Avian Influenza A Virus (H5N1) RT-PCR Detection Kit   Product Insert
Product # 35400

INTENDED USE
Norgen’s Avian Influenza A Virus (H5N1) RT-PCR Detection Kit is used for the purification and detection of the Avian Influenza A virus (H5N1) of avian origin that is an emerging influenza virus with potential pandemic threat. The kit contains two parts: (1) an Avian Influenza A virus (H5N1) RNA purification kit based on Norgen’s proprietary spin column technology, and (2) a ready-to-use detection kit for rapid and accurate detection of Avian Influenza A virus (H5N1) by end-point RT-PCR.

The kit is designed for the rapid extraction and qualitative detection of the H5 Hemagglutinin viral RNA transcript of Avian Influenza A virus (H5N1) in nasopharyngeal swabs, nasal swabs, throat swabs and nasal aspirates from human patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors. The kit can also be used from nasopharyngeal and cloacal swab obtained from birds or poultry. This kit is intended to be used in clinical labs with the ability to perform RT-PCR.

SUMMARY AND EXPLANATION
Influenza virus infection of birds, humans and other animals is a major public health problem worldwide. Influenza viruses are classified as either type A, B or C based on differences in their nucleoproteins and matrix proteins. The type A viruses are the most virulent human pathogens among the three influenza types and cause the most severe disease and epidemics. The different types can be further classified into subtypes based on antigenic differences in two surface glycoproteins; hemagglutinin and neuroaminidase. All known subtypes of influenza A can be found in birds (H1-H16, N1-N9), while a limited number of the subtypes have been found in humans (H1-H3, N1 and N2). However, over the past few years, various subtypes of Influenza A viruses, including H5N1, have been reported to infect humans (WHO, 2006). In addition, the coexistence of human influenza viruses and avian influenza viruses may provide an opportunity for genetic material to be exchanged between these viruses. This could potentially create a new virulent influenza strain that is easily transmissible and lethal to humans (Food Safety Research Information Office, 2006). Thus, there is the need for sensitive diagnostic tests to allow for the rapid and early detection of these H5 influenza virus infections, to help reduce the risk of epidemics or pandemics in both animals and humans.

PRINCIPLES OF THE PROCEDURES
Norgen’s Avian Influenza A Virus (H5N1) RT-PCR Detection Kit contains two parts: (1) an Avian Influenza A virus (H5N1) RNA purification kit based on Norgen’s proprietary spin column technology, and (2) a ready-to-use detection kit for rapid and accurate detection of the Avian Influenza A virus (H5N1) by end-point RT-PCR. In the first part, the provided RNA purification kit allows the extraction of viral RNA from the various acceptable respiratory specimens. Purification is based on spin column chromatography using Norgen’s proprietary resin. An Isolation control is provided for spike-in isolation for the subsequent determination of extraction efficiency. The H5N1 RNA isolated is of the highest quality and free of other cellular components. In the second part, the purified RNA is subjected to RT-PCR using the provided ready-to-use RT-PCR detection kit. The H5N1 2x RT-PCR Master Mix contains reagents and enzymes for the specific amplification of a 321 bp region of the viral genome. In addition, Norgen’s H5N1 RT-PCR Detection Kit contains a second Master Mix, the Control 2x RT-PCR Master Mix, which can be used to identify possible PCR inhibition and/or inadequate isolation via a separate RT-PCR reaction with the use of the provided PCR control (PCRC) or Isolation Control (IsoC), respectively. This kit is designed to allow for the testing of 24 samples.
Kit Components:

<table>
<thead>
<tr>
<th>Component</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis Solution</td>
<td>30 mL</td>
</tr>
<tr>
<td>Wash Solution (concentrate)</td>
<td>11 mL</td>
</tr>
<tr>
<td>Elution Buffer</td>
<td>2 mL</td>
</tr>
<tr>
<td>Spin Columns</td>
<td>24</td>
</tr>
<tr>
<td>Collection Tubes</td>
<td>24</td>
</tr>
<tr>
<td>Elution Tubes (1.7 mL)</td>
<td>24</td>
</tr>
<tr>
<td>H5N1 2x RT-PCR Master Mix</td>
<td>0.35 mL</td>
</tr>
<tr>
<td>Control 2x RT-PCR Master Mix</td>
<td>0.35 mL</td>
</tr>
<tr>
<td>Isolation Control (IsoC)</td>
<td>0.3 mL</td>
</tr>
<tr>
<td>H5N1 Positive Control (PosC)</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>Nuclease Free-Water</td>
<td>1.25 mL</td>
</tr>
<tr>
<td>Norgen's DNA Marker</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>Product Insert</td>
<td>1</td>
</tr>
</tbody>
</table>

*a* IsoC = Isolation Control; PosC= Positive Control
*b* The positive control is an in vitro transcribed H5 RNA fragments.
*b* The isolation control is an in vitro transcribed cloned RNA product.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 96-100% ethanol
- Thermocycler and or Real-Time PCR System
- Micropipettes with an accuracy range between 1-10 µL, 10-100 µL and 100-1000 µL
- Laminar flow hood for extractions
- Vortex
- Sterile, nuclease-free aerosol-barrier micropipettor tips
- Microcentrifuge tube rack
- Disposable latex gloves
- β-mercaptoethanol

Storage Conditions and Product Stability

- The Positive Control (H5N1 PosC, red cap) and Isolation Control (IsoC, orange cap) should be stored at -70°C. If needed, make aliquots of the controls according to the volume used in the protocol (10 µL of H5N1 PosC or 10 µL of IsoC) prior to freezing.
- The H5N1 2x RT-PCR Master Mix and the Control 2x RT-PCR Master Mix should be stored at -20°C upon receipt (-70°C for long-term). Make appropriate aliquots and store at -20°C if needed.
- All other kit components may be stored at room temperature
- The H5N1 2x RT-PCR Master Mix and the Control 2x RT-PCR Master Mix, Positive Control and Isolation Control should not undergo repeated freeze-thaw (a maximum freeze-thaw of three times).
- For RT-PCR:
  - Allow reagents to thaw at room temperature prior to use
  - When thawed, mix the components and centrifuge briefly
  - Work quickly on ice.
  - After thawed, mix the components and centrifuge briefly
  - After addition of RT-PCR Master Mix use within one hour
General Precautions

- Follow universal precautions. All patient specimens should be considered as potentially infectious and handled accordingly.
- Diagnostic laboratory work on clinical samples from patients who are suspected of having H5N1 influenza virus infection should be conducted in a BSL2 laboratory with BSL3 work practices. All sample manipulations should be carried out in a biosafety cabinet. Viral isolation on clinical specimens from patients who are suspected of having H5N1 influenza virus infection should be performed in a BSL2 laboratory following BSL3 work practices. (WHO Laboratory Biosafety Guidelines for Handling Specimens Suspected of Containing Avian Influenza A Virus; http://www.who.int/csr/disease/avian_influenza/guidelines/handlingspecimens/en/).
- Wear personal protective equipment, including gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the test.
- Do not smoke, drink or eat in areas where kit reagents and/or specimens are being used.
- Dispose of unused kit reagents and specimens according to local, provincial or federal regulations.
- Workflow in the laboratory should proceed in a uni-directional manner, beginning in the pre-amplification area(s) (i.e. specimen collection and RNA extraction) and moving to the amplification / detection area(s) (RT-PCR and gel electrophoresis).
- Do not use supplies and equipment across the dedicated areas of specimen extraction and sample preparation. No cross-movement should be allowed between the different areas.
- Supplies and equipment used for specimen preparation should not be used for pipetting or processing amplified DNA or other sources of target nucleic acids.
- All amplification supplies and equipment should be kept in the amplification / detection area at all times.
- Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- As contamination of patient specimens or reagents can produce erroneous results, it is essential to use aseptic techniques.
- Pipette and handle reagents carefully to avoid mixing of the samples.
- Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- Do not interchange reagent tube / bottle caps as this may lead to contamination and compromise test results.
- Only use the protocol provided in this insert. Alterations to the protocol and deviations from the times and temperatures specified may lead to erroneous results.

Quality Control
In accordance with Norgen’s ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen’s Avian Influenza A Virus (H5N1) RT-PCR Detection Kit, including the H5N1 2x RT-PCR Master Mix, the Control 2x RT-PCR Master Mix, Isolation Control and H5N1 Positive Control are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
Norgen’s Avian Influenza A Virus (H5N1) RT-PCR Detection Kit is designed for research purposes only. It is not intended for human or diagnostic use.

Product Warranty and Satisfaction Guarantee
NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information
Biosafety level 2 standards with Biosafety level 3 work practices are recommended for work involving clinical samples from patients who are suspected of having H5N1 influenza virus infection (WHO Laboratory Biosafety Guidelines for Handling Specimens Suspected of Containing Avian Influenza A Virus). Ensure the appropriate containment equipment and facilities are used for activities...
involving cultures or potentially infectious clinical materials. 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 online at [www.norgenbiotek.com](http://www.norgenbiotek.com).

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**CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.**

The *Lysis Solution* contains guanidine salts, and should be handled with care. Guanidine salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions. 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.

**INSTRUCTIONS FOR USE**

**Notes Prior to Use**

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the *Wash Solution* by adding 25 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated *Wash Solution*. This will give a final volume of 36 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Add 10 μL of β-mercaptoethanol (provided by the user) to each 1 mL of Lysis Solution required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- **Isolation Control (*IsoC*)**
  - An H5N1 Isolation Control (*IsoC*) is supplied. This allows the user to control the RNA isolation procedure. For this assay, add the Isolation Control (*IsoC*) to the lysate during the isolation procedure.
  - The Isolation Control (*IsoC*) must not be added to the sample material directly.
  - Do not freeze and thaw the Isolation Control (*IsoC*) more than 2 times.
  - The Isolation Control (*IsoC*) must be kept on ice at all times during the isolation procedure.
- The RT-PCR components of the H5N1 RT-PCR Detection Kit should remain at -20°C until RNA is extracted and ready for RT-PCR amplification.
- It is important to work quickly during this procedure.

**A. SPECIMEN COLLECTION AND LYSATE PREPARATION**

Acceptable specimen types include nasal swabs, nasopharyngeal swabs, throat swabs, cloacal swabs or nasal aspirates in viral transport medium tube or equivalent. If using swabs, use only sterile Dacron, nylon or rayon swabs with plastic shafts. Note: Do not use calcium alginate swabs as they may contain substances that are inhibitory to PCR.

This kit is also compatible with samples collected using Norgen’s Sample Collection Kit For Upper Respiratory Tract Infectious Agents (Cat #29100). Please follow the instructions provided with that kit for specimen collection and preservation.
i. Specimens previously collected using Norgen’s Sample Collection Kit For Upper Respiratory Tract Infectious Agents (Cat #29100)
   1) Transfer 300 µL of preserved specimen to an RNase-free microcentrifuge tube.
   2) Add 300 µL of the Lysis Solution and vortex for 10 seconds to mix.
   3) Add 10 µL of the Isolation Control (IsoC) to the lysate. Vortex for 10 seconds to mix.
   4) Add 300 µL of 95% ethanol to the lysate. Vortex for 10 seconds to mix.
   5) Proceed to RNA Isolation (Step B).

ii. Specimen collection from swab in media:
   1) If swab is transported in media the user should transfer 200 µL to an RNase-free microcentrifuge tube.
   2) Add 500 µL of the Lysis Solution and vortex for 10 seconds to mix.
   3) Add 10 µL of the Isolation Control (IsoC) to the lysate. Vortex for 10 seconds to mix.
   4) Add 400 µL of 95% ethanol to the lysate. Vortex for 10 seconds to mix.
   5) Proceed to RNA Isolation (Step B).

iii. Specimen collection directly from swab:
    Alternatively, dry swabs can be placed directly into an RNase-free microcentrifuge tube containing Lysis Solution.
    1) Aliquot 1 mL of Lysis Solution into an RNase-free microcentrifuge tube.
    2) Using sterile techniques, cut the tip where the nasal or throat cells were collected and place into the tube containing the Lysis Solution.
    3) Close the tube and vortex for 1 minute to release the virus particles.
    4) Using a sterile pipette transfer 400 µL of the lysate into another RNase-free microcentrifuge tube.
    5) Add 10 µL of the Isolation Control (IsoC) to the lysate. Vortex for 10 seconds to mix.
    6) Add 200 µL of 95% ethanol to the lysate. Vortex for 10 seconds to mix.
    7) Proceed to RNA Isolation (Step B).

B. SPECIMEN RNA PURIFICATION
Following the lysate preparation, viral RNA can be extracted from the patient specimens using the supplied buffers and solutions according to the following protocol:

1. Assemble a column with one of the provided collection tubes.
2. Apply the lysate with ethanol (up to 650 µL) to the column and centrifuge for 1 minute at 14,000 rpm.
   
   **Note:** Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed through, spin for an additional minute.

3. Discard the flowthrough and reassemble the spin column with its collection tube.
4. Depending on lysate volume, repeat steps B2 and B3.
5. Apply 400 µL of Wash solution and centrifuge for one minute at 14,000 rpm.
   
   **Note:** Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed through, spin for an additional minute.

6. Discard the flowthrough and reassemble the spin column with its collection tube.
7. Repeat steps B5 and B6 two more times.
8. Spin the column for 2 minutes to thoroughly dry the resin at 14,000 rpm. Discard the collection tube.
9. Place the column into a new 1.7 mL Elution tube.
10. Add 50 µL of Elution Solution to the column.
11. Centrifuge for 2 minutes at 2,000 rpm followed by a 2 minute spin at 14,000 rpm. Note the volume eluted from the column. If the entire 50 µL has not been eluted, spin the column for an additional minute at 14,000 rpm.
12. The purified RNA sample could be used immediately for RT-PCR as described below. It is recommended that samples be placed at -70°C for long term storage.

C. H5N1 RT-PCR Assay Preparation

Notes:
- Before use, suitable amounts of all PCR components should be completely thawed at room temperature, vortexed and centrifuged briefly.
- The amount of H5N1 2x RT-PCR Master Mix and Control 2x RT-PCR Master Mix provided is enough for up to 32 RT-PCR reactions (24 sample RT-PCR, 4 positive control RT-PCR and 4 no template control RT-PCR) each.
- For each sample, one RT-PCR reaction using the H5N1 2x RT-PCR Master Mix and one RT-PCR reaction using Control 2x RT-PCR Master Mix should be set up in order to have a proper interpretation of the result.
- For every RT-PCR run, one reaction containing H5N1 Positive Control (PosC) and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of RNA samples tested per RT-PCR run is 6.
- Using a lower volume from the sample than recommended may affect the sensitivity of H5N1 Limit of Detection.

1. Prepare the RT-PCR for sample detection (Set #1, using H5N1 2x RT-PCR Master Mix) and control detection (Set #2, using Control 2x RT-PCR Master Mix) as shown in Table 1 below. The recommended amount of sample RNA to be used is 2.5 µL. However, a volume between 1 and 5 µL of sample RNA may be used as template. Ensure that one H5N1 detection reaction and one control reaction is prepared for each RNA sample. Adjust the final volume of the RT-PCR reaction to 20 µL using the Nuclease-Free Water provided.

<table>
<thead>
<tr>
<th>RT-PCR Components</th>
<th>Volume Per RT-PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>H5N1 2x RT-PCR Master Mix</td>
<td>10 µL</td>
</tr>
<tr>
<td>Or Control 2x RT-PCR Master Mix</td>
<td></td>
</tr>
<tr>
<td>Sample DNA</td>
<td>2.5 µL</td>
</tr>
<tr>
<td>Nuclease-Free Water</td>
<td>7.5 µL</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>20 µL</strong></td>
</tr>
</tbody>
</table>

2. For each RT-PCR run, prepare one positive control RT-PCR as shown in Table 2 below:

<table>
<thead>
<tr>
<th>PCR Components</th>
<th>Volume Per RT- PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>H5N1 2x RT-PCR Master Mix</td>
<td>10 µL</td>
</tr>
<tr>
<td>Or Control 2x RT-PCR Master Mix</td>
<td></td>
</tr>
<tr>
<td>H5N1 Positive Control (PosC)</td>
<td>10 µL</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>20 µL</strong></td>
</tr>
</tbody>
</table>
3. For each RT-PCR run, prepare **one** no template control RT-PCR as shown in Table 3 below:

<table>
<thead>
<tr>
<th>RT-PCR Components</th>
<th>Volume Per RT-PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>H5N1 2x RT-PCR Master Mix</td>
<td>10 µL</td>
</tr>
<tr>
<td>Or Control 2x RT-PCR Master Mix</td>
<td></td>
</tr>
<tr>
<td>Nuclease-Free Water</td>
<td>10 µL</td>
</tr>
<tr>
<td>Total Volume</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

Therefore, at a minimum, each RT-PCR run will contain 6 separate RT-PCR reactions.

D. H5N1 RT-PCR Assay Programming

1. Program the thermocycler according to the program shown in Table 4 below.
2. Run RT-PCR.

<table>
<thead>
<tr>
<th>PCR Cycle</th>
<th>Step</th>
<th>Temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td>Step 1</td>
<td>50°C</td>
<td>25 min</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>Step 1</td>
<td>95°C</td>
<td>5 min</td>
</tr>
<tr>
<td>Cycle 3 (40x)</td>
<td>Step 1</td>
<td>94°C</td>
<td>15 sec</td>
</tr>
<tr>
<td></td>
<td>Step 2</td>
<td>60°C</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td>Step 3</td>
<td>72°C</td>
<td>45 sec</td>
</tr>
<tr>
<td>Cycle 4</td>
<td>Step 1</td>
<td>72°C</td>
<td>5 min</td>
</tr>
<tr>
<td>Cycle 5</td>
<td>Step 1</td>
<td>4°C</td>
<td>∞</td>
</tr>
</tbody>
</table>

E. H5N1 RT-PCR Assay Results Interpretation

1. For the analysis of the RT-PCR data, the entire 15-20 µL RT-PCR Reaction should be loaded on a 1X TAE 1.7% Agarose RNA gel along with 10 µL of Norgen’s RNA Marker (provided). Prepare enough agarose gel for running one set of RT-PCR of H5N1 detection and one set of RT-PCR for controls detection.
2. The RT-PCR products should be resolved on the 1X TAE 1.7% Agarose gel at 150V for 30 minutes (Gel running time will be vary depending on an electrophoresis apparatus).
3. Sample results are provided below:
Figure 1: A representative 1X TAE 1.7% agarose gel showing the amplification of H5N1 (H5N1 Target) using the H5N1 2x RT-PCR Master Mix. The size of the H5N1 target amplicon corresponds to 321 bp as represented by the provided DNA Marker (M). NC = Negative Control.

Figure 2: A representative 1X TAE 1.7% agarose gel showing the amplification of Isolation Control and PCR Control under different conditions using the Control 2x RT-PCR Master Mix. The size of the Isolation Control amplicon and PCR Control amplicon correspond to 499 bp and 150 bp, respectively, as represented by the provided DNA Marker (M). Lanes 1 to 5 showed detection of both Isolation Control and PCR Control, suggesting that the RNA isolation as well as the RT-PCR reaction was successful. Lane 6 showed only the detection of PCR Control suggesting that while the RT-PCR was successful, the isolation failed to recover even the spiked-in Isolation control. NC = Negative Control.
Table 5. Interpretation of One-Step RT-PCR Assay Results

<table>
<thead>
<tr>
<th>Input Type</th>
<th>Target reaction</th>
<th>Control Reaction</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H5N1 Target</td>
<td>H5N1 IsoC</td>
<td>H5N1 PCR</td>
</tr>
<tr>
<td></td>
<td>Band (321 bp)</td>
<td>Band (499 bp)</td>
<td>Band (171 bp)</td>
</tr>
<tr>
<td>Positive Control</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Negative Control</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Sample</td>
<td>X</td>
<td>X</td>
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<td>Sample</td>
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<tr>
<td>Sample</td>
<td>X</td>
<td>X</td>
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</tbody>
</table>

** For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.

F. H5N1 RT-PCR Assay Specificity

- The specificity of Norgen’s Avian Influenza A Virus (H5N1) RT-PCR Detection Kit is first and foremost ensured by the selection of the H5-specific primers, as well as the selection of stringent reaction conditions. The primers were checked for possible homologies to all in GenBank published sequences by sequence comparison analysis. The specific detectability of all relevant strains has thus been ensured by a database alignment and by PCR amplification with the following commonly found seasonal flu viruses:
  - Novel H5N1 (Swine Origin)
  - Seasonal H1N1
  - H3N2
  - Influenza B

G. H5N1 RT-PCR Assay Specificity

- The linear range (analytical measurement) of Norgen’s Avian Influenza A Virus (H5N1) RT-PCR Detection Kit was determined by analysing a dilution series of a H5N1 quantification standard ranging from $1 \times 10^7$ copies/µl to $1 \times 10^9$ copies/µl.
- Each dilution has been tested in replicates (n = 4) using Norgen’s Avian Influenza A Virus (H5N1) RT-PCR Detection Kit on 1X TAE 2% Agarose gel.
- The linear range of Norgen’s Avian Influenza A Virus (H5N1) RT-PCR Detection Kit has been determined to cover concentrations from $5 \times 10^5$ copies/mL to at least $1 \times 10^9$ copies/mL.
Frequently Asked Questions

1. **How many samples should be included per RT-PCR run?**
   - Norgen’s Avian Influenza A Virus (H5N1) RT-PCR Detection Kit is designed to test 24 samples. For every 6 samples, a non-template control and a Positive Control must be included. It is preferable to pool and test 6 samples at a time.

2. **How can I interpret my results if neither the PCR control nor the Isolation Control amplifies?**
   - If neither the PCR control nor the Isolation Control amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, whereas if the Positive control did not amplify, therefore the Problem has occurred during the setup of the PCR assay reaction.

3. **How should it be interpreted if only the PCR control showed amplification but neither the H5N1 target nor the Isolation control amplificed for a sample?**
   - This indicates a poor isolation. The isolation procedure must be repeated.

4. **How should it be interpreted if only the Isolation Control was amplified in a sample?**
   - The sample tested can be considered as H5N1 negative.

5. **How should it be interpreted if only the H5N1 target and the H5N1 PCR control were amplified in a sample?**
   - The sample tested can be considered as H5N1 positive.

6. **How should it be interpreted if only the H5N1 target was amplified in a sample?**
   - The sample tested should be considered as H5N1 positive. At high H5N1 copies input, the H5N1 amplicon will be predominant and thus the H5N1 PCR control as well as the H5N1 Isolation control may not amplify as they compete for PCR resources.

7. **How should it be interpreted if only the H5N1 PCR control and the H5N1 Isolation control showed amplification in a sample?**
   - The sample tested can be considered negative

8. **What If I forgot to do a dry spin after my second wash?**
   - Your RNA elution will be contaminated with the Wash Solution. This may dilute the RNA yield in your elution and it may interfere with the RT-PCR detection, as ethanol is known to be a PCR inhibitor.

9. **What If I forgot to add the H5N1 Isolation control during the Isolation?**
   - It is recommended that the isolation is repeated.
References

Food Safety Research Information Office. 2006. A Focus on Avian Influenza.


Technical Assistance

NORGEN’s Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen’s Avian Influenza A Virus (H5N1) RT-PCR Detection Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362, or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.