features:

Broad-Spectrum Plant Sample Compatibility: The kit is formulated to handle a diverse array of plant species, including those with high levels of secondary metabolites such as polyphenols and polysaccharides, which are notoriously difficult for DNA extraction. This adaptability makes the kit suitable for research in agricultural genetics, plant pathology, and environmental biotechnology.

High-Efficiency DNA Isolation: The kit's lysis buffer and binding conditions are meticulously developed to maximise the release and subsequent capture of high-quality DNA. This includes the optimal balance of lysis agents and chaotropic salts, ensuring that DNA is efficiently liberated from the cell matrix and bound to the silica membrane.

Streamlined Protocol: The protocols associated with the kit are designed to minimise handling steps and reduce the potential for sample loss or contamination. This streamlined approach not only saves time but also maintains the integrity of the DNA throughout the extraction process.

Purity and Integrity of DNA: The DNA extracted using the AmpliMax kit exhibits high purity with typical A260/A280 ratios between 1.8 and 2.0, indicating minimal contamination by proteins or other organic compounds. This level of purity is crucial for sensitive downstream applications such as quantitative PCR (qPCR), cloning, and next-generation sequencing.

Protocol Versatility: The kit includes detailed protocols that can be adjusted based on the specific requirements of the sample and the researcher's needs. This flexibility allows for the optimization of extraction conditions for specific types of plant tissues or for the recovery of DNA from challenging samples.

Scale and Throughput: Available in multiple configurations to accommodate different sample sizes and throughput requirements, the AmpliMax Plant DNA Extraction Kit can efficiently process individual samples or batches, making it suitable for both small-scale laboratory studies and high-throughput industrial applications.

Automation Compatibility: The kit is designed to be compatible with standard laboratory equipment and, for higher throughput needs, can be adapted to work with automated liquid handling systems. This compatibility reduces manual intervention and increases the consistency and reproducibility of the DNA extraction process.

## 5. HARDWARE AND CONSUMABLES (SUPPLIED BY THE USER)

### 5.1 Hardware

Automated Liquid Handling System: Essential for the kit's operation, this system automates the addition, mixing, and transfer of fluids across different stages of the DNA extraction process. It is vital for ensuring consistency and reproducibility, particularly in high-throughput settings.

Magnetic Stand: Used in conjunction with magnetic bead technology, the magnetic stand facilitates the separation of the magnetic beads (with bound DNA) from the supernatant during the wash and elution steps.

Heating Block or Thermomixer: Critical for the proper execution of the cell lysis and elution steps. These devices ensure that samples are maintained at the optimal temperatures necessary for effective lysis and for the elution of DNA from the magnetic beads.

Centrifuge: Used for spinning down samples and reagents, ensuring complete mixing and settling of contents, which is crucial for the homogeneity of samples and the removal of air bubbles.

Vortex Mixer: Necessary for resuspending and thoroughly mixing reagents, especially the magnetic beads, to maintain a uniform suspension that is crucial for the efficiency of the DNA binding process.

\*Tissue Lyser: Effective DNA extraction begins with thorough tissue disruption. A TissueLyser or similar mechanical disruptor is essential for breaking down tough plant cell walls, which is particularly critical for fibrous or woody samples. This equipment uses high-speed shaking with beads or steel balls to pulverize the plant material, ensuring that the cellular contents are fully accessible for subsequent chemical lysis.

\*Mortar and Pestle: Used along with liquid nitrogen to manually crush plant tissues into a fine powder prior to the DNA extraction process.

## 5.2 Consumables

Pipettes and Pipette Tips: Accurate pipetting is essential for the precision of liquid transfers. Use of sterile, disposable tips prevents cross-contamination between samples and reagents.

Reaction Tubes and Plates: Specific to the type of automated system used, these must be compatible with both the hardware and the magnetic bead technology. They serve as vessels in which all reactions take place.

Sealing Films or Caps: Used to cover reaction tubes or plates to prevent evaporation and contamination during incubation steps.

Ethanol (96-100%) and Isopropanol (95%), molecular grade: These solvents are crucial in the washing steps to effectively remove contaminants and impurities from the DNA. It is important that these solvents are of molecular biology grade to ensure optimal results.

\*Liquid Nitrogen: Used as it has a very low temperature of  $-176^{\circ}\text{C}$  which can help to pulverize the hard cell wall of plants to turn the sample into powder. This ensures that the subsequent buffers are able to interact with as much of the plant material during the extraction as possible. It also helps to deactivate DNAases that may digest the DNA and lead to poor yield and quality.

### \* Optional step in the extraction process

## 6. QUICK VIEW PROTOCOL

| Steps                     | Procedure                                                                                                                                                                                                                                                                                             | Details                                                                                                                                                                                                                                        |
|---------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Sample Preparation</b> | Place 25-30 mg of plant tissue material into a microcentrifuge tube with 4 mm steel balls. Crush in a Tissue Lyser until fully homogenized OR Grind up 25 - 30 mg of plant tissue using liquid nitrogen and a mortar and pestle                                                                       | Mechanical lysis step                                                                                                                                                                                                                          |
| <b>Lysis</b>              | Add 400 uL of Lysis Buffer to the sample > Incubate @ 60 for 45 mins                                                                                                                                                                                                                                  | Vortex intermittently during incubation step                                                                                                                                                                                                   |
| <b>Binding</b>            | Centrifuge for 1 minute @ 10 000 rpm to clear lysate > Transfer lysate to a clean microcentrifuge tube > Add 300 uL of Binding Buffer + 20 uL of Booster Compound + 25 uL of Magnetic Beads > Shake for 5 minutes at room temperature and place on magnetic stand for 2 minutes > discard supernatant | Pipette buffer off slowly to ensure you do not aspirate the beads                                                                                                                                                                              |
| <b>Washing</b>            | Add 400 uL of Wash Buffer A to the beads > Incubate for 2 minutes on the bench > Place tubes on magnetic stand for 2 minutes > Discard supernatant > Repeats steps for Wash Buffer B and C                                                                                                            | <p>Pipette buffer off slowly to ensure you do not aspirate the beads.</p> <p>Vortex during the incubation step to ensure beads do not clump together</p> <p>An additional wash step with 80% EtOH can be performed to increase DNA purity.</p> |
| <b>Elution</b>            | Add 100 uL of preheated Elution Buffer > Incubate for 2 minutes on the bench > Place tubes on the magnetic stand for 2 minutes > Carefully pipette supernatant and transfer to a clean microcentrifuge tube > Discard the magnetic beads > eluted DNA is suitable for immediate processing or storage | <p>Adjust the volume of the Elution Buffer depending on desired concentration.</p> <p>Store eluted DNA at 4-8 for short term storage or at -20 to -80°C for long term storage.</p>                                                             |

## 7. KIT SPECIFICATIONS

| Parameter                | Specification                          |
|--------------------------|----------------------------------------|
| Format                   | Spin Column                            |
| Sample Material          | Plant tissues                          |
| Typical Yield            | Up to 100 µg (depending on the sample) |
| Purity Ratio (A260/A280) | 1.8 - 2.0                              |
| Elution Volume           | 50 - 100 µL                            |
| Preparation Time         | Approx. 30 minutes                     |
| Binding Capacity         | Up to 100 µg DNA per column            |

## 8. WORKFLOW TIPS

For the AmpliMax Plant DNA Extraction Kit, proper collection and storage of plant materials are essential to maintain the integrity of the extracted DNA. Below are comprehensive guidelines for handling plant samples effectively:

### COLLECTION AND STORAGE OF STARTING MATERIAL

**Fresh Plant Samples:** It's imperative to process fresh plant samples immediately after collection to minimize the degradation of nucleic acids. If processing immediately is not feasible, move to the next step.

Quickly freeze the samples in liquid nitrogen post-collection. This step is critical for samples that cannot be processed immediately to preserve the nucleic acids.

Store the frozen samples at temperatures ranging from a 90°C to 65°C. Consistent temperature maintenance is crucial to prevent freeze-thaw cycles that can lead to DNA degradation.

**Dried or Lyophilized Samples:** Ensure complete drying of plant samples within 24 hours of collection using a lyophilizer, or air-dry in a clean, dry environment.

Keep the dried samples at room temperature in airtight containers with desiccants to absorb residual moisture, protecting against moisture and humidity that can promote fungal growth and degrade DNA.

**Sample Containers:** Use cryovials for frozen samples, designed to endure low temperatures without cracking. For dried samples, opt for airtight containers equipped with desiccants.

## SAMPLES SIZE CONSIDERATIONS

The AmpliMax Plant DNA Kit accommodates typical sample volumes needed for effective plant DNA extraction. Following the recommended sample volume relative to the weight of the starting material is essential for optimal extraction:

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

**Volume Guidelines:** The kit is designed to effectively process sample volumes typically ranging from 100  $\mu$ L to 200  $\mu$ L of prepared plant extract. This volume has been optimized for the kit's binding capacity and ensures maximal DNA yield and purity.

Adhering to this volume is crucial for effective lysis and DNA binding, facilitating optimal interaction with the kit's reagents and silica membrane technology.

**Weight of Starting Material:** Ensure the weight of the starting material is proportional to the recommended sample volume to avoid overloading, which can negatively impact yields and purity.

**General Weight Recommendations:** For leaf and other soft tissues, it is recommended to use between 20 mg to 30 mg. For harder, fibrous materials like roots or bark, limiting the sample to 10 mg to 15 mg is advisable to prevent overloading the silica columns.

**Proportional Scaling:** Always scale the amount of buffer and other reagents proportionally to the sample weight to ensure complete lysis and efficient DNA binding. This balancing act helps prevent issues like incomplete lysis or reduced yield and purity.

**Consistency in Sampling:** When dealing with heterogeneous plant tissues, particularly those with high levels of secondary metabolites like polyphenols and polysaccharides, ensure that the sample piece used is representative of the entire specimen. This consistency is crucial for maintaining uniform DNA yield and quality across multiple extractions.

## **9. PREPARING BUFFERS AND EQUIPMENT**

### BEFORE STARTING

**Pipette Calibration and Maintenance:** Regular calibration and maintenance of pipettes are mandatory to ensure precise volume dispensing. Accurate pipetting is vital for the correct addition of reagents and buffers, which is crucial for the efficiency and reliability of DNA extraction.

**Workspace Preparation:** Ensure that the work area is free from contaminants and organized. A decontaminated and well-organized workspace is essential to prevent sample cross-contamination and to promote an efficient workflow.

**Reagent and Solution Preparation:** Confirm that all reagents are within their expiration dates and have been stored according to the manufacturer's instructions. Expired or improperly stored reagents may lead to suboptimal DNA yield or quality.

**Prepare Fresh Solutions:** Some solutions, such as the ethanol mixture for washing, should be prepared freshly on the day of the extraction to ensure their effectiveness.

**Centrifuge and Incubator Verification:** Confirm that all equipment, including centrifuges and incubators, are functioning correctly and set to the appropriate temperatures and speeds as outlined in the protocol.

**Lysis Buffer Preparation:** Before each use, inspect the Lysis Buffer to ensure it is clear and free of any precipitates. This check is crucial as precipitates can interfere with the lysis of plant cells. If precipitates are observed, gently warm the buffer to about 37°C to dissolve them, ensuring the buffer returns to room temperature before use.

**Elution Buffer Preparation:** For optimal DNA recovery, preheat the Elution Buffer to 50°C. This higher temperature helps in efficiently eluting the DNA from the silica membrane. Consider warming the Elution Buffer at the same time as the Lysis Buffer to streamline the workflow.

**Magnetic Bead Suspension:**

Ensure that the magnetic beads are well-suspended before use. Vortex the beads and verify homogeneity to guarantee efficient DNA binding.

## 10. COMPLETE PROTOCOL

### 1. Tissue Homogenisation:

With Liquid Nitrogen:

- 1.1. Place 20 - 30 mg of plant tissue into a mortar and pestle and add liquid nitrogen.
- 1.2. Grind the tissue up until the sample is completely homogenized into a fine powder.
- 1.3. Place into a clean microcentrifuge tube and proceed to the lysis step.

With a Tissue Lyser:

- 1.3. For tough-to-lyse samples, place 20-30 mg of plant tissue into a microcentrifuge tube with 4mm steel balls and 100 uL of Lysis Buffer and place into a Tissue Lyser.
- 1.4. Crush the sample until thoroughly homogenized.
- 1.5. Proceed to the lysis step.

### 2. Lysis

- 2.1. Add 400 uL lysis buffer to the crushed sample.

2.2. Incubate the sample at 60°C for 45 minutes and vortex intermittently during the incubation step.

2.3. Centrifuge for 1 minute at 10 000 rpm to clear the lysate.

2.4. Transfer 400 uL of lysate to a clean microcentrifuge tube.

### 3. Binding

3.1. To the lysate, add 300 uL of Binding Buffer and 20 uL of Booster Compound.

3.2. Vortex the mixture thoroughly.

3.3. Incubate or shake using a thermomixer at room temperature for 5 minutes.

3.4. Place the tubes onto the magnetic stand for 2 minutes. Allow the magnetic beads to pellet at the bottom of the tube. Remove the tube from the magnetic stand.

3.5. Discard the lysate by slowly pipetting the lysate off the magnetic beads without aspirating the beads.

### 4. Washing

4.1. Add 400 uL of Wash Buffer A while the tube is off the magnetic stand.

4.2. Vortex the tube and incubate on the bench for 2 minutes.

4.3. Place the tube back onto the magnetic stand for 2 minutes. Allow the magnetic beads to pellet at the bottom of the tube. Remove the tube from the magnetic stand.

4.4. Discard the lysate by slowly pipetting the lysate off the magnetic beads without aspirating the beads.

4.5. Add 400 uL of Wash Buffer B while the tube is off the magnetic stand.

4.6. Vortex the tube and incubate on the bench for 2 minutes.

4.7. Place the tube back onto the magnetic stand for 2 minutes. Allow the magnetic beads to pellet at the bottom of the tube. Remove the tube from the magnetic stand.

4.8. Discard the lysate by slowly pipetting the lysate off the magnetic beads without aspirating the beads.

4.9. Add 400 uL of Wash Buffer A while the tube is on the magnetic stand.

- 4.10. Let the tube sit for 2 minutes to allow the magnetic beads to pellet at the bottom of the tube. Remove the tube from the magnetic stand.
- 4.11. Discard the lysate by slowly pipetting the lysate off the magnetic beads without aspirating the beads.

Note: perform an additional wash step with 80% EtOH for increased purity.

## 5. Elution

- 5.1. Add 100 uL of Elution while the tube is off the magnetic stand.
- 5.2. Vortex the tube and incubate on the bench for 2 minutes.
- 5.3. Place the tube back onto the magnetic stand for 2 minutes. Allow the magnetic beads to pellet at the bottom of the tube. Remove the tube from the magnetic stand.
- 5.4. Pipette the supernatant into a clean, sterile microcentrifuge tube. This contains your eluted DNA which is ready for downstream processing and/or storage. Discard the magnetic beads.

## 11. TROUBLESHOOTING GUIDE

| Problem Description                                 | Possible Causes                                                                                                                                                                                                                             | Suggestions                                                                                                                                                                                                                                                                               |
|-----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Low DNA Yield</b>                                | <ul style="list-style-type: none"> <li>- Insufficient lysis of cells due to inadequate Proteinase K digestion.</li> <li>- Incomplete binding of DNA to magnetic beads.</li> <li>- Inefficient washing steps leading to DNA loss.</li> </ul> | <ul style="list-style-type: none"> <li>- Ensure proper incubation time and temperature for Proteinase K digestion.</li> <li>- Confirm the volume and ratio of DNA Binding Bead Mix used.</li> <li>- Repeat the wash steps carefully, ensuring complete removal of supernatant.</li> </ul> |
| <b>High DNA Contamination</b>                       | <ul style="list-style-type: none"> <li>- Cross-contamination during sample handling or processing.</li> <li>- Inadequate washing of magnetic beads.</li> </ul>                                                                              | <ul style="list-style-type: none"> <li>- Use separate, dedicated pipettes and tips for each sample to avoid cross-contamination.</li> <li>- Perform thorough washing steps, ensuring complete removal of supernatant.</li> </ul>                                                          |
| <b>Presence of Residual Mag Beads in Eluted DNA</b> | <ul style="list-style-type: none"> <li>- Incomplete drying of beads before elution.</li> <li>- Inadequate mixing of elution buffer and beads.</li> </ul>                                                                                    | <ul style="list-style-type: none"> <li>- Ensure that beads are air-dried for the recommended time.</li> <li>- Mix the elution buffer and beads thoroughly but gently to facilitate DNA elution without carrying over beads.</li> </ul>                                                    |
| <b>Inconsistent DNA Yield Across Samples</b>        | <ul style="list-style-type: none"> <li>- Variation in sample quality or quantity.</li> <li>- Inconsistent mixing or incubation during processing.</li> </ul>                                                                                | <ul style="list-style-type: none"> <li>- Standardize sample collection and processing techniques.</li> <li>- Use automated mixing or shaking to ensure uniform processing.</li> </ul>                                                                                                     |
| <b>Insufficient Eluted DNA Volume</b>               | <ul style="list-style-type: none"> <li>- Incomplete elution of DNA from magnetic beads.</li> <li>- Loss of DNA during transfer steps.</li> </ul>                                                                                            | <ul style="list-style-type: none"> <li>- Ensure that the elution buffer fully covers the beads during elution.</li> <li>- Carefully transfer eluate to a new container, ensuring no loss.</li> </ul>                                                                                      |
| <b>Cloudy Appearance of Eluate</b>                  | <ul style="list-style-type: none"> <li>- Presence of residual contaminants or salts from wash steps.</li> </ul>                                                                                                                             | <ul style="list-style-type: none"> <li>- Repeat the wash steps, ensuring thorough removal of contaminants.</li> </ul>                                                                                                                                                                     |
| <b>Beads Clumping Together During Processing</b>    | <ul style="list-style-type: none"> <li>- Use of incorrect buffer or reagent volumes.</li> <li>- Inadequate mixing of reagents or samples.</li> </ul>                                                                                        | <ul style="list-style-type: none"> <li>- Verify the accuracy of the buffer and reagent volumes used.</li> <li>- Ensure thorough, gentle mixing of reagents and samples.</li> </ul>                                                                                                        |
| <b>Buffer Precipitation</b>                         | <ul style="list-style-type: none"> <li>- Incorrect storage or preparation of buffers.</li> <li>- Use of expired or degraded reagents.</li> </ul>                                                                                            | <ul style="list-style-type: none"> <li>- Store buffers according to manufacturer instructions.</li> <li>- Ensure all reagents are within expiration date.</li> </ul>                                                                                                                      |
| <b>DNA Degradation</b>                              | <ul style="list-style-type: none"> <li>- Prolonged exposure to high temperatures.</li> <li>- Contamination with nucleases.</li> </ul>                                                                                                       | <ul style="list-style-type: none"> <li>- Follow the recommended incubation times and temperatures.</li> <li>- Use nuclease-free reagents and equipment.</li> </ul>                                                                                                                        |

|                                                    |                                                                                                                                                       |                                                                                                                                                                                                          |
|----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Low DNA Purity</b>                              | <ul style="list-style-type: none"> <li>- Contamination with proteins or other contaminants.</li> <li>- Incomplete removal of wash buffers.</li> </ul> | <ul style="list-style-type: none"> <li>- Repeat the wash steps, ensuring complete removal of supernatant.</li> <li>- Use fresh wash buffers and ensure they are at the correct concentration.</li> </ul> |
| <b>Incomplete Removal of Ethanol in Eluted DNA</b> | <ul style="list-style-type: none"> <li>- Inadequate drying of beads before elution.</li> <li>- Insufficient washing steps.</li> </ul>                 | <ul style="list-style-type: none"> <li>- Ensure that the beads are air-dried for the recommended time.</li> <li>- Repeat the wash steps, ensuring thorough removal of ethanol.</li> </ul>                |
| <b>No DNA Visible After Elution</b>                | <ul style="list-style-type: none"> <li>- Complete loss of DNA during processing.</li> <li>- Insufficient lysis of cells.</li> </ul>                   | <ul style="list-style-type: none"> <li>- Repeat the DNA extraction process using fresh samples.</li> <li>- Confirm adequate Proteinase K digestion.</li> </ul>                                           |
|                                                    | 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.                                                                                                               |

## 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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