

Primer Design Ltd

Z-Path-EHV-4-std

# Equid alphaherpesvirus 4

**Kit version: 1**

**Target region:**

ORF62-63 intergenic region

genesig<sup>®</sup> Standard Kit

150 tests

For general laboratory and research use only

GENESIG

Kits by Primerdesign

# Introduction to Equid alphaherpesvirus 4

Equid alphaherpesvirus (EHV-4) is a double stranded DNA virus which is responsible for acute febrile respiratory disease in horses upon primary infection and it is characterized by rhinopharyngitis and tracheobronchitis. It is a member of the genus Varicellovirus, which belongs to the subfamily Alphaherpesvirinae.

The genome of Equid alphaherpesvirus 4 is approximately 145kb in length. The linear DNA is composed of two regions, L (Long) and S(Short). The region S is flanked by two inverted repeat regions: an internal repeat sequence (IRS) and a terminal repeat sequence (TRS) which allow the generation of two equimolar isomers. Region L consists of a unique sequence flanked by small, inverted repeats (IRS and TRS).

Transmission occurs by direct or indirect contact with infective nasal discharges, aborted fetuses, and placental fluids. It is notable for its ability to target and attack three separate organ systems of the horse. Primary infection of the virus occurs at the vascular epithelium especially the nasal mucosa and lungs. It gains access to peripheral tissues via cell-associated viremia, which may manifest as abortion or neurologic disease. The infection damages the microvasculature of the central nervous system due to initiation of an inflammatory cascade, vasculitis, microthrombosis and extravasation of mononuclear cells resulting in haemorrhage. EHV-4 can establish latency in infected horses, but reactivation and shedding of the virus are generally less common than with EHV-1.

# Specificity

The genesig® Standard Kit for Equid alphaherpesvirus 4 (EHV-4) is designed for the in vitro quantification of EHV-4 genomes. The kit is designed to have a broad detection profile. Specifically, the primers will detect over 95% of sequences available on the NCBI database at the time of the most recent review.

The dynamics of genetic variation means that new sequence information may become available after the initial design. If you require further information or have a specific question about the detection profile of this kit, then please send an e-mail to [techsupport@primerdesign.co.uk](mailto:techsupport@primerdesign.co.uk) and our team will answer your question.

# Kit contents

- **1x EHV-4 primer/probe mix (150 reactions, BROWN)**  
FAM labelled
- **1x EHV-4 positive control template (RED)**
- **1x RNase/DNase free water (WHITE)**  
for resuspension of primer/probe mixes
- **2x Template preparation buffer (YELLOW)**  
for resuspension of positive control template and standard curve preparation

## Reagents and equipment to be supplied by the user

### Real-time PCR Instrument

#### Extraction kit

This kit is recommended for use with genesig® Easy DNA/RNA extraction kit or exsig®Mag. However, it is designed to work well with all processes that yield high-quality nucleic acid with minimal PCR inhibitors.

#### oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix

This kit is intended for use with oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix.

#### Pipettors and filter tips

#### Vortex and centrifuge

#### 1.5 ml microtubes

#### qPCR plates or reaction tubes

## Kit storage and stability

This kit is stable for shipping at ambient temperature but should be stored at -20°C upon arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

If a standard curve dilution series is prepared this can be stored frozen for an extended period. If you see any degradation in this serial dilution a fresh standard curve can be prepared from the positive control.

Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

## Suitable sample material

This kit can be used with all types of samples from various origins. Please ensure that the extracted nucleic acid samples are suitable in terms of purity, concentration, and DNA integrity.

## Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template. If running a positive control standard curve for a quantitative result, and an efficiency of between 90% to 110% is not achieved, then the run should be repeated with a freshly prepared standard curve.

# Principles of the test

## Real-time PCR

A target specific primer and probe mix is provided, and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan<sup>®</sup> principle. During PCR amplification, forward and reverse primers hybridize to the target DNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled with a 5'-dye and a 3'-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

## Positive control

For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template. This can be used to generate a standard curve of target copy number/Cq value. Alternatively, the positive control can be used at a single dilution where full quantitative analysis of the samples is not required. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

## Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. It is also known as a No Template Control or NTC.

# Resuspension protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting is performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

**1. Pulse-spin each tube in a centrifuge before opening.**

This will ensure lyophilised primer/probe mix or template is in the base of the tube and is not lost upon opening the tube.

**2. Resuspend the kit components in the RNase/DNase free water supplied, according to the table below.**

To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in water	Volume
<b>Pre-PCR pack</b>	
EHV-4 primer/probe mix ( <b>BROWN</b> )	165 µl

**3. Resuspend the positive control template in the template preparation buffer supplied, according to the table below:**

To ensure complete resuspension, vortex the tube thoroughly.

Component - resuspend in template preparation buffer	Volume
<b>Post-PCR heat-sealed foil</b>	
EHV-4 Positive Control Template ( <b>RED</b> )	500 µl

\* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

# qPCR detection protocol

1. For each DNA sample prepare a reaction mix according to the table below:  
Include sufficient reactions for positive and negative controls.

Component	Volume
oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix	10 µl
EHV-4 primer/probe mix ( <b>BROWN</b> )	1 µl
RNase/DNase free water ( <b>WHITE</b> )	4 µl
<b>Final Volume</b>	<b>15 µl</b>

2. Pipette 15µl of this mix into each well according to your qPCR experimental plate set up.
3. Pipette 5µl of DNA template into each well, according to your experimental plate set up.  
For negative control wells use 5µl of RNase/DNase free water (**WHITE**). For positive control wells use 5 µl of the positive control template (**RED**). The final volume in each well is 20µl.



#### 4. (Optional) Standard curve preparation for quantitative analysis.

For quantitative analysis of the samples, a standard curve dilution series can be prepared using the positive control template (**RED**). This is not required for qualitative analysis.

##### 4.1 Reaction mix preparation for the standard curve.

Include sufficient reactions for each dilution of the standard curve.

Component	Volume
oasig <sup>®</sup> lyophilised or PrecisionPLUS <sup>®</sup> 2X qPCR Master Mix	10 µl
EHV-4 primer/probe mix ( <b>BROWN</b> )	1 µl
RNase/DNase free water ( <b>WHITE</b> )	4 µl
<b>Final Volume</b>	<b>15 µl</b>

##### 4.2 Preparation of a 10-fold standard curve dilution series.

- a. Pipette 90µl of template preparation buffer (**YELLOW**) into 5 tubes and label 2-6.
- b. Pipette 10µl of Positive Control Template (**RED**) into tube 2.
- c. Vortex thoroughly.
- d. Change pipette tip and pipette 10µl from tube 2 into tube 3
- e. Vortex thoroughly.

Repeat steps d and e to complete the dilution series.

Standard Curve	Copy Number
Tube 1 Positive control ( <b>RED</b> )	2 x 10 <sup>5</sup> per µl
Tube 2	2 x 10 <sup>4</sup> per µl
Tube 3	2 x 10 <sup>3</sup> per µl
Tube 4	2 x 10 <sup>2</sup> per µl
Tube 5	20 per µl
Tube 6	2 per µl

##### 4.3 Pipette 5µl of standard template into each well for the standard curve according to your experimental plate set up.

The final volume in each well is 20µl.

# qPCR Amplification Protocol

Amplification conditions using oasig<sup>®</sup> lyophilised or PrecisionPLUS<sup>®</sup> 2X qPCR Master Mix.

	<b>Step</b>	<b>Time</b>	<b>Temp</b>
	Enzyme activation	2 min	95 °C
Cycling x50	Denaturation	10 s	95 °C
	DATA COLLECTION *	60 s	60 °C

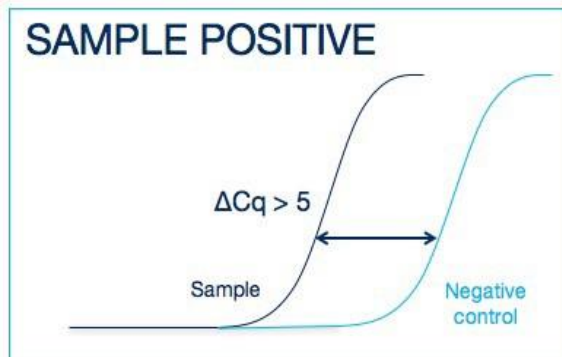
\* Fluorogenic data should be collected during this step through the FAM channel.

# Interpretation of results

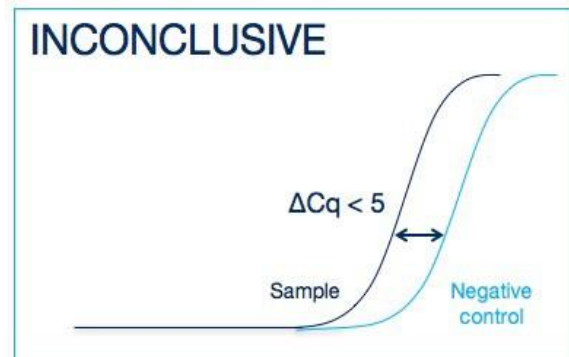
Target	Positive control	Negative control	Interpretation
+	+	-	<b>POSITIVE QUANTITATIVE RESULT</b> calculate copy number
-	+	-	<b>NEGATIVE RESULT</b>
+ / -	+	$\leq 35$	<b>EXPERIMENT FAILED</b> due to test contamination
+ / -	+	$> 35$	*
+ / -	-	+ / -	<b>EXPERIMENT FAILED</b>

Positive control template is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

\*Where the test sample is positive and the negative control is positive with a Cq  $> 35$ , the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies  $> 5$  Cq earlier than the negative control, then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies  $< 5$  Cq earlier than the negative control, then the positive sample result is invalidated, and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

# Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primer Design Ltd genesig® detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applied Biosystems Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc. and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

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