For in vitro diagnostic use only.
For professional use only.
Cat No: BS-TF-T-25/BS-TF-T-100

Tropical Fever RT-qPCR Panel

Package Insert

Table 1. Kit Content

Component	Intended Use	25 Reactions	100 Reactions
2X Prime Script Mix	Optimized ready-to-use mix for RT-qPCR assay	2 x 1000 µL	7 x 1250 µL
TF Oligo Mix 1	Specific nucleic acid amplification and detection: **FAM:** Crimean-Congo Hemorrhagic Fever virus (CCHFV) **HEX:** Human (IC-Internal Control)	1 x 125 µL	1 x 500 µL
TF Oligo Mix 2	**FAM:** Dengue virus (DENV)	1 x 125 µL	1 x 500 µL
TF Oligo Mix 3	**FAM:** Ebola virus **ROX:** Hantavirus **CY5:** Mayaro Virus	1 x 125 µL	1 x 500 µL
TF Oligo Mix 4	**FAM:** Rift Valley virus **ROX:** *Trypanosoma cruzi* **CY5:** *Plasmodium* spp.	1 x 125 µL	1 x 500 µL
TF Oligo Mix 5	**FAM:** *Brucella* spp. **ROX:** *Coxiella burnetii* **CY5:** *Burkholderia pseudomallei*	1 x 125 µL	1 x 500 µL
TF Oligo Mix 6	**FAM:** *Salmonella* spp. **HEX:** *Rickettsia* spp. **ROX:** *Leptospira* spp. **CY5:** *Leishmania* spp.	1 x 125 µL	1 x 500 µL
TF Oligo Mix 7	**FAM:** West Nile Virus (WNV) **HEX:** Zika virus (ZIKV) **CY5:** *Streptococcus pneumoniae*	1 x 125 µL	1 x 500 µL
TF Oligo Mix 8	**FAM:** Yellow fever virus **ROX:** Chikungunya virus (CHIKV) **CY5:** Japanese Encephalitis (JE) virus	1 x 125 µL	1 x 500 µL
PC-TF 1-8	Positive Control (PC)	1 x 100 µL	1 x 100 µL
NTC	Negative Control	1 x 1000 µL	1 x 1000 µL

Table 2. Transport Condition, Storage Condition, and Shelf Life of the Components

Component	Transport Condition	Storage Condition*	Shelf Life
2X Prime Script Mix	(-22) – (+8) °C	(-22) – (-18) °C	12 Months
Oligo Mix		(-22) – (-18) °C	
PC-TF 1-8		(+2) – (+8) °C	
NTC		(+2) – (+8) °C	

*Each reagent stored at storage temperature can be used until the expiration date indicated on the tube following the first opening. The kit's expiration date is determined by the expiration date of the reagents.

Table 3. Components Required but Not Included with The Test

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1. Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS) or/and CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad) Real-Time PCR systems
2. Micropipettes and compatible filtered pipette tips (nuclease-free) suitable for transferring 1-10, 10-100, and 100-1000 µL of liquid
3. A centrifuge or Mini-spin
4. Vortex
5. Reaction tubes and caps/films specific to qPCR instruments and compatible with reaction volume
6. Sigmoida Software

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Table 4. Intended Use, Test Principle, and Analytical Specifications

Function	Aid to diagnosis	**Sample Type(s)**	Table 5
Analyte(s)	Table 1	**Nucleic Acid Preparation Method(s)**	Table 5
Qualitative/Quantitative	Qualitative	**Validated PCR Instrument(s)**	Table 3
Test Principle	Reverse Transcription and Real-Time PCR (RT-qPCR)	**Results Interpretation and Reporting**	Automated (Sigmoida software)
Automated/Manual	Manual	**Inclusivity and Exclusivity**	Validated on the reference strains and the field isolates
Intended Users	Professional use	**Limit of Detection (LoD)**	Table 5
Target Population	Individuals with the suspected infection	**Sensitivity and Specificity**	98.55% and 99.21%

Table 5. Collection, Storage, and Transfer of Clinical Specimens / Nucleic Acid Preparation Methods and the Respected LoD Values

Sample Type**	Sample Transfer	Sample Storage	Nucleic Acid Preparation Method	LoD (cp/mL)
Whole blood, serum ve plasma	EDTA-treated blood tube	3 days at (+2) – (+8) °C 1 year at -70 °C	***RINA™ M14 Nucleic Acid Extraction Device*** (Robot Catalog No: RINA-M14-01, Kit Cat. No: RN-NA-101) ***Zybio EXM3000 Nucleic Acid Isolation System*** (Robot Model No: EXM3000, Kit Cat. No: ZFNAE01)	500-1000
Urine	Preservative-free sterile tubes/containers			1000-2000

**Clinical specimens should be collected by a healthcare provider in accordance with national/international clinical specimen collection regulations.

1. RT-qPCR Application Protocol

Before starting the assay, please consider the following:

1. The kit was validated only for the template nucleic acid volume which is 25% of the total qPCR volume.
2. The kit cannot be used with real-time PCR instruments without periodic maintenance records.
3. The kit for Bio-Rad Real-Time PCR systems has been validated with white reaction tubes specific to these systems. Clear reaction tubes result in 5-10 times lower fluorescence signal in Bio-Rad instruments compared to white reaction tubes. In addition, device-specific reaction tubes should be used in the BMS device. The kit's stated analytical performance can only be achieved using validated tubes.
4. To test for contamination, a negative control reaction containing NTC (Nuclease-free Water) must be set up in each run.

Program the qPCR device as follows and add the reagents into the qPCR tubes, close the tubes, place them into the qPCR instrument and start the run. (Table 6)

Table 6. RT-qPCR Program Details

Reaction Setup		RT-qPCR Program				QR Code for Thermal Protocol and Plate Setup
		CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx (Bio-Rad) **and** ***Magnetic Induction Cycler (Mic) (Bio Molecular System - BMS)***				
Reagent	**Volume/Rxn**	**Step**	**Cycle No.**	**Temperature**	**Duration**	
2X Prime Script Mix	10 µL	**Enzyme Activation**	1 Cycle	52 °C	3 min	
		Pre-Incubation	1 Cycle	95 °C	10 sec	
Oligo Mix	5 µL	**Denaturation**	12 Touchdown Cycles: 1 °C decrement in annealing temperature per cycle	95 °C	1 sec	
		Annealing and Extension		67 °C to 56 °C	15 sec	
Template Nucleic Acid/NTC/PC	5 µL	**Denaturation**	30 Cycles	95 °C	1 sec	
		Annealing and Extension		55 °C	15 sec	
Total Reaction Volume	**20 µL**	**Detection (Reading)**		(FAM-Green)/(HEX-Yellow)/(ROX-Orange)/(CY5-Red)		https://www.bioeksen.com.tr/files/L_TD_43P

WARNING: The RT-qPCR thermal programs (Bio-Rad and BMS-Mic) and the plate setup (Bio-Rad) file should be downloaded from the QR code or link above.

2. Interpretation of the Assay Results Using The "Sigmoida" Software

The data produced by the instruments must be evaluated and reported using the Sigmoida software. The result files opened with the "Sigmoida" software will be analyzed automatically. Below are examples of results that can be achieved with the Sigmoida software:

Negative: The sample tested is negative for the tested agent.
Positive: The sample tested is positive for the tested agent.
Contamination: Repeat the analysis paying attention to the "Warnings and Limitations" section.
Invalid: Sampling isn't successfully done, or there is a problem during the sample transportation. A new sample from the same patient should be collected and tested again.
Reagent Problem: Test the "PCs" (refer to Table 1) provided with the kit setting up the PC reactions as shown in Table 6. If the test result is positive, the run is valid. In case the software generates a "Reagent Problem" again, contact the manufacturer.

3. Warnings and Limitations

1. False-negative results may occur if inadequate numbers (lower than the LoD) of organisms are present in the specimen.
2. Mutations within the target regions could affect primer and/or probe binding, resulting in failure to detect the presence of agents.
3. A false-negative result may occur if a specimen is improperly collected, transported, or handled.
4. The clinical specimens shall be collected by a healthcare provider in accordance with the specimen collection guidelines.
5. Test procedures should be performed by personnel trained in the use of the kit.
6. Except for liquid transfers, sample tubes should always be kept closed.
7. Filtered and nuclease-free pipette tips should be used for sample transfer.
8. The components in the kit should not be used together with different lot numbers or chemicals of the same name but from different manufacturers.
9. ***The caps of the reaction tubes must not be opened after the PCR run.*** The PCR tubes should be placed in a bag and thrown away after the bag is tightly closed.
10. The surfaces of the workbenches should be wiped with freshly diluted 10% bleach (0.5% NaClO) at the beginning and end of each day.
11. Disposal of waste must be carried out in accordance with local, state, and federal regulations.

4. Explanation of Symbol

Symbol	Title of Symbol	Symbol	Title of Symbol	Symbol	Title of Symbol
CE	European Conformity CE Mark	LOT	Batch code		Keep away from sunlight
IVD	*In vitro* diagnostic *medical device*	REF	Catalogue number		Protect from heat and radioactive sources
	Manufacturer	NON STERILE	Non-*sterile*		Do not use if package is damaged and consult *instructions for use*
	Use-by date		Consult *instructions for use* or consult electronic *instructions for use*		Keep dry
CONTROL -	Negative control		Caution		Keep upright
CONTROL +	Positive control		Temperature limit	Σ	Contains sufficient for *<n>* tests
CONTROL	Control				

5. Manufacturer and Technical Support

Bioeksen AR GE Teknolojileri A.Ş
Address: Huzur Mah. Metin Oktay Cad. Nurol Life Sitesi D Blok No:3/31, 34396 Sarıyer/İstanbul-TÜRKİYE
Phone: +90 (212) 285 10 17, **Fax:** +90 (212) 285 10 18
Web: www.bioeksen.com.tr, **e-mail:** info@bioeksen.com.tr,
Technical Support: support@bioeksen.com.tr

Notice to User: Please inform us about product-related incidents at "**vigilance@bioeksen.com.tr**" within 24 hours.