VetqPCR-realtime™ BTV16

BioinGentech
VetqPCR - Real Time™

Bluetongue virus 16 - Real Time

50 / 100 /150 tests (Ready to use kit)

VetqPCR-realtime™ BTV16 Real Time Kit is a screening assay for the detection of Bluetongue virus 16.

Principles of the test:

The Bluetongue virus 16 specific primer and probe mix are provided and this can be detected through the yellow channel.
During PCR amplification, forwar and reverse primers hybridize to the Bluetongue virus 16. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled with a 5-dye Chods ZX™ and 3-quencher kurü Zy™. Internal control consists of a DNA probe labeled with a 5-dye kellú ZZ™ and a 3-quencher kurü Zy™.
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 realtime PCR platforms.

Principle and use:

This amplification kit has been manufactured by Bioingentech Ltd. Chile to detect Bluetongue virus 16 in real time PCR. This is a posibility absolute quantification or qualitative assay.
Real time PCR is based on fluorogenic dyes. Up to 36 Ct should be taken positive. Value between 36-40 Ct should be taken as marginal positive.
This kit needs RNA which can be isolated from blood, serum, faeces, respiratory fluid, digestive system, tissue and others.
See (PU-A003, PU-A004 & PU-A006) included in advanced formats.

Kit Components:

Pulse-spin each tube in a centrifuge before opening.
Completely thaw the components of the kit prior to use.
Homogenize the solutions for 5 seconds prior to pipetting.

Reactions	50 test	100 test	150 test
cDNA Synthesis Premixture	(1 vial)	(1 vial)	(1 vial)
VetqPCR-realtime™ BTV9	(1 vial)	(1 vial)	(1 vial)
Lig™ RNAse Inhibitor Solution	(1 vial)	(1 vial)	(1 vial)
Bio™ Transcriptase Solution	(1 vial)	(1 vial)	(1 vial)
PCR Grade water	(1 vial)	(1 vial)	(1 vial)
BTV16 Positive Control	(1 vial)	(1 vial)	(1 vial)
BTV16 Negative Control	(1 vial)	(1 vial)	(1 vial)
VetqPCR-realtime™ Internal Control	(1 vial)	(1 vial)	(1 vial)

Instrument Compatibility in:

* Ask us for information.

* ABI 7300	* LightCycler 2.0
* ABI 7500FAST	* LightCycler 480
* ABI 7900	* Mastercycler® ep realplex
* AB Step One	* Mx3000P QPCR System
* AB Step One Plus	* Mx3005P QPCR System
* Agilent Mx3005P	* RotorGene 3000
* CFX96 & CFX384	* RotorGene 6000
* ExiCyclerTM 96	* RorotGeneQ
* iQ5 & MyiQ Cycler	* SLAN® Real-Time PCR
* Illumina Eco	* Smartcycles II
* LightCycler Nano	* Applied 7300 and 7500

Procedure:

Please read through the entire procedure before starting.

Step 1

Preparation of First Strand cDNA Synthesis:

1) Prepare the reaction mixture for sample by combining the reagents as shown in the table 1.

Table 1. Components for cDNA synthesis

Kit components	samples
cDNA Synthesis Premixture	4.5 µL
PCR Grade water	4µL
RNA isolated from the sample	2µL

Sample:

2µl RNA (see PU-A004, PU-A005 or PU-A006)
4µl PCR Grade water
4.5µl cDNA Synthesis Premixture

Notes:

• The mineral oil is necessary, even when using a termal cycler that employs a top heating method.

Sample:

A Mineral Oil Drop
2µl RNA (see PU-A004, PU-A005 or PU-A006)
4µl PCR Grade water
4.5µl cDNA Synthesis Premixture

Total: 10,5 µL

• The reactive necessary to cDNA Synthesis should be 10.5µl.

Step 2

2) Place the tubes in a thermal cycler and perform Reverse Transcription according to the program outlined in Table 2.

Table 2. Reverse Transcription

			Temp	Time
Program 1	1 cycle	Initial denaturation	65 ºC	10 min.
	1 cycle	Stop	4 ºC	5 min.

Add 1.0 µl	Lig™ RNAse Inhibitor Solution
Add 1.0 µl	Bio™ Transcriptase Solution

Sample: 

2µL RNA (se PU-A004, PU-A005 or PU-A006)
4µL PCR Grade Water
4,5 µL cDNA Synthesis Premixture
1µL LigTm RNAse Inhibitor Solution
1µL BioTm Transcriptase Solution

Total: 13,5 µL

Then use this program

			Temp	Time
Program 2	1 cycle	Annealing	25 ºC	10 min.
	1 cycle	Extension	37ºC	60 min.
	1 cycle	Denaturation	70 ºC	10 min.

After this program achieved: cDNA from the sample.

Sample: 

cDNA from the sample

Step 3

Table 3. Reaction components for Real Time PCR

	Samples	Positive control	Negative control	Internal control
VetqPCR-realtime™ BTV11	5.5 µL	5.5 µL	5.5 µL	
VetqPCR-realtime™ Internal Control				5.5 µL
PCR Grade water	6µL	6µL	6µL	6µL
cDNA from the sample	2µL			2µL
BTV16 Positive Control		2µL		
BTV16 Negative Control			2µL	
Total Volumen	13.5µL	13.5µL	13.5µL	13.5µL

Visual explanation:

Sample: 

2µl cDNA from the sample
6µl PCR Grade water
5.5µl VetqPCR-realtime™ BTV16

Total: 13,5 µL

Positive Control: 

2µl BTV16 Positive Control
6µl PCR Grade water
5.5µl VetqPCR-realtime™ BTV16

Total: 13,5 µL

Negative Control: 

2µl BTV16 Negative Control
6µl PCR Grade water
5.5µl VetqPCR-realtime™ BTV16

Total: 13,5 µL

Internal Control: 

2µl cDNA from the sample
6µl PCR Grade water
5.5µl VetqPCR-realtime™ Internal Control

Total: 13,5 µL

Observation: homogenize solution 10 seconds.

3) Place the tubes in a Instrument and perform amplification according to the program outlined in Table 4.

Table 4. Real Time cycling parameters

Real Time PCR cycle		Temp.	Time
x 1 cycles	Initial Denaturation	94ºC	2 min.
x 45 cycles	Denaturation	95°C	15 seconds
	Annealing	60°C	60 seconds

Interpretation of the test

1) Qualitative analysis:

Ct (Threshold cycle) value of each sample can be read as follows.

Ct value result

> 40 negative	≤ 40 positive

2) Quantitative analysis:

Table 3. Preparation of standard curve dilution series. BTV16 positive control:

Average Positive Control Concentration	
BTV16	See quality control

Standar curve	Preparation series a fresh dilution	Concentration	Copy Number
Tube N°1:	2uL BTV16 Positive Control (0,3 ng/µl) + 18uL PCR Grade Water	See quality control	See quality control
Tube N°2:	2uL Tube N°1 + 18uL PCR Grade Water	See quality control	See quality control
Tube N°3:	2uL Tube N°2 + 18uL PCR Grade Water	See quality control	See quality control
Tube N°4:	2uL Tube N°3 + 18uL PCR Grade Water	See quality control	See quality control
Tube N°5:	2uL Tube N°4 + 18uL PCR Grade Water	See quality control	See quality control
Tube N°6:	2uL Tube N°5 + 18uL PCR Grade Water	See quality control	See quality control
Tube N°7:	2uL Tube N°6 + 18uL PCR Grade Water	See quality control	See quality control

Homogenize tube.

*** We will send a Quality Control report for each purchase**
**** If you want to obtain 13 DNA copies you must include a new dilution tube (Tube N° 8)**

Table 4. Reaction components for Real Time PCR standar curve.

	Tube A	Tube B	Tube C	Tube D	Tube E	Tube F	Tube G
VetqPCR BTV16	5.5 µl	5.5 µl	5.5 µl	5.5 µl	5.5 µl	5.5 µl	5.5 µl
PCR Grade Water	6.0 µl	6.0 µl	6.0 µl	6.0 µl	6.0 µl	6.0 µl	6.0 µl
Tube N°1	2.0 µl						
Tube N°2		2.0 µl					
Tube N°3			2.0 µl				
Tube N°4				2.0 µl			
Tube N°5					2.0 µl		
Tube N°6						2.0 µl	
Tube N°7							2.0 µl

2.1.-Assess the Ct value when amplification curve of Standard tube 1, 2, 3, 4, 5, 6 passes the threshold line. However four tubes is sufficient for standard curve. (tube1-tube4).

2.2.- Calculate quantitative value to compare with Ct value of unknown samples and curve of Standard tube 1, 2, 3, 4, 5, 6.

3) Test validation:

3.1.- Each Ct value standard should be as follows. Standard 1 > Standard 2 > Standard 3 > Standard 4> Standard 5 > Standard 6.

3.2.- R-value of standard curve should be 0.900~0.999.

3.3.- The standard result should be all negative.

Visual explanation:

Tube A

2.0µl Tube N°1 (Positive Control)
6.0µl PCR Grade Water
5.5µl VetqPCR-realtime™ BTV16

Total: 13,5 µL

Tube B

2.0µl Tube N°2
6.0µl PCR Grade Water
5.5µl VetqPCR-realtime™BTV16

Total: 13,5 µL

Tube C

2.0µl Tube N°3
6.0µl PCR Grade Water
5.5µl VetqPCR-realtime™ BTV16

Total: 13,5 µL

Tube D

2.0µl Tube N°4
6.0µl PCR Grade Water
5.5µl VetqPCR-realtime™ BTV16

Total: 13,5 µL

Tube E

2.0µl Tube N°5
6.0µl PCR Grade Water
5.5µl VetqPCR-realtime™ BTV16

Total: 13,5 µL

Tube F

2.0µl Tube N°6
6.0µl PCR Grade Water
5.5µl VetqPCR-realtime™ BTV16

Total: 13,5 µL

Tube G

2.0µl Tube N°7
6.0µl PCR Grade Water
5.5µl VetqPCR-realtime™ BTV16

Total: 13,5 µL

FAQ:

1.-Positive control:

The positive control assay uses a Chods Zx™ dye and should be detected through the Yellow channel of your real time PCR instrument.
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 Bluetongue virus 16 copy number / CT value. Alternatively the positive control can be used at a single dilution Bluetongue virus 16 on where full quantitative analysis of the sample 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 primer and probes for detecting the target Bluetongue virus 16 gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. sealing all other samples and negative controls before pipetting the positive control into the positive control well tube.

2.-Internal Control:

The internal control assay uses a kellú ZZ™ dye and should be detected through the Green channel of your real time PCR instrument. A positive result through the Green channel therefore indicates that PCR conditions are suitable for detection of the target pathogen gene. If a negative result is obtained through the Green channel the results should be ignored and the test repeated.

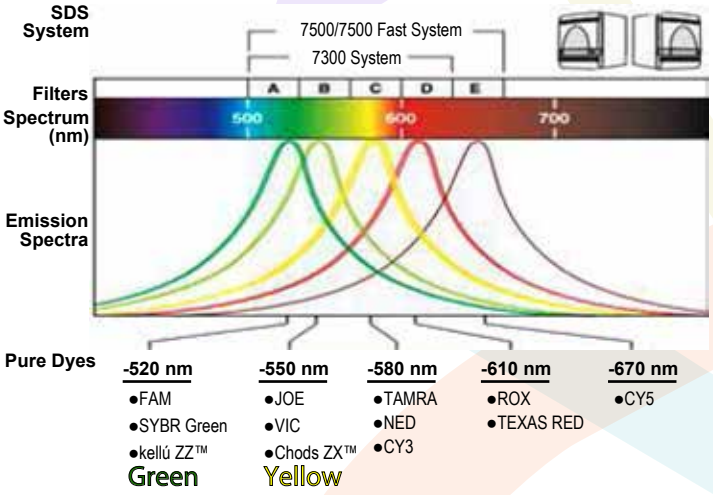
3.-Negative control:

To confirm absence of contamination a negative control reaction should be included every time the kit is used. In this instance the PCR grade water should be used in place of template. A negative result indicates that the reagents have not become contaminated. If a positive result (CT value less than 37) is obtained the results should be ignored and the test repeated.

You must use quencher and reporter dye to setup your software (see table 2) and run the following channel:

Table 7. Report Dye channel selection chart

Channel	Source	Detector	Dyes
Green	470 (Nm)	520 (Nm)	FAM, Sybr green1, Fluorescein, Eva green, Alerxa flour 488, kellú ZZ™
Yellow	530 (Nm)	550 (Nm)	Joe, Vic, Hex, Tet, Cal Fluorgold 540, YaKima Yellow, Chods ZX™
Orange	585 (Nm)	610 (Nm)	Rox, Cal Fluor Red 610, Cy3.5, Texas Red, Alexa Fluor 568
Red	625 (Nm)	660 (Nm)	Cy5, Quasar 670, Lightcycler, Red 640, Alexa Fluor 633.
Crimson	680 (Nm)	710 (Nm)	Quasar 705, Lightcycler Red 705, Alexa Fluor 680



Similarity of our fluorophores with HEX and FAM.

●kellú ZZ™	FAM
●Chods ZX™	HEX

Temperature

All our reagents are made through protein engineering and are stable at room temperature, the label temperature is just a recommendation after the product is open.

	Store temperature	The label temperature
	Shipping temperature	At room temperature

Table 8. Interpretation of Results

Target	Internal Control	Negative Control	Positive Control	Interpretation
+ive	+ive	-ive	+ive	+ive
+ive	-ive	-ive	+ive	+ive
+ive	+ive	+ive	+ive	*
+ive	-ive	+ive	+ive	*
-ive	+ive	-ive or +ive	+ive	-ive
-ive	-ive	-ive or +ive	-ive	Experiment fail
-ive	+ive	-ive or +ive	-ive	Experiment fail

Table 9. Products

Products		Code
Bioingentech - Genomic RNA Purification Kit	50 test	PU-A004
Bioingentech - Genomic RNA Purification Kit	100 test	PU-A005
Bioingentech - Genomic RNA Purification Kit	150 test	PU-A006
VetqPCR-realtime™ BTV16 Bluetongue virus 16 Real Time 50 tests (Ready to use kit) / Cat. No: RTq-V609-50R		
VetqPCR-realtime™ BTV16 Bluetongue virus 16 Real Time 100 tests (Ready to use kit) / Cat. No: RTq-V609-100R		
VetqPCR-realtime™ BTV16 Bluetongue virus 16 Real Time 150 tests (Ready to use kit) / Cat. No: RTq-V609-150R		