AMA EBV Real-Time qPCR Detection Kit

Product Manual

Version 2.0

This product is intended for research use only.

Contents

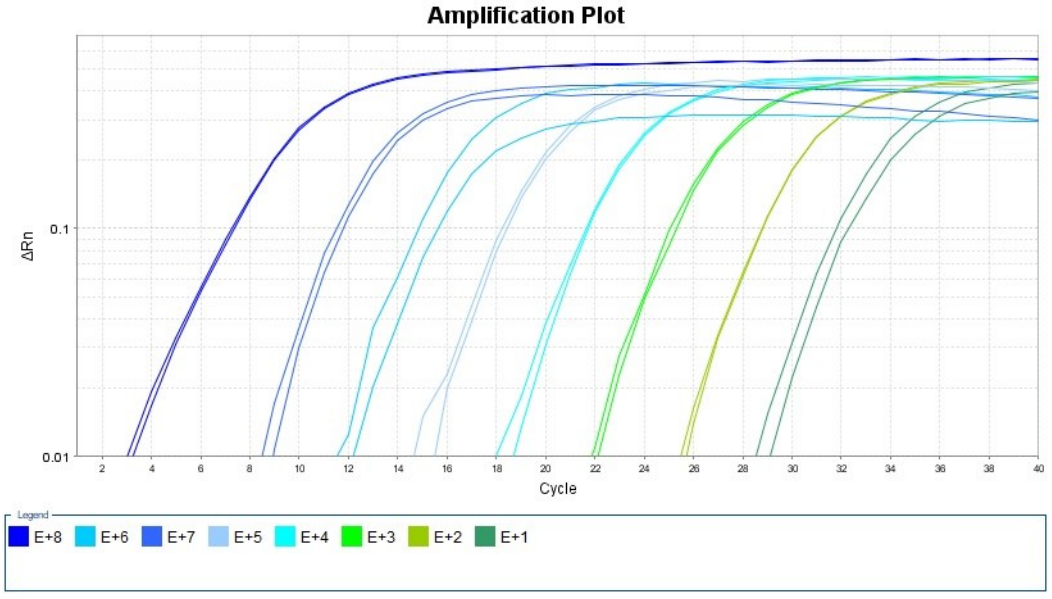
A. Introduction and Principles of the Test

This duplex, probe-based assay is intended for detection of the Epstein-Barr virus in human clinical samples. The assay simultaneously targets 73bp of the seven-repeat region of the viral EBNA1 gene and a 128bp human-specific DNA segment located on chromosome6. Both the EBV and human targets are intergenic, which eliminate the potential contribution of RNA in viral quantification.

Target	EBV (EBNA1)	IC (Human Chr6)
Detection Channel	FAM	HEX

Instead of using a synthetic DNA spike-in to serve as the internal control, our assay targets a human-specific DNA segment. Although synthetic spike-ins are sufficient controls for the extraction, amplification, and detection steps, they do not provide enough information about the state of the original sample during the collection and transportation steps. This could be problematic for degraded samples, which may be incorrectly called negative. By targeting human DNA, this assay overcomes this problem while providing all of the necessary controls that spike-ins provide.

100 million synthetic viral copy equivalents were serially diluted by a factor of 10 all the way down to 10 viral copy equivalents. Our assay showed robust, consistent performance across all dilutions, with PCR efficiency of 97% and R^2=0.999. See the figure below.



In-silico analysis shows that our assay has no cross-reactivity with any of the pathogens listed in the following table:

Pathogen Name (NCBI TaxID)		
Human coronavirus 229E (taxid:11137)	Enterovirus EV-A (taxid:138948)	Legionella pneumophila (taxid:446)
Human coronavirus OC43 (taxid:31631)	Enterovirus EV-B (taxid:138949)	Mycobacterium tuberculosis complex (taxid:77643)
Human coronavirus HKU1 (taxid:290028)	Enterovirus EV-C (taxid:138950)	Mycobacterium tuberculosis typus humanus (taxid:1773)
Human coronavirus NL63 (taxid:277944)	Enterovirus EV-D (taxid:138951)	Streptococcus pneumoniae (taxid:1313)
SARS coronavirus (taxid:694009)	Human enterovirus EV68 (taxid:42789)	Streptococcus pyogenes (taxid:1314)
MERS coronavirus (taxid:1335626)	Human respiratory syncytial virus (taxid:11250)	Bordetella pertussis (taxid:520)
Human adenovirus 1 (taxid:10533)	Human rhinovirus A (taxid:147711)	Bordetella pertussis 18323 (taxid:568706)
Human adenovirus 7 (taxid:10519)	Human rhinovirus B (taxid:147712)	Mycoplasma pneumoniae (taxid:2104)
Human metapneumovirus (taxid:162145)	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)	

B. 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. The qPCR Master Mix and Oligo Mix should not be exposed to light for extended periods of time.

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

C. Protocol

1. Extract DNA or total nucleic acids from each clinical sample, as in your laboratory's approved internal protocol. Nucleic acid should be used immediately after extraction (within 30 minutes) or stored at -20°C 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 a reaction master mix by mixing the following components (5% overage should be included to account for pipetting errors):

    a. *10 µL of qPCR Master Mix / reaction*
    b. *2 µL of the AMA Oligo Mix / reaction*

Example

Component	Per 1 reaction	X10 reactions (+5% overage)
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 controls 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	EBV	IC
Detection Channel	FAM	HEX

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

9. Run the Real-Time PCR thermal cycler for 40 cycles as follows:

Step1:	*3 minutes - 95°C (initial denaturation)*
Step2:	*15 seconds - 95°C (denaturation)*
Step3:	*60 seconds - 60°C (annealing/extension)* → ***DATA COLLECTION***
Step4:	*go to step2 (X39 times)*

10. Analyze the results according to your thermal cycler's software.

D. Data Interpretation

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 the following table, whereas the + sign indicates detection (Ct < 37) and the – sign indicates no detection (Ct ≥ 37):

It is important to note that acceptable cycle threshold of detection is Ct ≤ 37, which means that Ct > 37 should be neglected.

	EBV (FAM)	IC (HEX)	INTERPRETATION
CASE1	+	+/-	**Positive infection**
CASE 2	–	+	**No infection**
CASE 3	–	–	**Sample degraded**

Run validity

For the run to be valid, the Negative Control must return a negative infection result, whereas the Positive Control must return a positive infection result (see sample calling above). If not, the whole run is considered invalid.

E. Support

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

support@havensci.com

F. Quality Control

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