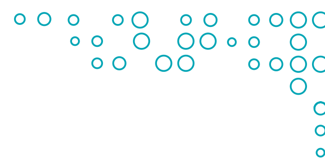
EURx® MOLECULAR BIOLOGY PRODUCTS



Duplex TBEV qRT-PCR Kit

Kit Components

Component	Cat. No. E0461-01 50 reactions of 20 µl	Cat. No. E0461-02 100 reactions of 20 µl
TBEV Buffer Mix brown tube	2 x 360 µl	4 x 360 µl
TBEV Enzyme Mix orange cap	45 µl	90 µl
IC-internal control blue cap	1 x 500 µl	2 x 500 µl
PC-positive control black cap	1 x 100 µl	1 x 200 µl
RNase free water transparent cap	1 x 500 µl	1 x 500 µl

Storage

Store the components at -20°C in the dark.
Repeated thawing and freezing (> 2x) should be avoided, as this may reduce the sensitivity.

Description

- Duplex TBEV qRT-PCR Kit is a ready-to-use system for the detection of TBEV RNA in animal tissue samples (tick, human).
- The kit detects all three TBEV subtypes: Western European, Siberian and Far Eastern subtype.
- Duplex TBEV qRT-PCR Kit contains reagents and primers for the amplification of a region of the TBEV genome. The detection of the TBEV amplicon is based on using the specific FAM-labelled probe.
- In addition, the kit contains a second heterologous amplification system to monitor the RNA isolation procedure and identify possible PCR inhibition simultaneously. The internal control system consists of: primers, the HEX-labelled probe present in TBEV Buffer Mix and the exogenous nucleic acid fragment (IC-internal control). The amplification of the inner control does not interfere with the amplification of the specific TBEV RNA and confirms RNA isolation and/or PCR reaction correctness.
- IC-internal control can be added at the RNA isolation stage (point a) - then it is both a control of the RNA isolation and PCR inhibition, or only at the PCR stage (point b) - to assess the quality of the purified RNA and possible PCR inhibition.

a. Add IC to the lysis buffer or to the mixture of lysis buffer and sample material at a ratio of 0.1 µl per 1 µl elution volume. For example, 5 µl of IC should be added initially using 50 µl elution volume. It is recommended to use carrier RNA when isolating viral RNA. Carrier RNA increases the efficiency of RNA binding to silica membranes in the presence of small amounts of genetic material in the sample and reduces viral RNA degradation.

b. Add 0.5 µl of IC for every 20 µl of PCR reaction.

- TBEV Buffer Mix contains optimized reaction buffer, dNTPs, primers, probes, and the ROX dye while TBEV Enzyme Mix contains smART Reverse Transcriptase and onTaq DNA Polymerase.
- onTaq DNA Polymerase is a modified “hot start” enzyme that is blocked at moderate temperatures and allows room temperature reaction setup.
- ROX passive reference dye included in the TBEV Buffer Mix allows fluorescence normalization on certain cyclers. The use of ROX passive reference dye is necessary for all real-time PCR cyclers from Applied Biosystems and optional for cyclers from Agilent. ROX compensates for variations of fluorescent signal between wells due to slight differences in reaction volume and fluorescence fluctuations. ROX is not involved in PCR reaction and does not interfere with real-time PCR on any instrument.
- RNA isolation from animal tissues should be performed with dedicated, commercially available kits using silica/magnetic beads technology.

This product is developed, designed and sold exclusively for in vitro use only.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Protocol

Preparation of PCR Reaction:

Component	Negative Amplification Control -NAC	Negative Extraction Control-NEC	Positive Extraction Control-PEC	Sample RNA	Positive Control TBEV-TPC
TBEV Buffer Mix	14.2 µl	14.2 µl	14.2 µl	14.2 µl	14.2 µl
TBEV Enzyme Mix	0.8 µl	0.8 µl	0.8 µl	0.8 µl	0.8 µl
IC-internal control (optional)	-	-	-	0.5 µl	0.5 µl
PC-positive control	-	-	-	-	5 µl
Sample of: extracted H_2O/	-	5 µl	-	-	-
extracted IC	-	-	5 µl	-	-
extracted RNA	-	-	-	5 µl	-
RNase free water	5 µl	-	-	-	-
Total volume	20 µl	20 µl	20 µl	20-20.5 µl	20-20.5 µl

Notes:

1. A reaction volume of 20 µl should be used with most real-time cyclers. Other reaction volumes may be used if recommended for a specific instrument.
2. Thaw, gently vortex, and briefly centrifuge all solutions. Keep TBEV Enzyme Mix and TBEV Master Mix on ice.
3. Set up PCR reactions at room temperature according to the table above. The use of Duplex TBEV qRT-PCR Kit allows room temperature reaction setup.
4. Add:

- 5 µl of water (negative amplification control-NAC),
- 5 µl of water purified according to the RNA isolation procedure (negative extraction control-NEC),
- 5 µl of IC purified according to the RNA isolation procedure (positive extraction control-PEC),
- 5 µl of purified RNA (sample RNA),
- 5 µl of PC (positive control, TBEV-TPC).

5. TBEV-TPC gives a signal in the FAM channel only when no IC-internal control was added to the reaction. To obtain a signal in both FAM and HEX channels, add 0.5 µl of IC to the reaction. Increasing the PCR reaction volume for the TBEV-TPC by 0.5 µl to 20.5 µl (when preparing one reaction mix for RNA samples and controls) does not adversely affect the efficiency of the PCR reaction.
6. Centrifuge briefly to settle down the reaction components and remove bubbles. Bubbles interfere with fluorescent detection.
7. Place the samples in the cycler and start the program.
8. Perform data analysis.

Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Reverse transcription Initial denaturation	50°C 95°C	10 min 10 min	1
Denaturation Annealing/Extension	95°C 60°C	15 s 60 s	45
Cooling	4°C	Indefinite	1

Notes:

1. Run PCR reactions using standard ramp rate (standard mode).
2. Fluorescence detection should be set at the end of the extension step and executed using FAM and HEX channels. FAM channel detects TBEV RNA and HEX channel detects the inner control.

Results Interpretation:

Sample type	FAM channel	HEX channel	Result
Negative Amplification Control -NAC	-	-	correct
Negative Extraction Control-NEC	-	-	correct
Positive Extraction Control-PEC	-	+	correct
Positive Control, TBEV-TPC (- IC) (+ IC)	 + +	 - +	correct
1	-	+	negative TBEV$^-$
2	+	+	positive TBEV$^+$
3	+	-	positive TBEV$^+$
4	-	-	incorrect

This product is developed, designed and sold exclusively for in vitro use only.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Notes:

1. A test can be considered valid/correct if:

- negative amplification control-NAC does not give a signal in both FAM and HEX channels,
- negative extraction control-NEC does not give a signal in both FAM and HEX channels,
- positive extraction control (when IC was purified according to the RNA isolation procedure) gives a signal only in the HEX channel,
- positive control, TBEV-TPC gives a signal only in the FAM channel when no IC was added to the reaction or in both FAM and HEX channels when IC was added to the reaction.

2. The sample is positive ($TBEV^+$) and the test is valid if:

- the conditions of point 1 are met,
- the sample gives a signal in the FAM channel regardless of any signal in the HEX channel.

  In some circumstances, when the reaction is strongly positive ($TBEV^+$), the sample may give a weak or no signal in the HEX channel. The reason for this is competition between TBEV and the internal control RNA amplification reactions.

3. The sample is negative ($TBEV^-$) and the test is valid if:

- the conditions of point 1 are met,
- the sample gives a signal only in the HEX channel.

4. The presence of a fluorescence signal in the HEX channel for a given sample indicates that the RNA isolation process and PCR reaction were correct (in the case of adding IC to the RNA isolation process), or only that the PCR reaction was not inhibited (in the case of adding IC to the PCR reaction mixture). Very high C_T values (> 34) in the HEX channel may indicate a partial inhibition of the PCR reaction. In turn, the complete absence of a signal in the HEX channel with the simultaneous lack of a signal in the FAM channel may indicate complete inhibition of the PCR reaction or failure to add RNA to the PCR reaction with the simultaneous addition of IC in the RNA isolation process. In this case, it is recommended to dilute the sample 10 times or repeat the RNA isolation and rerun the PCR reaction.
5. Lack of signal for the TBEV-TPC indicates an error in PCR preparation.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23