App-Hps-Mhyo DNA Test Kit

vetproof® App-Hps-Mhyo qPCR Kit

Revision A, April 2023

PCR kit for the detection of Actinobacillus pleuropneumoniae, Haemophilus parasuis & Mycoplasma hyopneumoniae DNA in porcine samples using qPCR.

Product No. KIT230365

Reagents for 100 reactions

Store at -20°C

For veterinary use only
For *in vitro* use

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1. Product Description

The **vet**proof® App-Hps-Mhyo DNA qPCR Kit (App-Hps-Mhyo qPCR) will detect the presence of DNA from App and/or Hps and/or Mhyo in extracts from swine samples (oral fluid, lung tissue and nasal swabs). Primers and probes are specific App and/or Hps and/or Mhyo; each probe is labelled with a specific fluorophore which is detected in a designated channel on the qPCR thermocycler. After extraction of the Nucleic Acids, samples are added to plates along with the dedicated Reaction Mix. The prepared wells are placed in the qPCR cycler for amplification and detection.

The App-Hps-Mhyo qPCR assay enables the simultaneous detection of:

- App; detected in CY-5 channel.
- Hps; detected in Texas Red channel.
- Mhyo; detected in FAM channel)
- Internal Control (IC; detected in HEX channel)

1.1 Number of Tests

The kit contains reagents for 100 PCR reactions with a final reaction volume of 25 µl each.

1.2 Storage and Stability

Upon receipt, store at -20°C.

1.3 Kit Contents

The vetproof® App-Hps-Mhyo qPCR Kit contains the following reagents:

1. **Master Mix**, 2 vials, liquid (2 x 675 µl) (yellow cap).
2. **Primer/Probe Mix & Internal Control (PP/IC)**, 2 vials, liquid (2 x 415 µl) (white cap).
3. **Control Template** (Positive Control), 1 vial, liquid (1 x 200 µl) (purple cap).
4. **H_2O PCR-grade** (Negative Control), 1 vial, molecular grade water (1 x 1000 µl) (clear cap).

Note: Control Template (Positive Control): Diluted App-Hps-Mhyo plasmid with cloned target sequence standardized to represent significant amounts of the 3 different targets.

1.4 Applicability Statement

This kit is compatible with all real-time PCR instruments suitable for detection of FAM, CY-5, Texas Red and HEX fluorophores.

1.5 Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for detection channels for FAM, CY-5, Texas Red and HEX
- DNA extraction method
- Heating block (optional)
- Vortex mixer (x2)
- Mini centrifuge (x2)
- PCR plate-spinner (recommended)
- Pipettes & disposable filter-tips for volumes of 1 – 1000 µl
- DNase/RNase free tubes for preparation of reaction mix
- Plates, strips (+ caps) or microtubes for RNA extraction
- Recommended plates for PCR reaction (suitable for use with your qPCR thermocycler)
- Heat resistant sealers for plate
- Disposable powder free gloves

1.6 Preparation of Samples

Samples

Swine – Oral fluid, lung tissue or nasal swabs.

DNA extraction from samples

Before running the PCR, DNA must be extracted from the sample.
The extraction method chosen to be used with the **vet**proof® App-Hps-Mhyo DNA qPCR Kit must be suitable for DNA extraction. Recommended are spin column extraction methods or magnetic bead extraction methods (e.g. **vet**proof® MagBead Extraction kit I – product number KIT230342).
Extracted DNA can be stored at -20°C prior to running the PCR.
Handle DNA extracts with great care due to the fragile nature of DNA.
It is recommended to validate the **vet**proof® App-Hps-Mhyo DNA qPCR Kit and chosen extraction method combined internally prior to generating results.

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.
- 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.
- 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 best results.
- Dedicate one airspace for kit storage/reagent preparation (Room 1, clean room) and another airspace (Room 2) for running the assay and sample preparation. A third airspace is optional (Room 3) for dedicated PCR amplification/running the assay.
- Never move any materials from Room 2 or 3 to Room 1.
- Decontaminate PCR laboratories with bleach or alternative nucleic acid decontaminant and UV light (optional) after testing.

2.2 Procedure

Recommended workflow protocol

When running complete assay including DNA extraction in 1 day

1. Start in Room 1 with reagent preparation.
2. Go to Room 2 for RNA extraction and running assay.
3. Never go from Room 2/3 to Room 1 during the same day.

When doing DNA extraction first

Day 1

1. Start in Room 2 with DNA extraction.

Day 2

1. Start in Room 1 with reagent preparation.
2. Go to Room 2/3 for running the assay.
3. Never go from Room 2/3 to Room 1 during the same day.

Reagent preparation (Room 1)

1. Defrost reagents at room temperature.
2. Vortex reagents thoroughly and briefly spin to remove any residues from the lid.
3. Calculate total volumes of Master Mix, PP/IC and Enzyme Solution required for all reactions (Reaction Mix). Do not forget to include reactions for controls (minimum one positive and one negative), and to compensate for dead volume, + 1 reaction for instance.

Reaction Mix (20 µl)	
Master Mix (yellow Cap)	12.5 µl
PP/IC (white cap)	7.5 µl

4. Put the total volume of required Master Mix into a clean microtube.
5. Add the total volume of required PP/IC to to the microtube.
6. Vortex microtube to mix thoroughly, and briefly spin to remove any residues from the lid.

Assay Preparation (Room 1)

1. Take a suitable qPCR plate and record location of samples on template.
2. Add 20 µl of Reaction Mix as prepared above for every sample. The plate does not need to be cooled.
3. Add 5 µl of H_2O PCR-grade (Negative Control) into control well. This is a reagent and environment control (optional in Room 1)
4. Cover plate and take into Room 2.

DNA Amplification (Room 2)

1. Add 5 µl of Control Template (Positive Control) into control well, the plate does not need to be cooled.
2. Add 5 µl of H_2O PCR-grade (Negative Control) into control well. This is an environment control.
3. Add 5 µl of DNA extract into each sample well.
4. Cover plate with heat resistant sealer.
5. Spin plate for 30-60 seconds at 200-1000 x g.
6. Place plate in qPCR thermocycler and run using the specified thermal cycler program in the table (qPCR program at normal ramp speed).

Temperature	Time	No. Of cycles
95 °C	3 min	1
95 °C	15 sec	40
60 °C	60 sec	
Data collection (@ 60 °C): FAM = Mhyo CY-5 = APP Texas Red = Hps HEX = IC		

Note: Do not use fast mode.

Note: Alternative channel names for the reporter dyes:

- FAM: no alternative name
- HEX: VIC, Yakima Yellow, CAL Fluor Orange 560, Alexa 532
- Texas Red: no alternative name
- CY-5: Quasar 670

2.3 Validation and Interpretation

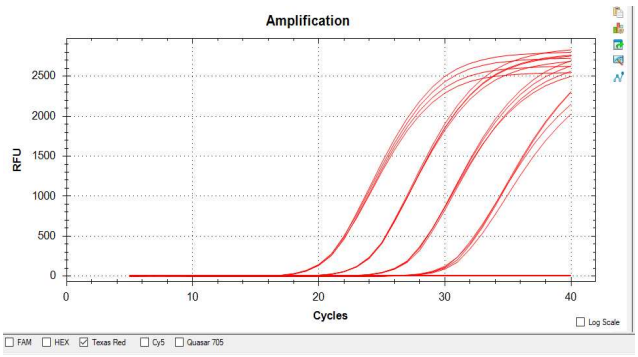
Analysis Settings

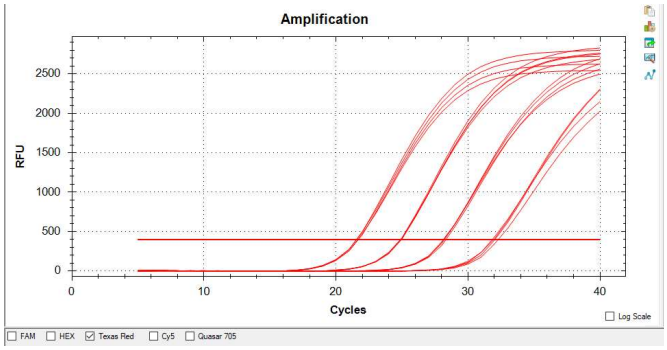
Bio-Rad CFX96™	Applied Biosystems® 7500	Stratagene Mx3005P™
Fluorescence drift correction: Yes	Passive reference: no	Amplification-based threshold
Cycles to analyze: 5 – 40	Baseline cycles: 3 – 12	Adaptive baseline

Note: Other PCR machines can be used, contact BioChek for further information regarding suitable qPCR thermocyclers.

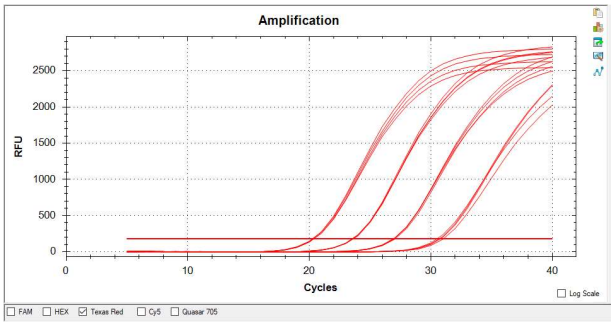
Setting thresholds in the cycler software

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 nr 20.
Look at which cycle the first formed curve goes up in 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 20 and 24, see picture below.



Repeat this process for the HEX, Texas Red and CY-5 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:

	Mhyo (FAM) Cq values	App (CY5) Cq values	Hps (Texas Red) Cq values	IC (CY5) Cq values	Interprataton
Positive Control	22-33	22-33	22-33	Not considered	Valid Control
Negative Control	N/A*	N/A*	N/A*	26-34	Valid Control

* No Cq value

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

Validation and interpretation of sample results: Always check validity of the amplification curves

Mhyo (FAM) Cq values	App (CY5) Cq values	Hps (Texas Red) Cq values	IC (CY5) Cq values	Interpretation
<40	<40	<40	Not considered	positive sample Mhyo, App and Hps
<40	<40	N/A*	Not considered	positive sample Mhyo and App
N/A*	<40	<40	Not considered	positive sample App and Hps
<40	N/A*	<40	Not considered	positive sample Mhyo and Hps
<40	N/A*	N/A*	Not considered	positive sample Mhyo
N/A*	<40	N/A*	Not considered	positive sample App
N/A*	N/A*	<40	Not considered	positive sample Hps
N/A*	N/A*	N/A*	26-34	negative sample
N/A*	N/A*	N/A*	N/A* or <26/>34	invalid well**

* No Cq value

** When a sample is negative and the internal control is out of range, the assay is invalid for this particular sample, and should be repeated with a 1/10 dilution of the extract or a new DNA extract.

Note: For sample results with a Cq between 38 and 40, it is recommended to check the amplification curve.

Note: For final diagnosis qPCR positive results should be considered presumptive and confirmed by standard reference methods or alternative tests for App-Hps-Mhyo.

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 BV.

Ordering Information

This kit and associated products are available from BioChek BV. 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 BV 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 BV does not warrant its product against any and all defects when: the defect is a result of material or workmanship not provided by BioChek BV; 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 only for a period of one year from the date of manufacture. There are no other warranties that extend beyond those described on the face of this warranty;
4. BioChek BV 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 BV;
5. BioChek BV 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 BV reserves the right to replace or allow credit for any modules returned under this warranty.

Regulatory Disclaimers

For veterinary research only. For *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 BV.

4. Revision Index

Version 1: New document
Revision A: Changed kit name, product code, kit content (reagents: name, color of caps, number of vials and volumes), layout product instructions and manufacturer information.

5. Supplier Information

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