Primerdesign

Z-Path-BTV-v3.0-std

Bluetongue Virus

Kit version: 3

Target region:

Nonstructural protein 3 (NS3) gene

genesig® Standard RNA Kit

150 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Product Description

This genesig® Standard qPCR detection kit targets the nonstructural protein 3 (NS3) gene from bluetongue virus (BTV). Bluetongue virus is an infectious, non-contagious, arthropod-borne viral disease of ruminants such as sheep, cattle, deer, goats and camels. The virus is a member of the genus Orbivirus in the family Reoviridae. There are 24 serotypes of BTV that have been identified worldwide but they do not exist together in any single geographical region. They are closely related to the epizootic haemorrhagic disease group.

BTV is primarily transmitted by midges of the genus Culicoides and fewer than 20 of the 1400 Culicoides species serve as vectors of bluetongue virus. The midges are prevalent in a wide geographical region across the equator and have spread to mainland Europe. Cattle are a major source of infection due to their extended viremia and the host preference by the midges.

The virus has a high affinity for red blood cells, although it can replicate in a number of cell types, and enters the host cells using receptor-mediated endocytosis which is mediated by the VP2 and VP5 proteins. The virus particles reside within these cells in the presence of antibodies thus prolonging viremia. They remain dormant during the winter season and reemerge as soon as the climate turns warm. Whilst the virus is not considered contagious, semen from infected bulls can act as a constant source of infection to cows through natural or artificial insemination.

The incubation period of BTV is usually 5 to 20 days and ruminants are usually infectious to the vector for several weeks. Clinical signs of the infection in sheep include fever, excessive salivation, lameness, depression, panting, oedema and necrosis. Ulcers and swellings in the oral cavity make it difficult for the animal to ingest food. The tongue becomes cyanotic, ‘blue tongue’, due to swelling and protrudes from the mouth. It also causes vascular endothelial damage which results in changes to capillary permeability and intravascular congestion. In cattle, the clinical signs are not apparent and in rare cases they suffer from vesicles or ulcers in the mouth, hyperaemia in the coronary band, cracks in hoofs leading to foot rot and temporary sterility in bulls. Infected cattle and sheep may abort or give birth to calves or lambs with hydranencephaly, porencephaly or cerebral cysts. Infections in goats are usually subclinical, and similar to disease in cattle. Certain species such as white-tailed deer may develop severe haemorrhages which can lead to sudden death.

Specificity

The kit is designed for the in vitro quantification of bluetongue virus (BTV) genomes and to have a broad detection profile. Specifically, the primers will detect over 95% of BTV sequences available on the NCBI database at the time of last review.

This assay is also predicted to detect epizootic haemorrhagic disease virus strains 2 and 6 (EHDV-2 & EHDV-6) which primarily infect wild ruminants such as white-tailed deer.

The dynamics of genetic variation mean that new sequence information may become available after the most recent review. 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 and our team will answer your question.

Kit contents

Quantity	Component	Tube	Cap Colour
1	**BTV v3.0 primer/probe mix (150 reactions)** FAM labelled		**BROWN**
1	**BTV v3.0 positive control template**		**RED**
1	**RNase/DNase free water** for resuspension of the primer/probe mix		**WHITE**
2	**Template preparation buffer** for resuspension of positive control template and standard curve preparation		**YELLOW**

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 OneStep or Precision Plus® OneStep 2X RT-qPCR Master Mix

This kit is intended for use with oasig® lyophilised OneStep or PrecisionPLUS® OneStep 2X RT-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 RNA 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/probe mix is provided, and this can be detected through the FAM channel.

The primer/probe mix provided exploits with the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target cDNA. 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 the 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/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, RNase/DNase-free water should be used instead of the template. A negative result indicates that the reagents have not become contaminated while setting up the run.

Resuspension Protocol

To minimise the risk of contamination with foreign RNA/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 that the 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 allow primer/probe mix to rehydrate for 10 minutes at room temperature. Vortex each tube thoroughly, followed by pipetting up and down 10 times. Failure to mix well can produce poor kit performance.

Component - resuspend in water	Volume
Pre-PCR pack	
BTV v3.0 primer/probe mix (**BROWN**)	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
Post-PCR heat-sealed foil	
BTV v3.0 Positive Control Template (**RED**) *	500 µl

* This component contains a 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.

OneStep RT-qPCR detection protocol

For optimum performance and sensitivity.
All pipetting steps and experimental plate set up should be performed on ice. After the plate is prepared proceed immediately to the OneStep RT-qPCR amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

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

Component	Volume
oasig® OneStep or Precision®PLUS OneStep 2X RT-qPCR Master Mix	10 µl
BTV v3.0 primer/probe mix (**BROWN**)	1 µl
RNase/DNase-free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15 µl of this mix into each well according to your qPCR experimental plate set-up.**

3. **Pipette 5 µl of RNA template into each well, according to your experimental plate set up.**
   For negative control wells use 5 µl 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 20 µl.

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® OneStep or Precision®PLUS OneStep 2X RT-qPCR Master Mix	10 µl
BTV v3.0 primer/probe mix (**BROWN**)	1 µl
RNase/DNase-free water (WHITE)	4 µl
Final Volume	**15 µl**

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

   a. Pipette 90 µl of template preparation buffer (YELLOW) into 5 tubes and label them 2-6. The neat positive control tube (**RED**) is considered tube 1.
   b. Pipette 10 µl of positive control template (**RED**) into tube 2.
   c. Vortex thoroughly.
   d. Change pipette tip and pipette 10 µl from tube 2 into tube 3.
   e. Vortex thoroughly.

   Repeat steps **d** and **e** across the tubes to complete the dilution series.

Standard Curve	Copy Number
Tube 1 Positive control (**RED**)	2 x 10^5 per µl
Tube 2	2 x 10^4 per µl
Tube 3	2 x 10^3 per µl
Tube 4	2 x 10^2 per µl
Tube 5	20 per µl
Tube 6	2 per µl

4.3 Pipette 15 µl of reaction mix and 5 µl of the respective standard into each well for the standard curve according to your plate set up.
The final volume in each well is 20 µl.

OneStep RT-qPCR Amplification Protocol

Amplification conditions using oasig® OneStep or Precision®PLUS OneStep 2X RT-qPCR Master Mix.

	Step	Time	Temp
	Reverse Transcription	10 min	55 °C
	Enzyme activation	2 min	95 °C
Cycling x50	Denaturation	10 s	95 °C
	DATA COLLECTION *	60 s	60 °C

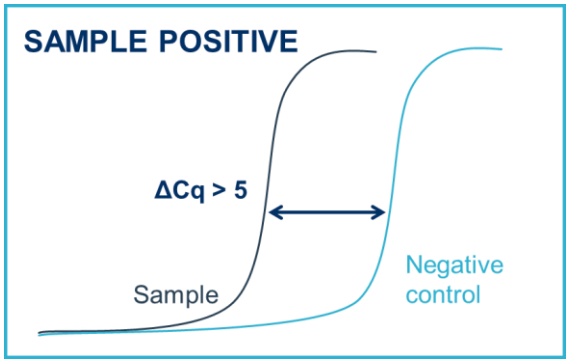
* Fluorogenic data should be collected during this step through the FAM channel

Interpretation of results

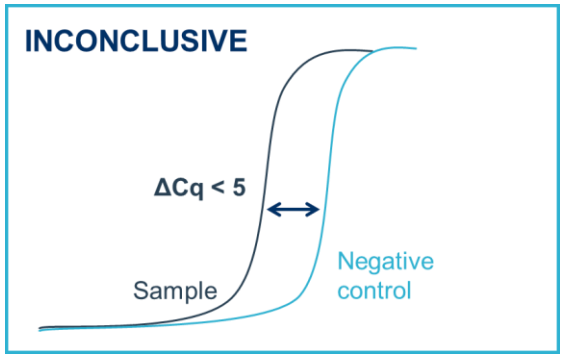
Target	Positive control	Negative control	Interpretation
+	+	-	**POSITIVE QUANTITATIVE RESULT** calculate copy number
-	+	-	**NEGATIVE RESULT**
+ / -	+	≤ 35	**EXPERIMENT FAILED** due to test contamination
+ / -	+	> 35	*
+ / -	-	+ / -	**EXPERIMENT FAILED**

The 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

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