



**Instruction for use**

**Kit for qualitative detection of  
Chikungunya, Dengue and Zika viruses RNA  
by Real-Time RT-PCR**

**Tropic-test**

## 1) Manufacturer

TestGene LLC, www.testgen.ru

## 2) Assignment

The kit is assigned for qualitative detection of Chikungunya, Dengue (1-4 serotypes) and Zika viruses RNA by Real-Time RT-PCR in a sample of nucleic acids isolated from human blood plasma or in a sample of plasma (without RNA-extraction) in patients with suspected infection with Chikungunya, Zika, and Dengue virus.

Type of analyzed sample: RNA samples extracted from blood plasma of patients only for configuration 1. Blood plasma of patients for configuration 2.

Analyzed targets are listed in table 1.

Table 1 – Analyzed targets

Fluorophore			
FAM / Green	ROX/Orange	Cy5/Red	HEX / Yellow
Dengue virus RNA	Zika virus RNA	Chikungunya virus RNA	IC (internal control)

Total time of PCR is 80–100 minutes depending of type of thermocycler (without preanalytical phase).

## 3) Reagent Kit Composition

### Configurations

#### a) Configuration 1

Composition: Reagent kit for RT-PCR «Tropic-test-Classic».

#### b) Configuration 2 (does not require extraction of RNA from biological material)

Composition: Reagent kit for RT-PCR «Tropic-test-Cito».

## 4) Description of reagents

The kit is intended for 96 reactions that correspond to analyzing of 94 samples, PTC, NTC in the case of parallel analyses of all samples. In the case of analysis of 1 sample per time, there 32 reactions could be analyzed (with PTC, NTC in each run).

The kit is validated on thermocyclers CFX96 (BioRad), QuantStudio 5 (Thermo Fisher Scientific), and Rotor-Gene Q (Qiagen). Kit composition is described in table 2 and table 3.

Table 2 – Reagent kit composition for configuration 1 «Tropic-test-Classic»

#	Reagent	Quantity and Volume
1.	RT-PCR-buffer 5x	1 tube, 480 ul
2.	Oligos mix	1 tube, 480 ul
3.	PTC (Positive Template Control)	1 tube, 480 ul
4.	NTC (Negative Template Control)	2 tubes, 1600 ul each
5.	IC (Internal Control)	1 tube, 940 ul

Table 3 – Reagent kit composition for configuration 2 «Tropic-test-Cito»

#	Reagent	Quantity and Volume
1.	RT-PCR-buffer-Cito 5x	1 tube, 480 ul
2.	Oligos mix	1 tube, 480 ul
3.	PTC (Positive Template Control)	1 tube, 480 ul
4.	NTC (Negative Template Control)	1 tube, 480 ul
5.	IC (Internal Control)	1 tube, 940 ul

## 5) Storage and transportation

### Storage

At the temperatures from or  $-18\text{ }^{\circ}\text{C}$  to  $-22\text{ }^{\circ}\text{C}$ . Storage and transportation at temperatures from  $+2\text{ }^{\circ}\text{C}$  to  $+6\text{ }^{\circ}\text{C}$  are allowed during 30 days. It is allowed to freeze/thaw the kit no more than 10 times.

## 6) Clinical samples

**Type of sample:** RNA samples extracted from blood plasma of patients only for configuration 1. Blood plasma of patients only for configuration 2.

**Taking peripheral blood:** in a test tube (vacuum tube) containing an EDTA solution, with a volume of 4- or 6-ml. Invert the tube 3-4 times immediately after taking blood to change the blood with EDTA. The use of heparin as an anticoagulant is strictly prohibited.

**Transportation and storage whole blood:** no more than 6 hours at a temperature from +2 °C to +8 °C; no more than 2 hours at room temperature.

Within 2 hours (when stored at room temperature) or 6 hours (when stored at a temperature from +2 °C to +8 °C) after taking the material, plasma should be taken, for which the blood tube is centrifuged at 800-1600 g for 20 minutes at room temperature. After centrifugation, the upper fraction (plasma) should be transferred to separate plastic tubes with a volume of 1.5 or 2.0 ml, free of DNase and RNase.

**Transportation and storage blood plasma:** it is allowed to store plasma at a temperature of +2 °C to +8 °C for up to 5 days, at a temperature of -18 °C to -22 °C for up to 3 months, at a temperature of -70 °C for a long time.

**RNA extraction (only for configuration 1):** according to instruction of the kit for nucleic acid extraction; the recommended kit: kit for RNA Extraction from Clinical Material «NA-Extra» (manufacturer: TestGen LLC).

**Important!** NTC (Negative Template Control) are also going through RNA-extraction only for configuration 1. Before extraction, 10 ul of IC (Internal Control) should be added to 100 ul of blood plasma.

## **7) Preparing of the kit components for configuration 1 «Tropic-test-Classic»**

Before PCR thoroughly mix the mixtures of the tubes with the extracted RNA, NTC (Negative Template Control), oligos mix, RT-PCR buffer 5x, PTC (Positive Template Control), turning each tube 10 times or vortexing at low speed for 3–5 seconds, and then precipitate drops from the lids of the tubes by short centrifugation.

It could be noted that the volumes of reagents for 1 reaction: RT-PCR buffer 5x – 5 ul, oligos mix – 5 ul, sample (examined RNA samples, PTC, NTC) – 15 ul. Total reaction volume is 25 ul.

### **PCR protocol**

- a) prepare the necessary number of PCR-tubes using the formula:  
1 x examined RNA samples<sup>1</sup> + 1 x PTC + 1 x NTC.
- b) mark the tubes;

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<sup>1</sup> To improve accuracy, it is recommended to analyze each sample in duplicate.

- c) using separate 1.5 ml tube prepare PCR-mix using the formula and components:  $(n+3) \times 5$  ul RT-PCR buffer 5x and  $(n+3) \times 5$  ul oligos mix, where n is a number of examined samples. Thoroughly mix the mixture using vortex for 3–5 s;
- d) add 10 ul of PCR-mix into all prepared PCR-tubes;
- e) add 15 ul of analyzed RNA template to corresponding tubes (not to tubes for PTC, NCT);
- f) add 15 ul of PTC to corresponding tube;
- g) add 15 ul of NTC (after DNA extraction without IC) to corresponding tube;
- h) place all tubes to thermocycler;
- i) use the amplification program of table 4; type of the analyses: quantitative with standards;
- j) launch PCR, after finishing of it start interpretation.

Table 4 – PCR protocol

Stage	Temperature, °C	Time, minutes:seconds	Detection	Number of cycles
1	52	25:00	–	–
2	95	02:00	–	–
3	95	00:15	–	5
	64	00:20		
4	95	00:15	FAM/Green, ROX/Orange, Cy5/Red, HEX/Yellow	45
	64	00:20		

### 8) Preparing of the kit components for configuration 2 «Tropic-test-Cito»

Before PCR thoroughly mix the mixtures of the tubes with the blood plasma, NTC (Negative Template Control), oligos mix, RT-PCR buffer-Cito 5x, IC (Internal Control), PTC (Positive Template Control), turning each tube 10 times or vortexing at low speed for 3–5 seconds, and then precipitate drops from the lids of the tubes by short centrifugation.

It could be noted that the volumes of reagents for 1 reaction: RT-PCR buffer-Cito 5x – 5 ul, oligos mix – 5 ul, sample blood plasma – 5 ul, IC (Internal Control) – 10 ul, PTC, NTC (without IC) – 15 ul. Total reaction volume is 25 ul.

## **PCR protocol**

- a) prepare the necessary number of PCR-tubes using the formula:  
 $1 \times \text{examined blood plasma samples}^2 + 1 \times \text{PTC} + 1 \times \text{NTC}$ .
- b) mark the tubes;
- c) using separate 1.5 ml tube prepare PCR-mix using the formula and components:  $(n+3) \times 5 \text{ ul RT-PCR buffer-Cito } 5x$  and  $(n+3) \times 5 \text{ ul oligos mix}$ , where n is a number of examined samples. Thoroughly mix the mixture using vortex for 3–5 s;
- d) add 10 ul of PCR-mix into all prepared PCR-tubes;
- e) add 5 ul of analyzed blood plasma template and 10 ul IC (Internal Control) to corresponding tubes (not to tubes for PTC, NCT);
- f) add 15 ul of PTC to corresponding tube;
- g) add 15 ul of NTC to corresponding tube;
- h) place all tubes to thermocycler;
- i) use the amplification program of table 4; type of the analyses: quantitative with standards;
- j) launch PCR, after finishing of it start interpretation.

## **9) Interpretation of results**

Before interpretation the threshold should be defined at the level of 10–20 % of a maximum fluorescence of PTC.

In a case of using Rotor-Gene 6000, Rotor-Gene 3000 and Rotor-Gene Q (Qiagen) thermocyclers, use the functions Dynamic Tube, Noise slope correction, set 10% Outlier Removal.

In a case of using CFX96 (BioRad) thermocycler, for correct baseline correction choose from 5 to 45 or 10 to 45 Cycles to Analyze.

At the beginning the results in PTC and NTC are analyzed, where Ct values should be as in table 5.

If the results for NTC are different from those at the table 5, all results are regarded as doubtful and possible contamination could be found. In this case it is necessary to carry out measures to eliminate possible contamination and repeat analysis.

If the results for PTC are different from those at the table 5, it is required to repeat the analysis. When re-obtaining values for PTC differ from those indicated in table 5 again, it is necessary to use another reagents.

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<sup>2</sup> To improve accuracy, it is recommended to analyze each sample in duplicate.

Table 5 – Correct results for PTC and NTC

Sample	Channels (fluorophores)			
	FAM / Green (Dengue virus RNA)	ROX/Orange (Zika virus RNA)	Cy5/Red (Chikungunya virus RNA)	HEX / Yellow (IC)
NTC	> 35 or absent	> 35 or absent	> 35 or absent	> 35 or absent
PTC	Ct ≤ 32	Ct ≤ 32	Ct ≤ 32	Ct ≤ 32

**NOTE:** The Ct values  $\leq 35$  are taken into account. If a Ct value  $> 35$  is obtained for analyzed samples (with a Ct value of IC  $\leq 32$ ), the result is considered as doubtful.

**NOTE:** Invalid result could be obtained because of inhibitors presented in sample or incorrectly conducted analysis. Doubtful or negative results could be obtained because of low concentration of RNA in a sample. In the cases of doubtful or invalid results, it is necessary to repeat analysis starting from material sampling. It is recommended for doubtful results to use large volume of blood plasma for RNA extraction.

Interpretation of results see table 6.

Table 6 – Interpretation of results

Channels (fluorophores)				Indicated Result
FAM / Green (Dengue virus RNA)	ROX/Orange (Zika virus RNA)	Cy5/Red (Chikungunya virus RNA)	HEX / Yellow (IC)	
-	-	-	Ct ≤ 32	Dengue, Zika, Chikungunya viruses RNA <b>not detected</b>
Ct > 35 or absent			Ct > 32 or absent	Invalid result
Ct ≤ 35			n/a	Virus RNA <b>detected</b> corresponding to the channel
Ct > 35			Ct ≤ 32	The result is doubtful for the target corresponding to the channel

Note: “n/a” – the value is not taken into account.

### 10) Analytical efficiency

**Analytical specificity:** Dengue (1–4 serotypes), Zika and Chikungunya viruses.

**Shown absence of cross-reactivity in vitro with:** human immunodeficiency virus 1 (HIV-1), human immunodeficiency virus 2 (HIV-2), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), *Plasmodium falciparum*; *in silico* with: *Bartonella quintana*, *B. henselae*, *Borrelia bisetti*, *B. garinii*, *B. japonica*, *B. spielmanii*, *Coxiella burnetii*, *Dobrava-Belgrade orthohantavirus*, Japanese Encephalitis virus, *Leptospira interrogans*, *L. kirshneri*, *L. borgpetersenii*, Puumala orthohantavirus, *Rickettsia conorii*, *R. hejlonjiangensis*, Tick Borne Encephalitis Virus (TBEV), *Treponema pallidum*, *Trypanosoma cruzi*, West Nile virus, and Yellow Fever virus.

**Analytical sensitivity:** Configuration form 1: at least 500 copies of Dengue, Zika, Chikungunya virus RNA per 1 ml of clinical material.

Configuration form 2: at least 1,000 copies of Dengue, Zika, Chikungunya virus RNA per 1 ml of clinical material.