Product Instructions

Influenza A Virus H5-H7 RNA Test Kit

vetproof® Influenza A Virus H5-H7 qPCR Kit

Product No. KIT250005

100 reactions

Store below -15°C

For veterinary in vitro use only

Contents

1 Product Description

The **vet**proof® Influenza A Virus H5-H7 qPCR Kit (InfA H5-H7 qPCR) will detect the presence of RNA from Influenza A Virus subtypes H5 and H7 in extracts from avian samples (e.g. tracheal, oropharyngeal or cloacal swabs, tissue samples, faeces, environmental samples, and FTA cards).
The kit contains all reagents and controls required for the detection of Influenza A Virus subtypes H5 and H7. Primers and probes are specific for Influenza A Virus subtypes H5 and H7; each probe is labelled with a specific fluorophore which is detected in a designated channel on the qPCR thermocycler. Influenza A Virus subtype H5 is detected in the FAM channel, subtype H7 is detected in the CY-5 channel. An Extraction Control (EC) is detected in the HEX channel to verify successful RNA extraction and amplification. For environmental samples or samples from wild/exotic birds (other than chickens, turkeys, ducks or geese), the EC is exogenous and requires the addition of EPC-Ext (Exogenous Extraction Control) to the extraction process. For samples from chickens, turkeys, ducks or geese, an endogenous control (housekeeping gene naturally present in the sample) is detected through the EC channel. Addition of EPC-Ext is not required for these samples.

In summary, the **vet**proof® Influenza A Virus H5-H7 qPCR Kit enables simultaneous detection of:

- Influenza A Virus subtype H5 (InfA H5; detected in FAM channel)
- Influenza A Virus subtype H7 (InfA H7; detected in CY-5 channel)
- Extraction Control (EC; detected in HEX channel)

1.1 Number of Tests

The kit is designed for 100 reactions with a final reaction volume of 25 µl each.

1.2 Storage and Stability

Upon receipt, store below -15°C. Store away from light.
Do not freeze-thaw reagents more than 3 times. It is recommended to aliquot reagents when lower sample numbers are processed and more than 3 freeze-thaw cycles for reagents are expected.

1.3 Kit Contents

Item No.	Component	Format	Contents / Function
1	PCR Mix (PCR MIX)	2 x 1000 µL vial with green cap	• Ready-to-use PCR Mix • For the amplification and detection of Influenza A Virus subtype H5- and H7-specific sequences • 20 µl per reaction
2	Control Template InfA H5-H7 (H5-H7 CTL+)	1 x vial (dehydrated) with purple cap	• Lyophilized • Needs to be reconstituted/rehydrated in H2O PCR • For use as a PCR run positive control for InfA H5 and H7
3	H2O PCR-grade (H2O PCR)	1 x 1000 µL vial with clear cap	• Ready-to-use nuclease-free, PCR-grade H2O • For use as a PCR run negative control • For reconstitution of H5-H7 CTL+
4	Exogenous Extraction Control (EPC-Ext)	1 x 1250 µL vial with white cap	• Ready-to-use • For use as extraction and amplification control • Add 5 µl/sample to the first lysis buffer used in the extraction protocol of environmental samples or wild/exotic bird samples

1.4 Applicability Statement

This kit is compatible with all real-time PCR instruments suitable for the detection of FAM, HEX, and CY-5 fluorophores, including the following: LightCycler® 96 (Roche Diagnostics), AriaMx® (Agilent Technologies), Applied Biosystems™ 7500 and QuantStudio™ 5 (Thermo Fisher Scientific) and CFX96™ (Bio-Rad Laboratories).

1.5 Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for the detection of FAM, HEX, and CY-5-labelled probes
- RNA extraction method (see: Preparation of Samples > RNA extraction)
- Mini-centrifuge for microtubes (x2)
- Vortex mixer (x2)
- PCR plate-spinner
- Pipettes & disposable filter-tips for volumes of 1 – 1000 µl
- Nuclease-free microtubes of 1.5 ml and 2 ml
- Plates for PCR reaction or PCR tubes (suitable for use with your real-time PCR cycler)
- Heat resistant plate sealers or cap strips
- Disposable powder-free gloves
- Refrigerator and freezer

1.6 Preparation of Samples

Sample material

Avian samples – Tracheal, oropharyngeal or cloacal swabs, tissue samples, faeces, environmental samples, and FTA cards.

RNA extraction

Before running the PCR, RNA must be extracted from the sample.

Extract RNA from the sample using an appropriate manual or automated extraction. Recommended are spin column methods or magnetic bead extraction methods (e.g. **vet**proof® MagBead Extraction Kit I (Product no. KIT230342)).

Extracted RNA can be stored at -20°C prior to running the PCR. Handle RNA extracts with care due to the fragile nature of RNA.

It is recommended to validate the chosen extraction method and the **vet**proof® Influenza A Virus H5-H7 qPCR Kit combined, internally, prior to generating results.

Exogenous Extraction Control (EPC-Ext)

During the extraction process, EPC-Ext must be added to the first lysis buffer used for the RNA extraction of environmental samples or samples from wild/exotic bird (other than chickens, turkeys, ducks or geese). This allows for verification of successful RNA extraction and amplification.

Aliquot EPC-Ext into appropriate volumes to avoid more than three freeze-thaw cycles. Store aliquots at temperatures below -15°C.

For use, add 5 µl of EPC-Ext per sample to the first lysis buffer used in the nucleic acid extraction procedure of environmental samples or wild/exotic bird samples.

Note: EPC-Ext must not be added for extracting RNA from chicken, turkey, duck or goose samples as these contain endogenous control RNA.

2 How to Use This Product

2.1 Good Laboratory Practices for PCR

- Assays must be performed by qualified laboratory personnel only.
- Wear disposable powder-free gloves at any stage of running the assay and/or sample preparation. Change gloves when changing work areas or if you suspect that they are contaminated.
- Handle all reagents with care.
- Do not thaw reagents more than 3 times.
- Treat all biological materials as potentially biohazardous, including all field samples.
- Never pipette anything by mouth. There must be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
- Avoid prolonged exposure of the PCR Mix to direct light.
- Keep the kit components separate from other reagents in the laboratory.
- Use nuclease-free lab ware (e.g., pipettes, pipette tips, reaction vials).
- To avoid cross-contamination of samples and reagents, use aerosol-preventive pipette tips.
- Strict adherence to the test protocol will lead to achieving the best results.
- Dedicate one airspace for kit storage/assay preparation (Room 1, Clean Room) and another airspace (Room 2) for running the assay and sample preparation/extraction (Room 2). A third airspace is optional (Room 3) for dedicated amplification/running the assay.
- Never move any materials and equipment from Room 2 or 3 to Room 1.
- Include positive and negative control in every run.
- Decontaminate PCR laboratories with bleach or an alternative nucleic acid decontaminant and UV light (optional) after testing.
- Do not use reagents after the expiration date or if the packaging is damaged.
- Do not mix reagents from different kit batches.
- Do not open the PCR wells after amplification and discard the plates/tubes safely avoiding breaking or leaking of the plates/tubes.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.

2.2 Procedure

Recommended workflow protocol
When running complete assay including RNA extraction in 1 day
1. Start in Room 1 with assay preparation.
2. Go to Room 2/3 for RNA extraction and running assay.
3. Never go from Room 2/3 to Room 1 during the same day.

When doing RNA extraction first
Day 1:
1. Start in Room 2 with RNA extraction.

Day 2:
1. Start in Room 1 with assay preparation.
2. Go to Room 2/3 to run the assay.
3. Never go from Room 2/3 to Room 1 during the same day.

Assay preparation (Room 1 – Clean Room)
1. Defrost PCR MIX at room temperature in the dark.
2. Vortex the vial(s) thoroughly and briefly spin to remove any residues from the lid.
3. Take a suitable qPCR plate and record location of samples on template.
4. Add 20 µl of PCR MIX to the plate wells for every sample plus controls.
5. Place the vial(s) with PCR MIX, after use, immediately back in the freezer at a temperature below -15°C.
6. Add 5 µl of H2O PCR-grade (Negative Control) into a control well. This is a reagent and environment control (Optional control for Room 1).
7. Cover plate and take into Room 2.

Positive Control preparation (Room 2/3 – Extraction/Amplification Room)
Control Template (H5-H7 CTL+)
1. Add 200 µl of H2O PCR-grade (H2O PCR) to the H5-H7 CTL+ tube and vortex for 20 seconds or longer, until the pellet is fully dissolved.
2. Aliquot this solution by 12 µl and store it at a temperature below -15°C.

RNA amplification (Room 2/3 – Extraction/Amplification Room)

1. Pipette 5 µl of the Control Template (H5-H7 CTL+; Positive Control) into a dedicated control well. This is a PCR run positive control for Influenza A Virus subtypes H5 and H7.
2. Add 5 µl of H2O PCR-grade (H2O PCR; Negative Control) into a dedicated control well. This is an environment control.
3. Add 5 µl of sample (RNA extract) into the appropriate sample wells.
4. Cover plate with heat resistant sealer or caps.
5. Spin plate for 30-60 seconds at 200-1000 x g (Optional).
6. Place plate in qPCR thermocycler and start the specified thermal cycler program as soon as possible.

<table>
<tr><th colspan="2">RNA Program</th></tr>
<tr><td colspan="2">10 min. 45°C</td></tr>
<tr><td colspan="2">10 min. 95°C</td></tr>
<tr><td>15 sec. 95°C</td><td rowspan="3">40 cycles</td></tr>
<tr><td>60 sec. 54°C</td></tr>
<tr><td>Data collection (@ 54°C):
FAM = InfA subtype H5
CY-5 = InfA subtype H7
HEX = Extraction Control</td></tr>
</table>

Note: Program the PCR instrument before preparing the PCR samples. For details on how to program the protocol, see the instrument operator's manual for your real-time PCR cycler.

Note: For some real-time PCR instruments, the type of probe quencher as well as passive reference dye must be specified. For the ABI7500, ROX should be specified as passive reference dye.

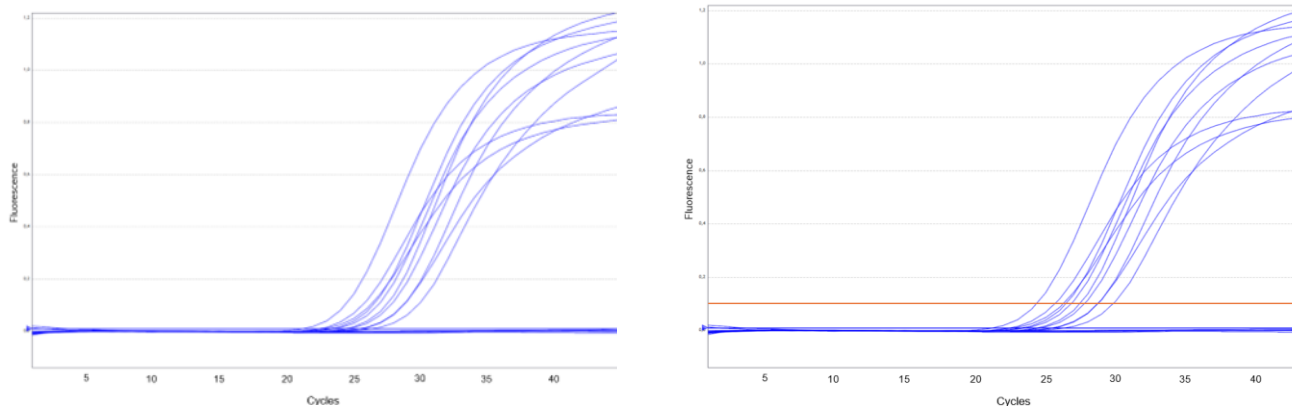
Note: Alternative channel names for reporter dyes:

- FAM: no alternative name
- HEX: VIC
- CY-5: Quasar 670

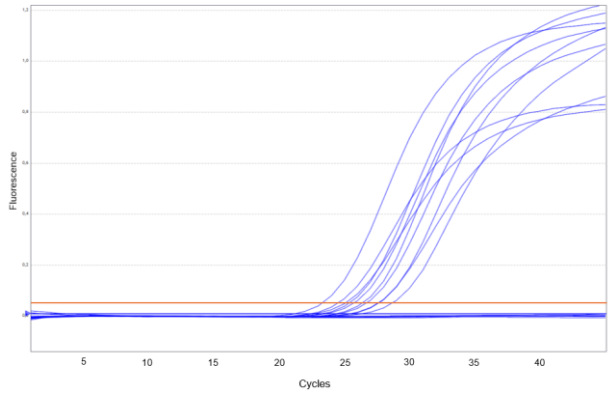
2.3 Validation and Interpretation

Setting thresholds in the PCR cycler software

Go to the part of the software where you can see the amplification curves. Select all wells on the plate, select linear view, and select the FAM channel. Turn off other channels.



Depending on the amount of samples the linear curves should look like the ones in the picture above on the left. To set the threshold look at which cycle the first curve starts to form, in this case around cycle no. 22. Look at which cycle the first formed curve goes up in a straight line, in this case around cycle 24. See picture above on the right. The threshold is placed for demonstration purposes at the point where the curve becomes a straight line.
The threshold should be set halfway between the fluorescence of cycles 22 and 24. See picture below.



Repeat this process for the CY-5 and HEX channel.

Note: After setting the thresholds for all channels, keep using those thresholds for all future PCR runs.

Validation of the PCR run

The following must apply for the PCR run to be valid:

	InfA H5 (FAM) Cq values	InfA H7 (CY-5) Cq values	EC (HEX) Cq values	Interpretation
Negative Control (H2O PCR)	N/A*	N/A*	N/A*	Valid Control
Positive Control/ Control Template (H5-H7 CTL+)	<40	<40	<40	Valid Control

**No Cq value*

Note: Repeat the PCR plate in the event of control failure.

Validation and interpretation of sample results

The amplification of Influenza A Virus subtype H5 RNA is analyzed in the FAM channel, subtype H7 in the CY-5 channel, the Extraction Control is analyzed in the HEX channel. Nucleic acids amplification are valid for each sample if at least one typical amplification curve is observed in FAM, CY-5 and/or HEX or equivalent.

Note: Always check the validity of the amplification curves.

InfA H5 (FAM)	InfA H7 (Cy5)	EC (HEX)	Interpretation
N/A*	N/A*	<40	Negative sample
<40	<40	Not considered	Positive sample: InfA H5 and H7
<40	N/A*	Not considered	Positive sample: InfA H5
N/A*	<40	Not considered	Positive sample: InfA H7
N/A*	N/A*	N/A*	Invalid well**

**No Cq value*

***The assay is invalid for this particular sample and should be repeated with a 1/10 dilution of the extract or a new RNA extract.*

Note: For final diagnosis qPCR positives should be considered presumptive and confirmed by standard reference methods or alternative tests for Influenza A Virus subtypes H5 and H7.

3 Supplementary Information

Symbols Glossary

REF	Product Reference Number		Expiry (Expiration) Date
	Kit Size/Reactions		Protect from Moisture
	Store at		Protect from Heat and Direct Sunlight
	Batch		Manufacturer

Quality Control

All products are monitored by our quality control on a batch-to-batch basis. A certificate of analysis (CoA) is available from BioChek.

Ordering Information

This kit and associated products are available from BioChek. For a complete overview and for more information, please visit our website at www.biochek.com.

Trademarks

vetproof® is a trademark of Hygiena Diagnostics GmbH.

Warranty and Disclaimer of Liability

"Limited Warranty" and "Disclaimer of Liability": BioChek B.V. warrants that this product is free from defects in materials and workmanship through the expiration date printed on the label and only if the following are complied with:

1. The product is used according to the guidelines and instructions set forth in the product literature;
2. BioChek B.V. does not warrant its product against any and all defects when: the defect is a result of material or workmanship not provided by BioChek B.V.; defects caused by misuse or use contrary to the instructions supplied, or improper storage or handling of the product;
3. All warranties of merchantability and fitness for a particular purpose, written, oral, expressed or implied, shall extend until the expiry date of the product. There are no other warranties that extend beyond those described on the face of this warranty;
4. BioChek B.V. does not undertake responsibility to any purchaser of its product for any undertaking, representation or warranty made by any dealers or distributors selling its products beyond those herein expressly expressed unless expressed in writing by an officer of BioChek B.V.;
5. BioChek B.V. does not assume responsibility for incidental or consequential damages, including, but not limited to responsibility for loss of use of this product, removal or replacement labor, loss of time, inconvenience, expense for telephone calls, shipping expenses, loss or damage to property or loss of revenue, personal injuries or wrongful death;
6. BioChek B.V. reserves the right to replace or allow credit for any modules returned under this warranty.

Regulatory Disclaimers

For veterinary in vitro use only.

Regulatory requirements vary by country; this product may not be available in your geographic area. For information on availability, please contact BioChek.

4 Revision Index

Revision A: New document.

5 Supplier Information

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