AMA AlKhurmah Virus Multiplex qPCR Detection Kit

This product is intended for research use only (RUO)

Made in
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Notice to Laboratories

Since AlKhurmah virus is a relatively new virus, chances of mutation through random evolution are considerable. Therefore, two AlKhurmah virus-specific genomic regions are targeted by this kit, so that if any mutations occur in one of them the other serves as a backup; if such case occurs, please contact our support and/or sales team so that the assay targets are re-evaluated and updated accordingly. We have already designed and validated assays for a number of other conserved regions on the viral genome.

Also, since the closely related KFDV virus shares a high degree of homology to AlKhurmah virus, the third, non-specific target (ALKFDV) in this kit enables laboratories to track both viruses.

Introduction and Principles of the Test

This is a fourplex RT-qPCR based assay for detecting AlKhurmah virus. Two AlKhurmah-specific viral genomics regions are targeted (Propep and NS4A/B), as well as a non-specific target (spanning NS1/NS2) that facilitates differentiation between AlKhurmah vs Kyasanur Forest disease virus (KFDV) infections, which we named ALKFDV target in this kit. The fourth target, the human β2M gene, serves as the internal control (IC) for extraction and PCR reaction conditions. Each sample is evaluated in one well in a 96-well format. So, a full 96 plate can run up to 94 samples (with 1 positive and 1 negative controls) in nearly 1 hour.

Target	ALKFDV (NS1/NS2)	AlKhurmah1 (NS4)	AlKhurmah2 (Propep)	IC (β2M)
Detection Channel	FAM	ROX	Cy5	HEX

By targeting the abundantly expressed human β2M gene as the internal control, instead of using a synthetic RNA spike-in, our kit provides complete control of all steps of the test, starting from sample acquisition and transportation and ending with fluorescent detection. This also reduces RNA extraction turnaround time as well as enabling testing of older or archived samples, to which a synthetic IC was not added.

To minimize the chances of carryover contamination by the viral PCR products, especially for labs with high throughput testing, dT is replaced with dU in the reaction mix and a UNG enzyme is added, which ensures degradation of any PCR product previously amplified by this kit.

Specificity

As of September 2022, there are 23 and 78 published full genomic sequences for AlKhurmah virus and AlKhurmah+KFDV, respectively in the NCBI Virus database. Our oligos target all of these sequences with perfect complementarity. In silico analysis reveals no cross reactivity with any flavivirus as well as a wide variety of other respiratory viruses (see Table1).

Table1: List of pathogens that have no cross-reactivity with our kit as revealed by in silico analysis.

Pathogen Name (NCBI TaxID)			
Flaviviridae (taxid:11050)	Enterovirus EV-A (taxid:138948)	Legionella pneumophila (taxid:446)	SARS-CoV-2 (taxid:2697049)
Enterovirus EV-B (taxid:138949)	Mycobacterium tuberculosis complex (taxid:77643)	Human coronavirus OC43 (taxid:31631)	Enterovirus EV-C (taxid:138950)
Mycobacterium tuberculosis typus humanus (taxid:1773)	Human coronavirus HKU1 (taxid:290028)	Enterovirus EV-D (taxid:138951)	Streptococcus pneumoniae (taxid:1313)
Human coronavirus NL63 (taxid:277944)	Human enterovirus EV68 (taxid:42789)	Streptococcus pyogenes (taxid:1314)	SARS coronavirus (taxid:694009)
Human respiratory syncytial virus (taxid:11250)	Bordetella pertussis (taxid:520)	MERS coronavirus (taxid:1335626)	Human rhinovirus A (taxid:147711)
Bordetella pertussis 18323 (taxid:568706)	Human adenovirus 1 (taxid:10533)	Human rhinovirus B (taxid:147712)	Mycoplasma pneumoniae (taxid:2104)
Human adenovirus 7 (taxid:10519)	Human rhinovirus C (taxid:463676)	Pneumocystis jirovecii (taxid:42068)	Human parainfluenza virus 1 (taxid:12730)
Human rhinovirus C1 (taxid:1219416)	Pneumocystis jirovecii RU7 (taxid:1408657)	Human parainfluenza virus 2 (taxid:1979160)	Human rhinovirus B14 (taxid:12131)
Candida albicans (taxid:5476)	Human parainfluenza virus 3 (taxid:11216)	Human rhinovirus B3 (taxid:44130)	Pseudomonas aeruginosa (taxid:287)
Human parainfluenza virus 4 (taxid:11203)	Human rhinovirus A1 (taxid:573824)	Pseudomonas aeruginosa group (taxid:136841)	Influenza A virus (taxid:11320)
Human rhinovirus NAT001 (taxid:992230)	Staphylococcus epidermis (taxid:1282)	Influenza B virus (taxid:11520)	Chlamydia pneumoniae (taxid:83558)
Streptococcus salivarius (taxid:1304)	Influenza A virus (A/PR 8/34 (H1N1)) (taxid:211044)	Haemophilus influenzae (taxid:727)	Human metapneumovirus (taxid:162145)

Kit Contents and Storage

The kit comes in a package of four tubes, as indicated in the following table. All components must be stored at -20°C. One-Step RT-qPCR Master Mix and Oligo Mix should not be exposed to light for extended periods of time.

Table2: Kit components.

Component	Volume (uL)
One-Step RT-qPCR Master Mix	1,000
AMA Oligo Mix	200
Positive Control	50
Negative Control	50

Protocol

1. Extract RNA or total nucleic acids from each sample, as in your laboratory's approved internal protocol. Nucleic acid should be used immediately after extraction (within 30 minutes) or stored for up to a week at -20°C if stored immediately after extraction

2. Thaw all components at room temperature then place them on ice. Mix each tube by gentle flicking followed by a quick spin down to collect the tube's component to the bottom of the tube. Do NOT exceed 3 freeze-thaw cycles!

3. Prepare the reaction master mix by mixing the following components (5% overage should be included to account for pipetting errors):
   a. **10 µL of One-Step RT-qPCR Master Mix / reaction**
   b. **2 µL of the AMA Oligo Mix / reaction**

*Sample calculation

Component	Per 1 reaction	X10 reactions (+5% overage)
One-Step RT-qPCR Master Mix	10 µL	105 µL
AMA Oligo Mix	2 µL	21 µL

NOTE: Don't forget to figure in the positive and negative samples in your calculations!

4. Transfer 12 µL of the reaction master mix (prepared in step3) into each designated well on a 96-well optical qPCR plate.

5. Add 8 µL of each sample's extracted nucleic acid to its designated well; add 8 µL of the positive and negative controls to their designated wells.

6. Seal the plate with optical cap strips or an optical adhesive seal.

7. On the Real-Time PCR thermal cycle software, define the detection filters as follows:

Target	ALKFDV	AlKhurmah1	AlKhurmah2	IC
Detection Channel	FAM	ROX	Cy5	HEX

8. On the software, define your Positive and Negative Controls.

9. Run the Real-Time PCR thermal cycler for 45 cycles as follows:

Step1: 2 minutes - 25°C (the UNG step)

Step2: 10 minutes - 50°C (the RT step)

Step3: 2 minutes - 95°C (initial polymerase activation)

Step4: 10 seconds - 95°C (denaturation)

Step5: 30 seconds - 60°C (annealing/extension) → DATA COLLECTION

Step6: go to step4 (X44 times)

10. Analyze the results according to your thermal cycler's software. Use your judgement to set/modify the threshold of detection for each filter and/or omit amplification artifacts (e.g. non-sigmoidal amplification curve).

Sample calling

Our assay merely provides Ct values, and interpretation of these values should be in accordance with the international standards. Here, we summarize our recommendations for sample calling in Table3.

It is important to note that acceptable cycle threshold of detection is Ct ≤ 40, which means that Ct > 40 should be neglected.

Table3: A guide for interpretation of the assay results. A (+) sign indicates a Ct ≤ 40 whereas a (–) sign indicates Ct>40 or no detection

	ALKFDV (FAM)	ALKHRMH1 (ROX)	ALKHRMH2 (CY5)	IC (HEX)	INTERPRETATION
CASE 1	+	+	+	+ or –	AlKhurmah infection
CASE 2	-	+	+	+ or –	AlKhurmah infection
CASE 3	+	-	+	+ or –	AlKhurmah infection
CASE 4	+	+	-	+ or –	AlKhurmah infection
CASE 5	-	-	+	+	AlKhurmah infection
CASE 6	-	+	-	+	AlKhurmah infection
CASE 7	+	-	-	+	KFDV infection
CASE 8	-	-	+	-	Presumptive AlKhurmah, retest
CASE 9	-	+	-	-	Presumptive AlKhurmah, retest
CASE 10	+	-	-	-	Presumptive KFDV, retest
CASE 11	-	-	-	+	No infection
CASE 12	-	-	-	-	Sample degraded

Run validity

This assay contains positive and negative controls. The three targets and internal control must all be detected in the positive control; if not, the whole plate is considered invalid. Similarly, the negative control must show detection of the internal control alone; any other result invalidates the whole plate.

Support

For questions, suggestions, or technical support, feel free to contact us by email on: support@havensci.com

Quality control

Each lot is tested against predetermined parameters to ensure consistent performance between all lots.

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AMA SARS – Cov – 2 Multiplex qPCR Detection Assay

Product Manual (PD115)
Version 2.0
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