Tick-borne encephalitis virus - Real Time RNA

BioinGentech Biotechnology

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

TBEV RNA Real Time PCR Kit is a screening assay for rapid and accurate detection of Tick-borne encephalitis virus.

Principles of the test:

One Step Bioingentech® qRT-PCR Kits provide components for onestep reverse transcription and quantitative PCR (qRT-PCR) in a convenient format that is compatible with both rapid and standard qPCR cycling conditions.
Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and either total RNA or mRNA. These one step qRT-PCR kits have been formulated for use with fluorogenic probe-based 5′ nuclease technology probes.

The One qPCR Enzyme Mix includes Reverse Transcriptase, Recombinant Ribonuclease Inhibitor in an optimized formulation, also include Bioingentech® Taq DNA polymerase and all reagents for an optimized qRTPCR.
The Tick-borne encephalitis virus specific primer and probe mix are provided in the kit and these can be detected through your real time platform by the 5′ nuclease PCR detection method. During PCR amplification, forward and reverse primers hybridize to the Tick-borne encephalitis virus target cDNA generated. Fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled with a 5-reporter FAM and 3-quencher which can be detected through green channel (see Table 8).
To confirm extraction of a valid biological template an Internal control primer and probe mix is included, consists of a DNA probe labeled with a 5-reporter HEX and a 3-quencher which hybridize inside a specific housekeeping endogenous target gene. During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated. As a result, a fluorescence increase can be detected on a range of real time PCR platforms through yellow channel (see Table 8).

Also our RNA Kits include an exogenous source of RNA template that is spiked into the lysis buffer. A separate qPCR primer/probe mix are supplied with this kit to detect an exogenous RNA using qPCR. The PCR primers are present at PCR limiting concentrations which consists of a DNA probe labeled with a 5-dye HEX and a 3-quencher that during PCR amplification a fluorescence increase can be detected through yellow channel of real time PCR platforms. Successful co-purification and qPCR for the control RNA indicates that PCR inhibitors are not present at a high concentration (see Table 8).
Our kits also include Positive and Negative Control which are details in FAQ section.

Principle and use:

This amplification kit has been manufactured by Bioingentech Ltd. Chile to detect Tick-borne encephalitis virus in real time PCR. This is a possibility relative/absolute quantification or qualitative assay. Real time PCR is based on fluorogenic dyes. Ct value between 12 – 36 should be taken positive. Value between 36-40 Ct should be taken as marginal positive. Ct above 40 must be considered as negative (for more details see Table 5).
This kit needs RNA as a template which can be isolated from blood, serum, faeces, respiratory fluid, cerebrospinal fluid, digestive system, tissue, Heptopancreas, Gills, Pleopods, Cloacal, Egg Yolk, Milk, swabs, Lee, bacterial cultures, cell lines and others. We have available detail procedures for RNA extraction from these samples.

Table 1. Kit Components:

Reactions Tubes	50 test	100 test	150 test
Universal qPCR Master Mix	(1 unit)	(1 unit)	(1 unit)
Primer, Probes and Internal Control Mix	(1 unit)	(1 unit)	(1 unit)
One qRTPCR Enzyme Mix	(1 unit)	(1 unit)	(1 unit)
TBEV Positive Control	(1 unit)	(1 unit)	(1 unit)
TBEV Negative Control	(1 unit)	(1 unit)	(1 unit)
PCR grade Water	(1 unit)	(1 unit)	(1 unit)
Exogenous RNA control	(1 unit)	(1 unit)	(1 unit)
Exogenous RNA control Primer and Probes Mix	(1 unit)	(1 unit)	(1 unit)

Table 2. Instrument Compatibility:

* 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.

Before Starting

• Pulse-spin each tube in a centrifuge before opening.
• Homogenize the solutions for 5 seconds prior to pipetting.
• You must consider use different tips in order to avoid cross contamination.
• Use only sterile, RNAses, DNAases and pyrogens free tips.
• Add Exogenous RNA Control to the lysis/binding solution concentrate that is used for the RNA isolation. You must add 2 µL of undiluted Exogenous RNA Control (20,000 copies) per desirable isolation.

Step 1

Prepare a Master mix according to the reaction table 3.

Table 3. Reaction components for PCR

Reaction Tubes	Sample and Internal Control	Positive Control	Negative Control	Exogenous RNA Control
Universal qPCR Master Mix	7,5µL	7,5µL	7,5µL	7,5µL
Primer, Probes and Internal Control Mix	0,6µL	0,6µL	0,6µL	0,6µL
One qRTPCR Enzyme Mix	0,5µL	0,5µL	0,5µL	0,5µL
Exogenous RNA control Primer and Probes Mix				3,5µL
PCR grade Water	2,9µL	2,9µL	2,9µL	2,9µL
RNA Sample	3,5µL			
TBEV Positive Control		3,5 µL		
TBEV Negative Control			3,5µL	
Total Volume	15µL	15µL	15µL	15µL

Step 2

Place the tubes in a thermal cycler and perform One Step qPCR according to the program outlined in Table 2.

Table 4. Six Step PCR Cycling table

	Steps	Time	Temp. (°C)
1 Cycle	Activation Step	2 min	25 °C
	Reverse Transcription	15 min	50 °C
	Denaturation	2 min	95 °C
40 Cycles	Denaturation	30 seg	95 °C
	Annealing	30 seg	60°C
	Extension	30 seg	72 °C
	Hold	----	4 °C

Notes: Hold Step is optional. Measure the fluorescence at the end of the Extension Step.

Interpretation of the test

1) Qualitative analysis:

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

Table 5. Ct value result

Ct value	Result
0 - 11	**Negative**
12 - 36	Positive
36 - 40	Marginal Positive
> 40	**Negative**

* Ct values over 40 are considered Negative result. If Ct value is in a 12 - 36 range, it must be considered as Positive result. This is depending of the sample initial concentration used for each reaction. You should consider that sample real concentration could be modify by the sample purity when it is quantified.
* For more technical information you must request the quality control for each kits. You can request more information by writing to our email kitpcr@bioingentech.com

2) Quantitative analysis:

Table 6. Preparation of standard curve dilution series. Tick-borne encephalitis virus positive control:

Average Positive Control Concentration	
Tick-borne encephalitis virus	See Quality Control

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

Note: Don't forget to homogenize the tubes.

* We will send a Quality Control report for each purchase.
** For reaction mix you must use Universal qPCR Master Mix.
*** If you want to obtain less DNA copies you must include a new dilution tube (Tube N° 8).
Note: Final DNA copy number will depend of the DNA concentration (you can see it in Quality Control Report).

Table 7. Standard curve set up

	Tube A	Tube B	Tube C	Tube D	Tube E	Tube F	Tube G
Universal qPCR Master Mix	7,5µl	7,5µl	7,5µl	7,5µl	7,5µl	7,5µl	7,5µl
Primer, Probes and Internal Control Mix	0,6µl	0,6µl	0,6µl	0,6µl	0,6µl	0,6µl	0,6µl
Enzyme Mix	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl
PCR grade Water	2,4µl	2,4µl	2,4µl	2,4µl	2,4µl	2,4µl	2,4µl
Tube N° 1 (Positive Control)	3,5µl						
Tube N° 2		3,5µl					
Tube N° 3			3,5µl				
Tube N° 4				3,5µl			
Tube N° 5					3,5µl		
Tube N° 6						3,5µl	
Tube N° 7							3,5µl
Total Volume	15µl	15µl	15µl	15µl	15µl	15µl	15µl

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 are 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.
2.3.- When you visualized result in the Real Time PCR platform you must see just one amplification curve for Positive Control. You must not see an Internal Control amplification curve.

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. R-value represent how well the experimental data fit the regression line. A significant difference in observed Ct values between replicates will lower the R-value.
3.3.- The standard curve slope result should be all negative.
3.4.- The desired amplification efficiencies vary from 90% to 110%. The theoretical maximum of 100% indicates that the polymerase enzyme is functioning at its maximum capacity. Low reaction efficiencies may be caused by poor primer design or by suboptimal reaction conditions. Reaction efficiencies >110 may indicate pipetting error in your serial dilutions or coamplification of nonspecific products, such as primer-dimers.

Visual explanation FAQ:

1.-Positive control:

The Positive control assay uses a FAM dye and should be detected through the Green 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 Tick-borne encephalitis virus copy number / Ct value.
Alternatively, the positive control can be used at a single dilution Tick-borne encephalitis virus 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. Particularly, due to amount of this reagent, you should run a positive control for each 12 samples. A positive result indicates that the primer and probes for detecting the target Tick-borne encephalitis virus gene worked properly in that particular experimental scenario. If a negative result is obtained the test results should be invalid and must be repeated (see Table 10). Sealing all other samples and negative controls before pipetting the positive control into the positive control well tube.

2.-Internal Control:

The internal control is included in Primer, Probes and Internal Control Mix along to the target pathogen detection.
In order to interpreted results, read the yellow channel. The internal control assay uses a HEX dye and should be detected through the Yellow channel of your real time PCR instrument and gives a Ct value of 28 (+/-5) depending on the level of sample dilution and concentration. A positive result through the Yellow channel therefore indicates that PCR conditions are suitable for detection of the target pathogen gene. If a negative result is obtained through the Yellow channel the results should be analyzed by combination of result, follow the Table 10 data.

3.-Negative control:

To confirm absence of contamination a negative control reaction should be included every time the kit is used. Particularly, due to amount of this reagent, you should run a negative control for each 12 samples. In this instance the Tick-borne encephalitis virus Negative Control should be used in place of template. A negative result indicates that the reagents have not become contaminated. If a positive result and Ct value less than 36 is obtained, the results should be analyzed and check if a correct amplification curve was obtained. When you obtain a clear amplification curve you should consider repeat your assay due to probably the sample was contaminated (see Table 10).

* Remember: Run a positive control and negative control for each 12 samples. For reaction mix you must use Universal qPCR Master Mix.

4.-Internal RNA control:

This control is an exogenous source of RNA template, of which its cDNA amplification of this control does not interfere with detection of the target cDNA, even at low copy numbers.

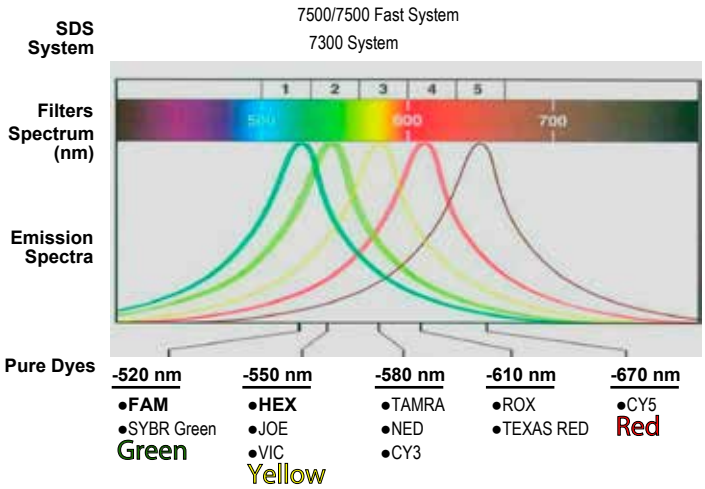
The Internal control is detected through the Green channel and gives a Ct value up to 30 depending on the level of sample dilution. Successful co-purification and qPCR for the control RNA also indicates that PCR inhibitors are not present at a high concentration. To confirm this, Internal RNA control should be included every time the kit is used.

Particularly, due to amount of this reagent, we recommend including and running one Internal RNA control for each 12 samples.

* Exogenous RNA control is just referential and its negative result should not interpreted as a failed assay when the sample and internal control give a positive result (see Table 10).
* In order to setup your software to read the probes, see Table 8 and run the following channels:

Table 8. Fluorogenic probes, Channels and Dyes

Channel	Source	Detector	Dyes
Green	470 (Nm)	520 (Nm)	**FAM**, Sybr green1, Fluorescein, Eva green, Alexa flour 488.
Yellow	530 (Nm)	550 (Nm)	Joe, Vic, **HEX**, Tet, Cal Fluorgold 540, YaKima Yellow.
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



Important Note:

Probes for the sample and controls mentioned in this manual are just referential and the purchased kits might have different ones. This information will be detailed in your Certificate of Analysis upon purchasing one of our PCR Kits.

We can develop special requests for other pathogens or multiplex detection according to the client specifications.

We strongly recommend to not use or combine our products with reagents from another kits or unknown procedence.
We cannot assure good results if incompatibility problems occur.

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.

Table 9. Store Temperature Kits

	Store temperature	The label temperature
	Shipping temperature	At room temperature

Table 10. Interpretation of Results

Sample	I.C	N.C	P.C	eRNA Control	Result
+	+	-	+	+	POSITIVE
+	-	-	+	+	POSITIVE
+	+	-	+	-	POSITIVE
+	-	-	+	-	POSITIVE
+	+	+	+	+	Check Ct and Consider repeat assay*
+	-	+	+	+	Check Ct and Consider repeat assay*
+	+	+	+	-	Check Ct and Consider repeat assay*
+	+	-	-	-	Check Ct and Consider repeat assay*
+	+	+	-	-	Check Ct and Consider repeat assay*
+	+	-	-	+	Exp. Fail
+	+	+	-	+	Exp. Fail
+	-	-	-	-	Exp. Fail
+	-	-	-	+	Exp. Fail
+	-	+	-	-	Exp. Fail
-	+/-	+/-	+/-	+/-	NEGATIVE

* Sometimes amplification curves for Negative or Internal control with Ct < 30 can be seen, however it might not be a Positive result. If the amplification curve is not sigmoid, you should consider it as a Negative result.

Table 11. Products Specifications

Technology	5' nuclease probe based real time PCR assay
Type of nucleic acid Kit	RNA
Kit storage	Shipped at room temperature, the label temperature is just a recommendation after the product is open.
Detection Limit	See Quality Control file. **Request it!**
Sensitivity & Specify	Ct value between 12 – 36 should be taken positive. Value between 36-40 Ct should be taken as marginal positive. Ct values above 40 must be considered as negative.
Controls included	Internal control, Internal RNA extraction control, Positive control and Negative control
Channels	FAM Green channel detects pathogen amplicons. HEX Yellow channel detects internal control amplicons. FAM Green channel detects Positive Control. FAM Green channel detects Exogenous RNA control.