IsoBind HPV Extraction Kit

Cat No. IB-HPV-100

Cat No. IB-HPV-200

System: Silica spin columns (manual workflow)

Sample types: cervical swabs, transport media

USER MANUAL

Website www.gene-vantage.com

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In Vitro Diagnostic Device

Table of Contents

Technical support: info@gene-vantage.com

1. KIT CONTENTS

The Gene Vantage HPV Extraction Kit is meticulously designed to provide superior performance in extracting high-quality nucleic acid from pap smear samples. These kits are engineered for optimal compatibility with manual workflows.

Component	Description/ Function	Volume per Sample	Short Term Storage	Long Term Storage	Total for 100 samples	Total for 200 samples
Lysis Buffer S	Lyses cells to release nucleic acid. Specially formulated to ensure complete lysis of cellular material for optimal yield.	200 µl	Room temperature	Room temperature	20 ml	40 ml
Proteinase K	Enhances lysis by breaking down proteins, facilitating more efficient nucleic acid release.	20 µl	4-8°C	-20°C	2 ml	4 ml
Binding Buffer * Add Isopropanol (95%, molecular grade) prior to use	Facilitates binding of nucleic acids to the silica membrane.	300 µl	Room temperature	4 - 8°C	30 ml	60 ml
Wash Buffer A * Add EtOH (96-100%, molecular grade) prior to use	Removes impurities such as cellular debris and proteins without stripping away the bound nucleic acids.	500 µl	Room temperature	Room temperature	100 ml	200 ml

Wash Buffer B * Add EtOH (96-100%, molecular grade) prior to use	Removes residual salts without stripping away the bound nucleic acids.	500 µl	Room temperature	4 - 8°C	100 ml	200 ml
Wash Buffer C * Add EtOH (96-100%, molecular grade) prior to use	Final wash used to remove remaining traces of chaotropic agents	500 µl	Room temperature	4 - 8°C	100 ml	200 ml
Elution Buffer	Elutes purified nucleic acid from the column	60 µl	Room temperature	4 - 8°C		
Spin Columns	Silica matrix that selectively binds nucleic acids while allowing other compounds to pass through	1 column per sample + collection tube	Room temperature	In zip lock bag at 4 - 8°C	100 columns	200 columns

 Buffers contain skin irritants

 Wear gloves

2. IMPORTANT NOTES

Before beginning your work with the Gene Vantage DNA Isolation Kit for HPV Screening, please take a moment to review these important notes. Adhering to these guidelines will ensure optimal results and efficiency throughout your DNA extraction process.

Handling of Samples: Handle all samples with care to prevent degradation or contamination. Use clean, sterile equipment at every step to maintain sample integrity.

Solubility Check: Some buffers, such as the Lysis Buffer, may form precipitates upon storage. Before use, inspect all buffers for any signs of precipitation. If present, gently warm the buffer to dissolve precipitates.

Buffer Preparation: Ensure that all buffers are prepared according to the instructions, paying special attention to any buffers that require dilution or mixing prior to use, such as the wash buffers. Proper preparation is essential for optimal binding and washing performance.

Centrifugation Parameters: Use the specified centrifugation speeds to ensure efficient separation of the supernatant from the pelleted material. Incorrect speeds may result in incomplete separation, leading to lower yield and purity.

Maximum Capacity: Do not exceed the maximum loading volume of the silica spin columns. Overloading can result in overflow or incomplete purification, which may contaminate the eluted DNA or reduce yield.

Concentration vs. 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.

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.

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 for 2 minutes on the bench 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 HPV Extraction 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 HPV Extraction Kit is engineered to efficiently isolate high-quality nucleic acids from pap smear samples using a robust process grounded in the solid-phase extraction principle. This process is facilitated by silica-based spin columns specifically designed to maximize nucleic acid yield and purity, crucial for subsequent HPV detection and genotyping. Below is a detailed explanation of each step involved in the DNA extraction process:

Cell Lysis: The first crucial step involves the breakdown of cell membranes to release nucleic acid into the solution. Effective lysis is key to ensuring that all nucleic acid is accessible for subsequent binding. This kit uses Lysis Buffer S, which contains a combination of surfactants and a buffering agent that disrupts cellular and nuclear membranes. The addition of Proteinase K, an enzyme, aids in digesting proteins that could otherwise bind nucleic acids and interfere with the extraction process. This enzymatic treatment further ensures that nucleic acids are fully liberated from the cellular membrane. The lysis reaction is enhanced by incubating the sample mixture at 56°C which optimises the activity of Proteinase K and ensures complete lysis.

DNA Binding: Following lysis, the free nucleic acids must be selectively captured or bound while other cellular debris and impurities are excluded. Nucleic acid in the lysate binds to a silica membrane within the spin column when in the presence the Binding Buffer. This environment promotes the adherence of nucleic acid to the silica surface due to the formation of hydrogen bonds between the negatively charged phosphate groups of the nucleic acids and the positively charged surface of silica. This step is critical as it determines the yield of nucleic acid.

Washing: Clean nucleic acid is essential for sensitive applications like PCR. The kit contains three sequential wash buffers (Wash A, B, and C), each designed to efficiently remove different types of contaminants. Wash Buffer A primarily removes proteins and other large organic molecules, Wash Buffer B is designed to eliminate smaller molecules and salts, and Wash Buffer C ensures that no residual wash buffer remains on the spin column matrix. Each wash involves adding a specific volume of buffer, followed by centrifugation to pull the liquid through the column while the nucleic acid remains bound to the silica membrane. This ensures that only purified nucleic acid remains on the column.

Elution: The final step is to release the purified nucleic from the silica membrane for use in downstream applications. Elution is achieved by applying an Elution Buffer, which disrupts the hydrogen bonds between the nucleic acid and the silica, allowing the nucleic to be released into the buffer. The elution buffer is pre-warmed to enhance the efficiency of nucleic acid recovery. The nucleic acid 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 nucleic acids while efficiently removing contaminants. This results in nucleic acid with high purity, characterised by optimal A260/A280 ratios, indicating minimal protein contamination and readiness for sensitive downstream applications.

Comprehensive Cell Disruption: The Lysis Buffer S and Proteinase K combination effectively disrupts cell types found in pap smear samples, ensuring complete release of nucleic acid.

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 optimised for pap smear samples, the kit's robust lysis and binding conditions are effective in isolating high quality nucleic acid for HPV screening, making it a useful tool in both clinical diagnostic and research settings.

Time efficiency: The entire nucleic acid extraction process can be completed in approximately 45 minutes for 12-24 samples, which is ideal for labs seeking to maintain their turnaround times without compromising on the quality of results.

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 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 RNA 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 RNA. 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 RNA within the solution. This ensures maximum contact between the RNA and the silica binding surface, increasing the efficiency of RNA 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 RNA extraction protocol.

5.2 Consumables

Microcentrifuge Tubes (1.5 mL)

Used for sample preparation and for collecting the eluted RNA.

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 RNase-free to prevent the degradation of RNA by residual enzymatic activity.

Ethanol (96-100%, molecular grade)

Added to wash buffers to help in washing away impurities without stripping the RNA from the column.

Isopropanol (95%, molecular grade)

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

Saline Solution:
Purpose: Used for resuspending the cell pellet.
Note: Saline is preferred over PBS to avoid potential interference with downstream PCR applications. Ensure the saline is sterile and free of contaminants.

Glycogen:
Purpose: Acts as a co-precipitant to enhance nucleic acid yield, especially valuable in samples with low nucleic acid content.
Note: Use only molecular biology grade glycogen to prevent contamination.

6. QUICK VIEW PROTOCOL

Step	Procedure	Details
Sample Preparation	Vortex samples > transfer 1ml to a microcentrifuge tube > Spin @ 14 000 rpm 8 mins > Discard supernatant > Resuspend pellet in 100 µl saline solution	Do not PBS for resuspension of pellet. If pellet is too small then spin down an additional 1 ml of sample. If pellet is clear then reduce the amount of saline added to 50 µl
Lysis	Add 200 µl Lysis Buffer S + 20 µl Proteinase K to the resuspended pellet > Vortex > Incubate @ 56°C for 30 minutes	Vortex intermittently during incubation
Binding	Add 1 volume of Binding Buffer to 1 volume lysate + 20 µl Glycogen > Vortex > Incubate on bench for 2 mins > Load onto spin column placed in a collection tube > Spin @ 8000 rpm for 1 min. > Discard the flow through and reuse the collection tube	Volume of lysate to Binding Buffer should be a 1:1 ratio ie. 500 µl of lysate : 500 µl of Binding Buffer

Step	Procedure	Details
Washing	Add 500 µl of Wash Buffer A > Incubate on bench for 2 mins. > Spin @ 8000 rpm for 1 min > Discard flow through and reuse the collection tube. Repeat steps for Wash Buffer B and Wash Buffer C	Ensure all wash buffer passes through the column
Dry Centrifuge	Place spin column back into collection tube and spin @ max speed for 2 minutes	This step ensures that all residual buffer is removed from the column
Elution	Place spin column into a new microcentrifuge tube > Add 60 µl of preheated Elution Buffer to the column > Incubate for 2 mins on the bench > Spin @ 8000 rpm for 1 min	Warm elution buffer helps with yield

7. KIT SPECIFICATIONS

Specification	Detail
Format	Silica-based spin column
Sample Material	Pap smear samples
Typical Yield	5-10 µg
Purity Ratio (A260/A280)	1.7-1.9
Elution Volume	60 µl
Preparation Time	Approx. 45 minutes
Binding Capacity	Up to 50 µg DNA per column

8. WORKFLOW TIPS

To maximise the effectiveness and reliability of the IsoBind HPV Extraction Kit, it is crucial to consider additional aspects of the extraction process that impact both the quality of the nucleic acid 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

Proper collection and storage of Pap smear samples are crucial for preserving the integrity and quality of DNA in preparation for extraction with the IsoBind HPV Extraction Kit. Here are detailed guidelines to ensure optimal conditions:

Immediate Processing: If processing cannot be done immediately after collection, store the sample in its transport media at 4°C to minimise enzymatic activities and microbial growth that can degrade nucleic acid. This is particularly important for ensuring that the nucleic acid remains stable and intact for extraction.

Transport Media Considerations: BD SurePath: Contains ethanol and methanol which must be removed by centrifugation as these chemicals will interfere with the extraction process.
ThinPrep or PreservCyt: Contains methanol and water, which also require removal for the same reasons.

Long-Term Storage: If samples cannot be processed right away, they should be stored at 4°C in their original collection vials processing. However, it's essential that this temperature is strictly maintained to prevent any fluctuations that could affect sample stability. Avoid long-term storage as the preservatives in the transport medium may begin to degrade or alter the nucleic acid over time, storing samples in their original collection vials at 4°C is typically safe for a few days, but this can vary based on the specific preservatives used in the transport media.

Resuspension and Lysis: Initial Resuspension: Start by resuspending the cell pellet in 100 µl of saline. The use of saline instead of PBS (Phosphate Buffered Saline) is crucial to avoid phosphate ions that might interfere with nucleic acid extraction and downstream PCR reactions.

Adjusting the Pellet Size: If the initial pellet is too small, reloading additional sample volume and re-centrifuging is a recommended approach. This can be crucial for ensuring that enough cellular material is available for nucleic acid extraction.

Pellet Visibility: For pellets that are mostly clear or difficult to see, reducing the volume of saline to 50 µl is an effective strategy to concentrate the nucleic acid. This adjustment helps in maximising the yield from samples with low cell counts.

Minimising Contamination:
Use sterile pipettes and filter tips for sample handling. Cross-contamination can introduce extraneous DNA or degrade the sample quality.

Documentation and labelling: Carefully ;Abel all samples with the date of collection, sample type, and specific storage requirements. Maintain detailed records of storage duration and conditions to help correlate sample quality with experimental outcomes.

SAMPLE CONSIDERATIONS

Scaling Buffer Volumes: If additional sample volume is required to increase the size of the pellet, adjust the volumes of Lysis Buffer S and Binding Buffer proportionally. For instance, doubling the sample volume should also double the volume of Lysis Buffer S from 200 µl to 400 µl. It is essential that the volume of each buffer matches the sample size to ensure complete lysis, optimal binding, and effective washing.

Handling Larger Sample Volumes: For samples exceeding the standard 1 ml size, it may be necessary to process the material in multiple batches or adjust the protocol to accommodate larger volumes, which includes scaling up the volumes of all reagents and possibly using multiple spin columns.

Sample Homogeneity: Ensure that cell pellets are fully resuspended in saline. This is crucial for consistent lysis across samples and significantly impacts the yield and purity of the extracted nucleic acid.

Considerations for High Metabolite Content: Transport media components like ethanol or methanol can interfere with nucleic acid extraction. Adequately removing these through centrifugation and discarding the transport medium before adding the lysis buffer is vital.

Concentration and Yield: Ensure that the Elute buffer is pre-warmed to help release the nucleic acid more effectively from the silica matrix. Adjust the volume of the elution buffer based on the required concentration of nucleic acid; smaller volumes yield more concentrated nucleic acid, which might be crucial for sensitive downstream applications like PCR.

Special Considerations for Low Biomass Samples: In cases where the sample biomass is low, take extra precautions to minimise loss during transfers and spins. Use low-retention pipette tips and ensure all centrifugation steps are precisely timed to avoid losing precious nucleic acid material.

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.

Pipette Calibration: Regular calibration of the centrifuge is crucial for achieving the precise speeds necessary for optimal RNA isolation. Inaccuracies in speed can lead to inefficient separation of phases, potentially contaminating the RNA 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 RNA 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 RNase-free environment. Use RNase decontamination solutions and maintain clean bench practices throughout the procedure.

10. COMPLETE PROTOCOL

1. Sample Collection:
   1.1. Remove the swab from the clear plastic pouch and swab the side of the participants' cervical canal.

   1.2. Rotate the swab 360 degrees five times in a clockwise direction.

   1.3. Place the swabs into a vial containing transport media. This medium helps preserve the cellular material and DNA until processing.

   1.4. Seal the tube and label it with the patient details.

Notes on storage of samples:

- Room Temperature: Samples in transport media can typically be stored at room temperature for short periods (a few days).
- Refrigeration: For longer storage (up to a month), refrigerating the samples at 4-8°C can help preserve the integrity of the DNA.

2. Sample Preparation
   2.1. Preheat an incubator to **56°C.** Place Elution Buffer in oven to heat it.
   2.2. Vortex the sample tube thoroughly.
   2.3. Transfer **1 ml** of liquid sample to a microcentrifuge tube and centrifuge at **14,000 rpm for 8 minutes** to separate the supernatant and pellet.
   2.4. Discard the supernatant as this contains compounds such as ethanol and methanol which may affect the extraction process and result in decreased yield and quality.
   2.5. Resuspend the pellet in 1**00 µl of saline solution** (not PBS).

- Note on pellet size: If the pellet is small, reload **1 ml** of sample and spin down again to increase the size of the pellet. If the pellet is mostly clear/invisible, only use **50 ul of saline** and **100 ul of Lysis Buffer S**.

3. Lysis:
   3.1. Add **200 µl of Lysis Buffer S** and **20 µl of Proteinase K** to the resuspended pellet.
   3.2. Vortex thoroughly.
   3.3. Incubate the samples at **56°C for 30 minutes**, vortexing intermittently.

4. Binding:
   4.1. Remove the samples from the oven/incubator.
   4.2. Add **300 µl of Binding Buffer and 20 ul Glycogen** to the lysate.
   4.3. Vortex thoroughly.
   4.4. Incubate for **2 minutes** on the bench.
   4.5. Transfer the lysate with the Binding buffer and Glycogen to the Gene Vantage silica spin column.

- Note: Do not transfer more than 700 uL of volume per column.

4.6. Place the spin columns into collection tubes.
4.7. Centrifuge the samples at **8000 rpm for 1 minute** and discard the flow-through. Reuse collection tube.

5. Washing:
5.1. Add **500 µl of Wash Buffer A** to the spin.
5.2. Centrifuge at **8000 rpm for 1 minute** and discard the flow-through. Reuse the collection tube.
5.3. Add **500 uL of Wash Buffer B** to the spin column.
5.4. Centrifuge at **8000 rpm for 1 minute** and discard the flow-through. Reuse the collection tube. 5.5. Add **500 uL of Wash Buffer C** to the column.
5.6. Centrifuge at **8000 rpm for 1 minute** and discard the flow-through. Reuse the collection tube.
5.7. Centrifuge tubes at **10 000 rpm for 2** min to dry the column and ensure there is no excess buffer.
5.8. Place the spin column in a new, sterile microcentrifuge tube.

6. Elution:
6.1. Add **60 µl of warm Elution Buffer** to the spin column.
6.2. Incubate for **2 minutes on the bench**.
6.3. Centrifuge at **8000 rpm for 1 minute** to collect the eluted nucleic acid.

7. Storage:
7.1. Eluted DNA is now ready for downstream applications.
7.2. Store the eluted nucleic acid at **4-8°C short term storage or at -20 to -80°C** for long term storage.

11. TROUBLESHOOTING GUIDE

Problem Description	Possible Causes	Suggestions
Low yield	Incomplete pelleting of cells in step 2	Ensure complete centrifugation at 14,000 rpm for 8 minutes. Consider repeating the centrifugation to improve cell recovery.
	Insufficient lysis	Increase incubation time with Lysis Buffer S and ProK, or vortex more frequently during the 30-minute incubation.
Contamination in the sample	Residual transport medium	Ensure complete removal of transport medium after spinning down the sample. Carefully pipette to avoid aspirating the pellet.
	Cross-contamination between samples	Use new pipette tips for each step and sample to prevent cross-contamination.
Insufficient recovery of cells	Pellet too small or difficult to see	Reload the sample, spin down again to increase pellet size. Use recommended volume of saline for resuspension based on pellet visibility.
Presence of inhibitors in PCR	Residual ethanol from transport media (BD surepath, ThinPrep, or PreservCyt)	Ensure complete removal of all transport media and wash buffers during the procedure. Do a second step with Wash C if necessary.
	Residual salts from wash buffers	Ensure complete removal of wash buffers by performing an extended spin during the wash steps.
Variable concentrations across samples	Inconsistent sample volume or handling	Standardize the volume of the sample processed and the handling technique across all samples.
	Inefficient lysis of cells	Verify that the Lysis Buffer S and ProK are thoroughly mixed with the cell pellet and that the incubation conditions are met.
Buffer Precipitation	Cold storage of buffers that should be at room temperature.	Warm the buffers to dissolve precipitants before use. Store buffers according to the manufacturing instructions.

12. PRODUCT USE RESTRICTION / WARRANTY

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