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# Pathogen/Viral Nucleic Acid Isolation Kit (Magnetic Bead System) – High Throughput

**Product Insert**

**Product # 72950**

Norgen's Pathogen/Viral Nucleic Acid Isolation Kit (Magnetic Bead System) – High Throughput 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) – High Throughput allows extraction of DNA and RNA from up to 200 µL of different bodily fluids and transport mediums such as saliva, urine, blood, plasma as well as 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. 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 #72950 (2000 samples) |
|---|---|
| Lysis Buffer B | 1 x 130 mL<br>1 x 500 mL |
| Solution WN | 5 x 190 mL |
| Elution Buffer F | 210 mL |
| Proteinase K in Storage Buffer | 21 mL |
| Nucleic Acid Magnetic Beads | 42 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 Safety Data Sheets (SDSs). 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 240 mL of 96-100 % ethanol (provided by the user) to the supplied bottle. This will give a final volume of 430 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.

***Notes Prior to Use***

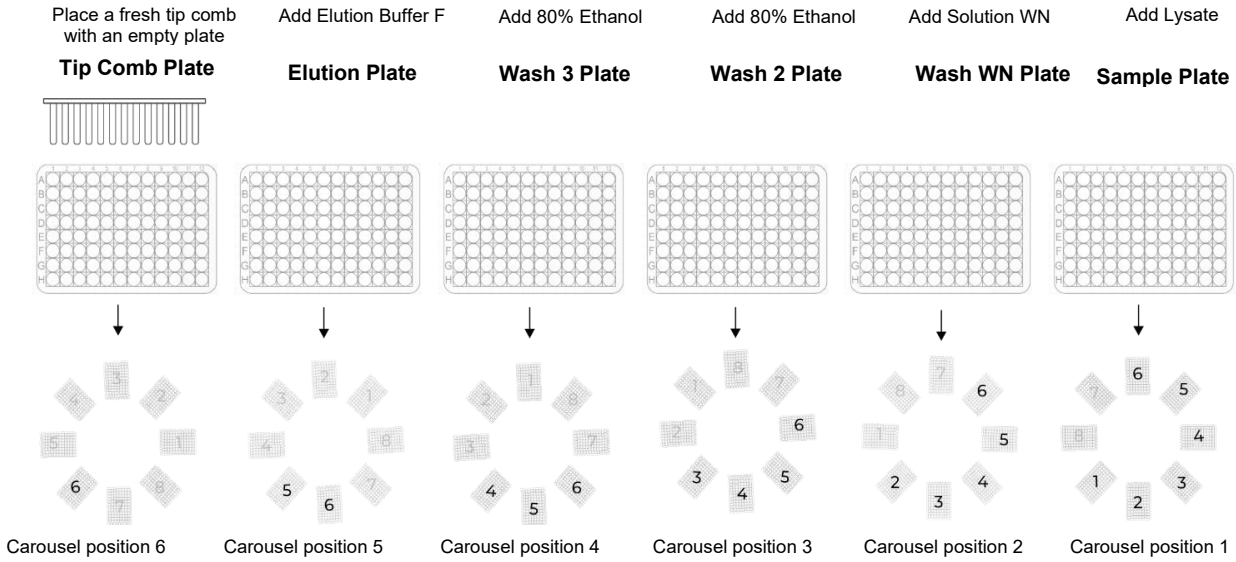
- The procedure provides information and steps to follow to extract Nucleic Acid using automated methods using KingFisher™ Flex platform. KingFisher instruments come with heat blocks and the scripts provided include the incubation at required temperature.
- **Section 1A** describes automated purification of Nucleic Acid from pathogen without gram-positive bacteria while **Section 1B** describes automated purification of Nucleic Acid from gram-positive bacteria.
- 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.
- **Always** vortex the **Nucleic acid Magnetic Beads** and **Proteinase K** before use.
- **For samples containing Gram positive bacterial pathogens**, prepare a 30 mg/mL stock solution (approximately 1.7 x $10^7$ units/mL) of lysozyme as per supplier's instructions
- Prepare a working concentration of the **Solution WN** by adding 96 - 100 % ethanol (not provided) to the supplied bottle containing the concentrated **Solution WN**. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- The isolation can be performed by using the following combination of heating blocks and plates

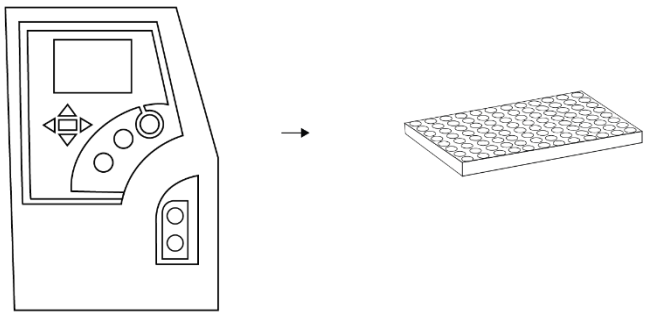
| Instrument | Heat Block | Plate type | Elution Plate type |
|---|---|---|---|
| **KingFisher Flex** | Deep Well heating block* | deep-well 96 plate (95040450 or 95040460) | deep-well 96 plate (95040450 or 95040460) |
| | 96 heating block | | deep-well 96 plate (95040450 or 95040460) or 96 KF plate (97002540) |

**Deep Well heating block is not compatible with 96 KF plate. Selecting the correct heating block and plates is important as elution step has a heating step. Using incompatible plates can lead to reduced DNA yield.*

## Flow Chart

**Procedure for using Norgen's Pathogen/Viral Nucleic Acid Isolation Kit (Magnetic Bead System) – Kingfisher™ Flex**



## Run Instrument

Place Sample at position 1 and continue to run program.

Run Complete. Take Elution plate and store NA.

# Flow Chart

**Procedure for using Norgen's Pathogen/Viral Nucleic Acid Isolation Kit (Magnetic Bead System) –Manual method**

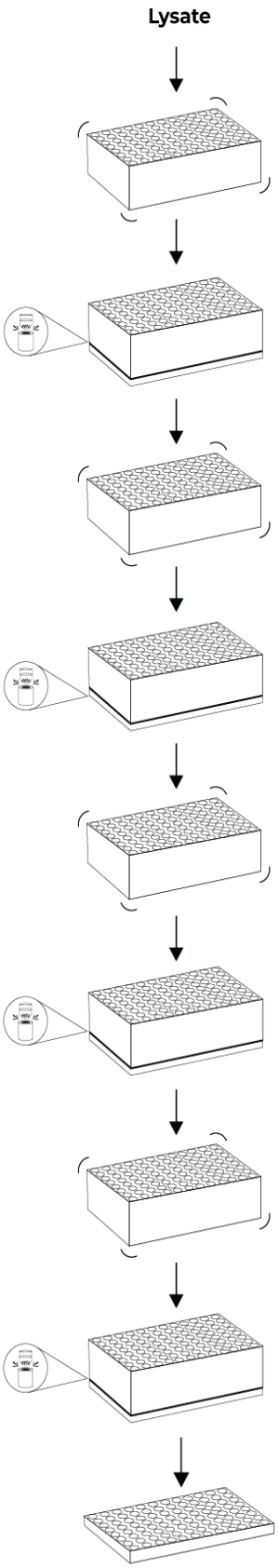


**Table 1. Set-up for Nucleic Acid Extractions using Kingfisher™ Flex System**

Prepare plates as per the volumes mentioned in the table below. Detailed description of step-wise process can be found in **Section 1.**

**Table 1a.** Setup of plates while using KingFisher™ Flex with a 96 Deep-well Head and a deep well heating block.

| Set-up while using Deep well heating block with 96 Deep-Well Head* | | | | |
|---|---|---|---|---|
| **Component** | **Plate ID** | **Location on Carousel** | **Plate Type** | **Amount** |
| **Lysate** | Sample | 1 | deep-well | 530 µL |
| **Solution WN** | Wash WN | 2 | deep-well | 1000 µL |
| **80% Ethanol** | Wash 2 | 3 | deep-well | 1000 µL |
| **80% Ethanol** | Wash 3 | 4 | deep-well | 500 µL |
| **Elution Buffer F** | Elution | 5 | deep-well | 50-100 µL |
| **Tip Comb** | Tip Comb | 6 | Place a 96-well Deep-well Tip Comb in a deep-well or a KF plate | |

**Refer to the table (1a or 1b) based on the heating block and plates being used. Deep Well heating block is not compatible with 96 KF plate. Selecting the correct heating block and plates is important as elution step has a heating step. Using incompatible plates can lead to reduced NA yield.*

**Table 1b.** Setup of plates while using KingFisher™ Flex with a 96 Deep-well Head and a 96 heating block.

| Set-up while using 96 heating block with 96 Deep-Well Head* | | | | |
|---|---|---|---|---|
| **Component** | **Plate ID** | **Location on Carousel** | **Plate Type** | **Amount** |
| **Lysate** | Sample | 1 | deep-well | 530 µL |
| **Solution WN** | Wash WN | 2 | deep-well | 1000 µL |
| **80% Ethanol** | Wash 2 | 3 | deep-well | 1000 µL |
| **80% Ethanol** | Wash 3 | 4 | deep-well | 500 µL |
| **Elution Buffer F** | Elution | 5 | KF plate or deep-well | 50-100 µL |
| **Tip Comb** | Tip Comb | 6 | Place a 96-well Deep-well Tip Comb in a deep-well or a KF plate | |

**Refer to the table (1a or 1b) based on the heating block and plates being used. Deep Well heating block is not compatible with 96 KF plate. Selecting the correct heating block and plates is important as elution step has a heating step. Using incompatible plates can lead to reduced NA yield.*

## Section 1. Automated Nucleic Acid Isolation

### Section 1A - Automated Nucleic Acid Isolation for Pathogens except gram positive

**Note: Ensure that the correct program is loaded KF_VNA_96. If you are specifically targeting gram positive pathogens follow section 1B.**

1. Set up all the plates according to **Table 1a or 1b.** Place a clean tip comb in an empty plate **(Tip Comb plate).**
2. Add 50-100 µL of **Elution Buffer F** to the **Elution plate.**
3. Add 1000 µL **Solution WN** (added with ethanol) to **Wash WN plate**.
4. Add 1000 µL **80% ethanol** to **Wash plate 2** and 500µL of **80% ethanol** to **Wash plate 3.**
5. Transfer 200-400 µL of sample to the **Sample plate**.

6. Add 20 µL of **Proteinase K** and 300 µL of **Lysis Buffer B** to the well with sample.
7. Alternatively, prepare a mixture of 20 µL of **Proteinase K** and 300 µL of **Lysis Buffer B** to the sample. We recommend a 10% overage while making mixture for multiple samples, to compensate for pipetting errors. Make sure to mix by inverting the bulk before adding it to the lysate (do not vortex). It is recommended to prepare the bulk fresh every time a new isolation is performed.
8. After setting up, run the program **VNA_96_KF**.
9. Place the plates as promoted by the KingFisher™ Flex instrument.
10. Prepare a mixture of 500 µL **96-100% ethanol** and 20 µL **Nucleic Acid Magnetic Beads** for the number of samples extracted. (For example, if Nucleic acid is to be extracted from 10 samples, mix 5 mL of **96-100% ethanol** with 400 µL **Nucleic Acid Magnetic Beads**) A fresh bulk mixture can be made every time isolation is performed. Make 10% extra volume to avoid volume loss due to pipetting.
11. After a few minutes of incubation, the instrument will pause and allow the user to add mixture prepared in **step 10**. Open the instrument carousel door and remove **Sample plate** from the instrument and add 520 µL of **96-100% ethanol** and **Nucleic Acid Magnetic Beads** mixture that was prepared in **step 10**. Make sure to vortex this mixture before adding to the sample, to maintain a homogenous mixture.
12. Put the plate back into the instrument and follow the instrument prompt. The instrument will continue to perform the extractions.
13. The instrument will prompt after the procedure is complete. Remove the **Elution plate** from the instrument and the Nucleic Acid is now ready for further downstream processing.
14. The purified Nucleic Acid sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long-term storage.

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

**Note: Ensure that the correct program is loaded VNApos_96_KF if you are specifically targeting gram positive pathogens.**

1. Setup all the plates according to **Table 1a or 1b.** Place a clean tip comb in an empty plate **(Tip Comb plate).**
2. Add 50-100 µL of **Elution Buffer F** to the **Elution plate.**
3. Add 1000 µL **Solution WN** (added with ethanol) to **Wash WN plate**.
4. Add 1000 µL **80% ethanol** to **Wash plate 2** and 500µL of **80% ethanol** to **Wash plate 3.**
5. Transfer 200-400 µL of sample to **Sample plate**.
6. Prepare a 40 mg/mL stock solution (approximately 1.7 x 107 units/mL) of lysozyme as per supplier's instructions and add 10 µL of Lysozyme to the **sample plate.**
7. Place the plates as prompted by the KingFisher™ Flex instrument.
8. After setting up, run the program **VNA_96_KF**.
9. After a few minutes of incubation, the instrument will pause and allow the user to add Lysis buffer and Proteinase K. Open the instrument carousel door and remove **Sample plate** from the instrument and add 20 µL of **Proteinase K** and 300 µL of **Lysis Buffer B** to the well with sample.
10. Alternatively, prepare a mixture of 20 µL of **Proteinase K** and 300 µL of **Lysis Buffer B and add** to the sample. We recommend a 10% overage while making mixture for multiple samples, to compensate for pipetting errors. Make sure to mix by inverting the bulk before adding it to the lysate (do not vortex). It is recommended to prepare the bulk fresh every time a new isolation is performed.

11. Put the plate back into the instrument and follow the instrument prompt. The instrument will continue to perform the extraction.
12. Prepare a mixture of 500 µL **96-100% ethanol** and 20 µL **Nucleic Acid Magnetic Beads** for the number of samples extracted. (For example, if Nucleic Acid is to be extracted from 10 samples, mix 5 mL of **96-100% ethanol** with 400 µL **Nucleic Acid Magnetic Beads**) A fresh bulk mixture can be made every time isolation is performed. Make 10% extra volume to avoid volume loss due to pipetting.
13. After a few minutes of incubation, the instrument will pause and allow the user to add mixture prepared in **step 10**. Open the instrument carousel door and remove **Sample plate** from the instrument and add 520 µL of **96-100% ethanol** and **Nucleic Acid Magnetic Beads** mixture that was prepared in **step 10**. Make sure to vortex this mixture before adding to the sample, to maintain a homogenous mixture.
14. Put the plate back into the instrument and follow the instrument prompt. The instrument will continue to perform the extraction.
15. The instrument will prompt after the procedure is complete. Remove the **Elution plate** from the instrument and the Nucleic Acid is now ready for further downstream processing.
16. The purified Nucleic Acid sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long-term storage.

## Section 2. Manual Nucleic Acid Isolation

### *2A* Nucleic Acid Isolation for Pathogens except gram positive Bacteria

1. Add 20 µL of **Proteinase K** (vortex before use) to a **96-Well plate** (provided by user).
2. Transfer 200-400 µL of sample to the tube containing **Proteinase K**.
3. Add 300 µL of **Lysis Buffer B** to the sample.
4. Seal the plate with an Adhesive Tape (provided by user) and mix the plate on an orbital shaker at 1000 rpm for 2 minutes. If an orbital shaker is not available, mix the lysate 10 times using a pipette.
5. Incubate at 65ºC for 10 minutes.
6. Briefly spin the plate to collect any drops of liquid.
7. Remove the Adhesive Tape, add 500µL **96-100% Ethanol** and 20 µL of **Nucleic Acid Magnetic Beads** (vortex prior to use) to the lysate.
8. Seal the plate with an Adhesive Tape (provided by user) and mix the plate on an orbital shaker at 1000 rpm for 5 minutes. If an orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 5 minutes.
9. Place the 96-Well Plate on the magnetic plate. Allow to sit for 1 minute. Incubate for more time if the solution is not clear.
10. Remove the Adhesive Tape. Aspirate and discard supernatant without touching the magnetic beads.
11. Remove the 96-Well Plate from the magnetic plate and gently add 1000 µL of **Solution WN (ensure ethanol was added).**
12. Seal the plate with an Adhesive Tape (provided by user) and mix the plate on an orbital shaker at 1000 rpm for 1 minute. If an orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 1 minute.
13. Place the 96-Well Plate on the magnetic plate and allow you to sit for 1 minute. Incubate for more time if the solution is not clear.
14. Aspirate and discard supernatant without touching the magnetic beads.
15. Add 1000 µL of freshly prepared **80% ethanol**.

16. Seal the plate with an Adhesive Tape (provided by user) and mix the plate on an orbital shaker at 1000 rpm for 1 minute. If an orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 1 minute.
17. Place the 96-Well Plate on the magnetic plate and allow you to sit for 1 minute. Incubate for more time if the solution is not clear.
18. Aspirate and discard supernatant without touching the magnetic beads.
19. Repeat **Steps 15-18 from this section** for a second wash step with 500 µL of 80% ethanol instead of 1000 µL.

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

20. Incubate the 96-Well plate at room temperature for 5 to 10 minutes to air-dry the magnetic beads. Make sure not to over-dry the magnetic beads as this might affect the DNA yield.
21. Add 50-100 µL of **Elution Buffer F**. Mix by gently pipetting and incubate at 65˚C for 10 minutes.
22. Briefly mix by pipetting and place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute. Incubate for more time if the solution is not clear.
23. Carefully transfer the elution to a **96-Well Elution Plate** (provided) without touching the magnetic beads.
24. The purified Nucleic Acid sample may be stored at -20°C for a few days. The provided Adhesive Tape can be used to seal the **96-Well Elution Plate** for storage of the DNA. It is recommended that samples be placed at –70°C for long-term storage.

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

***Notes prior to use:***

- Prepare a 400 mg/mL stock solution (approximately 1.7 x $10^7$ units/mL) of lysozyme as per supplier's instructions.

**Sample Preparation for Blood Containing Gram Positive Bacterial Pathogens**

a) Add 10 µL of Lysozyme to a **96-Well Plate** and transfer 200 µL - 400 µL of blood sample to the **96-Well Plate** containing Lysozyme. Cover the plate by using Adhesive Tape (provided by user)**.**
b) Mix well by pipetting and shake the plate 2 minutes on an orbital shaker at 1000 RPM. Alternatively mix by pipetting 10 times.
c) Incubate at Room Temperature for 10 Minutes. (Note: 0.5 to 2 hours incubation time can be used depending on the bacterial strain being lysed).
d) After incubation, add 20 µL of Proteinase K (vortex before use) to the **96-Well Plate** and proceed to Section 2A **Step 3.**

## 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 # |
|---|---|
| Thermo Scientific™ KingFisher™ Flex purification System with 96 KF head | 5400620 |
| Thermo Scientific™ KingFisher™ Flex purification System with 96 Deep Well head | 5400630 |
| Thermo Scientific™ KingFisher tip comb | 97002070 |
| Thermo Scientific™ 96 Deep Well plate, v-bottom | 95040450 |
| Thermo Scientific™ Tip Comb and Plate | 97002820 |
| Thermo Scientific™ Sterile 96 Deep Well Plate | 95040460 |
| Thermo Scientific™ KingFisher™ Plastics for 96 standard and PCR formats | 97002540 |
| Norgen Biotek Adhesive Tape | 28394 |

## 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. |

**Technical Support**
Contact our Technical Support Team between the hours of 9:00 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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