# Pathogen/Viral Nucleic Acid Isolation Kit (Magnetic Bead System) Product Insert

**Product # 1111-72900**

Norgen's Pathogen/Viral Nucleic Acid Isolation Kit (Magnetic Bead System) is designed to extract nucleic acid (DNA and RNA) from viruses and easily lysable bacteria found in body fluids and transport mediums. The purification is based on Norgen's magnetic bead-based technology that is designed for efficient high throughput extraction of high-quality DNA as well as RNA in a single elution. The kit is compatible with automation on KingFisher™ Flex Purification System and any other automation platform that supports magnetic bead-based extraction technology (e.g. IsoPure systems).

The Pathogen/Viral Nucleic Acid Isolation Kit (Magnetic Bead System) allows extraction of DNA and RNA from up to 200 µL of different bodily fluids and transport mediums such as saliva, urine, blood, plasma, VTM, Norgen's Total Nucleic Acid preservative and more. An optional step in the protocol also allows for the isolation of nucleic acids from gram positive bacteria by incorporating a lysozyme incubation. The nucleic acids are preferentially purified from the cellular proteinaceous components, followed by binding to the magnetic beads and co-elution of nucleic acids in an elution volume of 50-100 µL. The purified RNA is of the highest integrity and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays. The genomic DNA is of the highest quality, and can be used in PCR reactions, sequencing, Southern blotting, methylation studies and SNP analysis.

## Kit Components

| Component | Product # 72900 (96 samples) |
|---|---|
| Lysis Buffer B | 40 mL |
| Solution WN | 55 mL |
| Elution Buffer F | 15 mL |
| Proteinase K in Storage Buffer | 1.2 mL |
| Nucleic Acid Magnetic Beads | 2.5 mL |
| Product Insert | 1 |

## Advantages

- Isolate DNA/RNA from bodily fluids and transport medium tubes.
- Fast and easy processing using magnetic bead technology.
- High throughput and compatible with automation.
- Recovered DNA/RNA is compatible with various downstream applications.

## Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment. The kit contains a ready-to-use Proteinase K, which is dissolved in a specially prepared storage buffer. The buffered Proteinase K is stable for up to 2 years after the date of shipment when stored at room temperature.

## Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files upon request.

The **Lysis Buffer B** and **Solution WN** contain guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

**Customer-Supplied Reagents and Equipment**

- Micropipettors
- Multi-channel Micropipettors
- Magnetic stand – 96 wells
- Digital Microplate Shaker
- 80% ethanol (prepare fresh)
- 96 - 100% ethanol
- 65°C water bath or incubator
- Nuclease free water
- Lysozyme

## Procedure

### Extraction of Nucleic Acid from Various Sample Types

***Notes Prior to Use:***

- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Prepare a working concentration of the **Solution WN** by adding 73 mL of 96-100 % ethanol (provided by the user) to the supplied bottle. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare 200 mL of the 80% Ethanol by mixing 160 mL of 96 - 100% ethanol (provided by the user) and 40 mL of nuclease free water.
- Preheat a water bath or heating block to 65°C.
- Always vortex the Proteinase K before use.

### Section 1A - Automatic Nucleic Acid Isolation

### 1. Set-up the Instrument

1. Ensure that the correct program is loaded IsoPure_VNA or IsoPure_VNApos if you are specifically targeting gram positive pathogens.

### 2. Set up the Processing Plates for Washing and Elution

1. Set up the Solution WN, Elution and Tip Comb plates on the laboratory bench according to Table 1 below.
2. Load the plates on to the machine

Table 1 - Set-up of the IsoPure Processing Plates

| Component | Location | | Amount |
|---|---|---|---|
| | IsoPure 16 | IsoPure 96 | |
| **Solution WN** | Column 2 / Column 8 | Plate 4 | 1 mL |
| **80 % Ethanol** | Column 3 / Column 9 | Plate 5 | 1 mL |
| **80 % Ethanol** | Column 4 / Column 10 | Plate 6 | 500 µL |
| **Elution Buffer F** | Column 6 / Column 12 | Plate 8 | 50-100 µL |

### 3. Prepare the Sample Plate and Digest with Proteinase K

1. Prepare the lysis-bead binding mixture as per Table 2.

a) Calculate the number of samples that you are going to process.
b) Mix the magnetic beads by vortexing to make a homogeneous mixture.
c) Prepare a bulk mix of Lysis/Binding buffer as shown in Table 2. We suggest calculating a 10 % overage to compensate for pipetting errors and inaccuracies.

2. Add the sample, proteinase K, and lysis-bead mix to the plate as in the Table 3 below.
3. Select the program and press 'run'.

Table 2 - Preparation of Lysis-Bead Mix

| Solution | Volume per sample µL |
|---|---|
| Nucleic Acid Magnetic Beads | 20 µL |
| Lysis Buffer B | 300 µL |

Table 3 - Sample Digestion

| Solution | Location | | Amount |
|---|---|---|---|
| | IsoPure 16 | IsoPure 96 | |
| Sample | Column 1 / Column 7 | Plate 2 | 200 µL |
| Lysis-bead binding mixture | Column 1 / Column 7 | Plate 2 | 320 µL |
| Proteinase K in Storage Buffer | Column 1 / Column 7 | Plate 2 | 10 µL |

## 4. Purify the Nucleic Acids

1. When prompted by the machine (~ 10 minutes) remove the Sample Plate and add 500 µL of 96-100 % ethanol to all wells containing lysate.
2. Immediately place the plate back onto the machine and follow the prompts on the instrument to resume sample processing.
3. At the end of the run, promptly remove the plate from the instrument and transfer the eluate to the final plate or tube for final storage.

## Section 1B - Automatic Nucleic Acid Isolation for Gram Positive Pathogens

### 1. Set-up the Instrument

1. Ensure that the correct program is loaded IsoPure_VNApos if you are specifically targeting gram positive pathogens.

### 2. Set up the Processing Plates for Washing and Elution

1. Set up the Solution WN, Elution and Tip Comb plates on the laboratory bench according to Table 4 below.
2. Load the plates on to the machine

Table 4 - Set-up of the IsoPure Processing Plates

| Component | Location | | Amount |
|---|---|---|---|
| | IsoPure 16 | IsoPure 96 | |
| **Solution WN** | Column 2 / Column 8 | Plate 4 | 1 mL |
| **80 % Ethanol** | Column 3 / Column 9 | Plate 5 | 1 mL |
| **80 % Ethanol** | Column 4 / Column 10 | Plate 6 | 500 µL |
| **Elution Buffer F** | Column 6 / Column 12 | Plate 8 | 50-100 µL |

### 3. Prepare the Sample Plate and Digest with Enzymes

1. Add the sample and the lysozyme (30 mg/mL in TE buffer, provided by the customer) to each well of the sample plate as per Table 5.

Table 5 - Lysozyme Sample Incubation

| Solution | Location | | Amount |
|---|---|---|---|
| | IsoPure 16 | IsoPure 96 | |
| Sample | Column 1 / Column 7 | Plate 2 | 200 µL |
| Lysozyme (30 mg/mL) | Column 1 / Column 7 | Plate 2 | 10 µL |

2. Prepare the lysis-bead binding mixture as per Table 6.
   a) Calculate the number of samples that you are going to process.
   b) Mix the magnetic beads by vortexing to make a homogeneous mixture.
   c) Prepare a bulk mix of Lysis/Binding buffer as shown in Table 6. We suggest calculating a 10 % overage to compensate for pipetting errors and inaccuracies

Table 6 - Preparation of Lysis-Bead Mix

| Solution | Volume per sample µL |
|---|---|
| Nucleic Acid Magnetic Beads | 20 µL |
| Lysis Buffer B | 300 µL |

3. When prompted, remove the sample plate and add the proteinase K and lysis-binding mix as per Table 7 below.

Table 7 - Sample Proteinase K Digestion

| Solution | Location | | Amount |
|---|---|---|---|
| | IsoPure 16 | IsoPure 96 | |
| Lysis-bead binding mixture | Column 1 / Column 7 | Plate 2 | 320 µL |
| Proteinase K in Storage Buffer | Column 1 / Column 7 | Plate 2 | 10 µL |

a) Select the program and press ‘run’.

### 5. Purify the Nucleic Acids

1. When prompted by the machine (~ 10 minutes) remove the Sample Plate and add 500 µL of 96-100 % ethanol to all wells containing lysate.
2. Immediately place the plate back onto the machine and follow the prompts on the instrument to resume sample processing.
3. At the end of the run, promptly remove the plate from the instrument and transfer the eluate to the final plate or tube for final storage.

## Section 2 - Manual Nucleic Acid Isolation

## 2A Regular Method for Most Viral/Bacterial Pathogens

### 1. Lysate Preparation

1. Combine 300 µL of Lysis Buffer B, 200-400 µL of sample and 10 µL of Proteinase K (vortex before use)**.** Mix well by gentle vortexing.
2. Incubate at 65°C for 10 minutes.
3. Add 500 µL of 96 – 100% ethanol and 20 µL of Magnetic Bead Suspension (vortex prior to use) to the lysate collected above.
4. Incubate at room temperature for 5 minutes. Occasionally invert the tube.

## 2. Binding and Washing of Nucleic Acids

1. Assemble a magnetic separation rack and place the sample tube in the magnetic rack. Allow to sit for 1 minute and/or until the solution becomes clear.
2. Aspirate and discard supernatant without touching the magnetic beads.
3. Remove the sample tube from the magnetic rack and gently add 1000 µL of **Solution WN** (ensure ethanol was added). Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
4. Place the sample tube on the magnetic rack and allow to sit for 1 minute.
5. Aspirate and discard supernatant without touching the magnetic beads.
6. Remove the sample tube from the magnetic rack and gently add 1000 µL of freshly prepared **80% ethanol**. Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
7. Place the sample tube on the magnetic rack and allow to sit for 1 minute.
8. Aspirate and discard supernatant without touching the magnetic beads.
9. Repeat steps 10-12 for a second wash step with 500 µL of 80 % ethanol.
   a) **Note:** Remove as much of the 80% ethanol in the sample tube as possible by pipetting.
10. Incubate the open tube at 65˚C for 5 minutes to dry the magnetic beads.

## 3. Elution of Nucleic Acids

1. Remove the sample tube from the magnetic rack and add 50-100 µL of **Elution Buffer F**. Mix by vortexing and incubate at 65˚C for 10 minutes.
2. Remove the tube from heat and allow it to cool down by keeping it at ambient temperature for 5 minutes.
3. Briefly vortex and place sample tube on the magnetic rack and allow to sit for 1 minute.
4. Carefully transfer the elution to a fresh 1.7 mL elution tube (provided) without touching the magnetic beads.

## 2B Method Specific for Gram-Positive Bacterial Pathogens

## 1. Lysate Preparation

1. Combine 300 µL of Lysis Buffer B, 200-400 µL of sample and 10 µL of **lysozyme** (vortex before use). Mix well by gentle vortexing.
2. Incubate at room temperature for 10 minutes.
3. Add 10 µL of **Proteinase K** (vortex before use) to the sample mixture. Mix well by gentle vortexing.
4. Incubate at 65˚C for 10 minutes.
5. Add 500 µL of 96 – 100% ethanol and 20 µL of Magnetic Bead Suspension (vortex prior to use) to the lysate collected above.
6. Incubate at room temperature for 5 minutes. Occasionally invert the tube.

## 2. Binding and Washing of Nucleic Acids

1. Assemble a magnetic separation rack and place the sample tube in the magnetic rack. Allow to sit for 1 minute and/or until the solution becomes clear.
2. Aspirate and discard supernatant without touching the magnetic beads.
3. Remove the sample tube from the magnetic rack and gently add 1000 µL of **Solution WN** (ensure ethanol was added). Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
4. Place the sample tube on the magnetic rack and allow to sit for 1 minute.
5. Aspirate and discard supernatant without touching the magnetic beads.
6. Remove the sample tube from the magnetic rack and gently add 1000 µL of freshly prepared **80% ethanol**. Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
7. Place the sample tube on the magnetic rack and allow to sit for 1 minute.
8. Aspirate and discard supernatant without touching the magnetic beads.
9. Repeat steps 12-14 for a second wash step with 500 µL of 80 % ethanol.

a) **Note:** Remove as much of the 80% ethanol in the sample tube as possible by pipetting.

10. Incubate the open tube at 65˚C for 5 minutes to dry the magnetic beads.

### 3. Elution of Nucleic Acids

1. Remove the sample tube from the magnetic rack and add 50-100 µL of **Elution Buffer F**. Mix by vortexing and incubate at 65˚C for 10 minutes.
2. Remove the tube from heat and allow it to cool down by keeping it at ambient temperature for 5 minutes.
3. Briefly vortex and place sample tube on the magnetic rack and allow to sit for 1 minute.
4. Carefully transfer the elution to a fresh 1.7 mL elution tube (provided) without touching the magnetic beads.

## Section 3. Storage of Purified DNA/RNA

- The purified RNA/DNA may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

| Related Products | Product # |
|---|---|
| IsoPure96, Automated Purification System, 115V | 72705 |
| IsoPure96, Automated Purification System 230V | 72710 |
| IsoPure16, Automated Purification System, 115V | 72715 |
| IsoPure16, Automated Purification System, 230V | 72720 |
| 96 Deep-Well Plates for IsoPure, Pack of 50 | 72725 |
| 96 Well Elution Plates for IsoPure, Pack of 100 | 72730 |
| Tip Comb for IsoPure96, Pack of 50 | 72735 |
| 6-Well Sample Strip for IsoPure16, Pack of 50 | 72740 |
| 8-Place Magnetic Tip Comb for IsoPure16, Pack of 50 | 72745 |

## Troubleshooting Guide

| Problem | Possible Cause | Solution and Explanation |
|---|---|---|
| Magnetic beads were accidently pipetted up with the supernatant. | The pipette tip was placed too close to the magnetic beads while pipetting. | Return the magnetic beads and the supernatant back into the sample tube. Mix well, and place the tube back onto the magnetic separation rack for the specified time. Carefully remove the supernatant without touching the magnetic beads. |
| The yield of nucleic acid is low. | Incomplete lysis of cells. | Ensure that correct lysis protocol was applied to the sample. Ensure Proteinase K and/or lysozyme is added properly. |
| | Amount of magnetic beads added was not sufficient. | Ensure that the magnetic bead suspension is mixed well prior to use to avoid any inconsistency in DNA isolation. |
| | DNA concentration in the sample being used is low. | Some samples contain very little target DNA. This varies from individual to individual based on numerous variables. Extend the incubation time of Proteinase K digestion or reduce the amount of tissue or cells used for lysis. |
| Very gelatinous prior to adding the Magnetic bead and Ethanol. | The lysate solution mixture is not homogeneous. | To ensure a homogeneous solution, vortex for 10-15 seconds before adding the magnetic beads to the lysate. |
| | Maximum number of cells exceeds kit specifications. | Refer to specifications to determine if amount of starting material falls within kit specifications. |
| DNA does not perform well in downstream applications. | DNA was not washed with 80% Ethanol. | Traces of salt from the binding step may remain in the sample if the magnetic beads are not washed with 80% Ethanol. Salt may interfere with downstream applications, and thus must be washed from the magnetic beads. |
| | Ethanol carryover. | Ensure that the drying step after the 80% ethanol wash steps is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications. |