



## Chikungunya Virus (CHKV) Real Time RT-PCR Kit

Product Catalogue No.: BP805

Storage temperature: -15°C to -25°C

**For In Vitro Diagnostic Use for Research and Investigational Use Only**

### **1. Intended Use**

Biocan Chikungunya Virus (CKV) Real Time RT-PCR Kit is used for detection of Chikungunya virus in serum, plasma and mosquito sample by using Real Time PCR system.

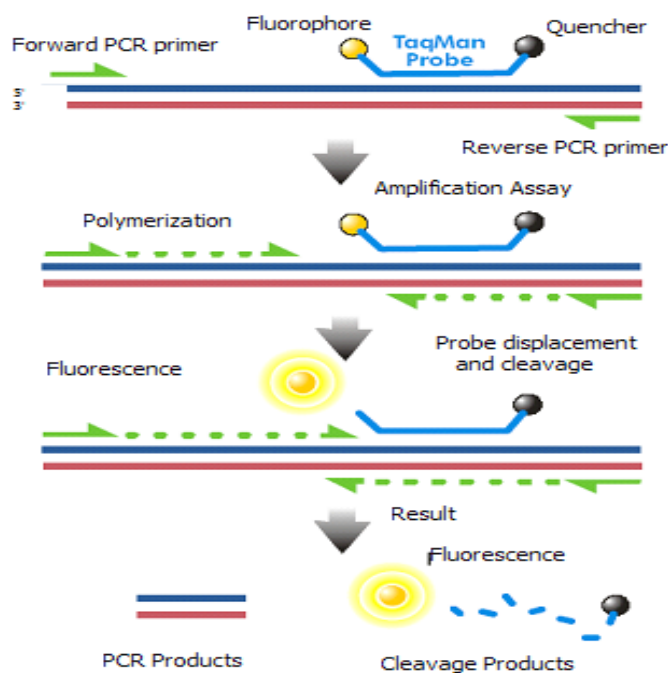
### **2. Background**

Chikungunya virus (CKV) is a member of the *Alphavirus* genus of the family *Togaviridae*. The alphaviruses consist of 30 species of arthropod-borne viruses, which are further subgrouped into seven serocomplexes based on serological data (Porterfield, 1980; Strauss & Strauss, 1994; Van Regenmortel *et al.*, 2000). CHIK was first isolated from the serum of a febrile patient during a dengue epidemic that occurred in the Newala district, Tanzania, in 1953 (Ross, 1956).

CKV is an important human pathogen that causes a disease syndrome characterized by fever, headache, rash, nausea, vomiting, myalgia and arthralgia. Its transmission to humans is mainly through *Aedes* species mosquito.

### **3. Principle of the Assay**

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after amplification.



#### 4. Product Description

The chikungunya real time RT-PCR kit contains a specific ready-to-use system for the detection of the chikungunya virus (for genotype I~IV) using RT-PCR (Reverse Transcription Polymerase Chain Reaction) in the real-time PCR system. The master contains a Super Mix for the specific amplification of the chikungunya virus RNA. The reaction is done in one step real time RT-PCR. The first step is a reverse transcription (RT), during which the dengue virus RNA is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by means of PCR (polymerase chain reaction). Fluorescence is emitted and measured by the real time systems' optical unit during the PCR. The detection of amplified chikungunya virus DNA fragment is performed in fluorimeter **channel FAM** with the fluorescent quencher BHQ1. In addition, the kit contains a system to identify possible PCR inhibition by measuring the HEX/VIC/JOE fluorescence of the internal control (IC). An external positive control defined as  $1 \times 10^7$  copies/ml is supplied which allow the determination of the gene load.

#### 5. Kit Components

Ref.	Reagent	Number of vials	Volume
1	CHKV Super Mix	1	480µl
2	RT-PCR Enzyme Mix	1	28µl
3	Internal Control (IC)	1	30µl
4	CHKV Positive Control ( $1 \times 10^7$ copies/ml)	1	30µl
5	Molecular Grade Water	1	400µl

## 6. Kit Storage

- Biocan RT-PCR Kit is shipped on dry ice and contents of the kit should arrive frozen.
- All the Reagents should be stored between -15°C to -25°C.
- Repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity of the assay.
- Reagents must be kept cool during the assay.
- DFV Super Mix should be stored in the dark.

## 7. Additional Devices and Materials Required but not provided:

- Compatible Real Time PCR System:
  - *ABI Prism 7000/7300/7500/7900,*
  - *Step One Plus, iCycler iQ 4/ iQ 5, Smart Cycler II,*
  - *Bio-Rad CFX 96, Rotor gene 6000, Mx3000P,*
  - *MJ-Option2/Chromo4*
- Biological Cabinet
- Microcentrifuge with at least 13000 RPM and 1.5ml – 2ml tube capacity.
- Vortex Mixer
- Real time PCR reaction tubes or appropriate 96 well reaction plates with optical adhesive tape
- Pipettes (1ul- 1000ul) and Sterile Filter tips
- Disposable powder free gloves
- Biohazard Waste Container
- Refrigerator and Freezer
- Tube racks
- Cryo-Container
- RNA Extraction Kit

## 8. General Consideration and Precaution

- For in vitro diagnostic use only.
- Read the Instructions for Use carefully before using the kit.
- Lab Personnel must be familiar with the protocol and instruments used.
- Maintain separate areas, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips, gowns and gloves) for assay reagent setup, handling of extracted nucleic acids, Real-time RT-PCR amplification.
- Workflow must always proceed unidirectional from the RNA extraction/reagent preparation area to the PCR amplification room in order to avoid contamination of clinical samples with amplified nucleic acids.
- Wear clean, previously unworn, disposable gowns and new, powder-free gloves during assay reagent setup and handling of extracted nucleic acids. Change gloves whenever you suspect they may be contaminated.
- Store primer/probes and enzyme master mix at appropriate temperatures (see package inserts). Do not use reagents beyond their expiration dates.
- Keep reagent tubes and reactions capped as much as possible.
- Use DNase/RNase-free disposable aerosol barrier (filter) pipette tips only.
- Use alcohol and 10% aqueous bleach to clean the working area
- Do not bring extracted nucleic acid or PCR-amplified material into the assay setup area.
- Do not pipette by mouth. Do not eat, drink, and smoke in laboratory.

## 9. Sample Collection, Storage and Transportation

- Collect samples in sterile tubes
- Specimens can be extracted immediately or frozen at -20 C to -80 C
- Transportation of clinical specimens must comply with local regulation for the transportation of etiologic agents.

## 10. Procedure

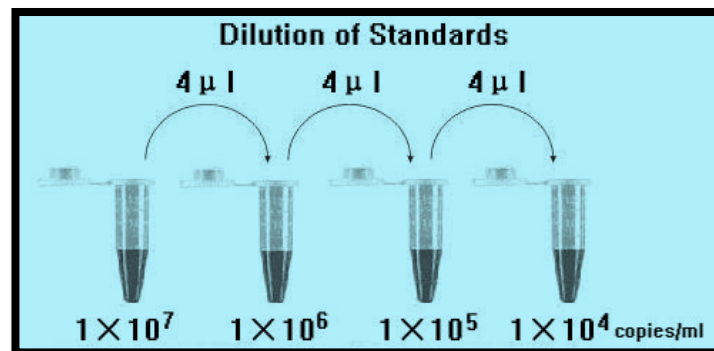
### 10.1 RNA Extraction

- The quality of the extracted RNA has a profound impact on the performance of the entire experiment. It has to be ensured that the method used for nucleic acid extraction is compatible with real-time PCR technology.
- RNA extraction kits are available from various manufacturers. You may use your own extraction method or commercial kits based on the yield.
- The recommended extraction kit is as follows:

Nucleic Acid extraction Kit	Catalogue #	Manufacturer
Biocan Viral RNA extraction kit	BR501	Biocan
QIAamp Viral RNA Mini extraction Kit	52904 (50 extractions)	QIAGEN

### 10.2 Quantitative and Qualitative Assay

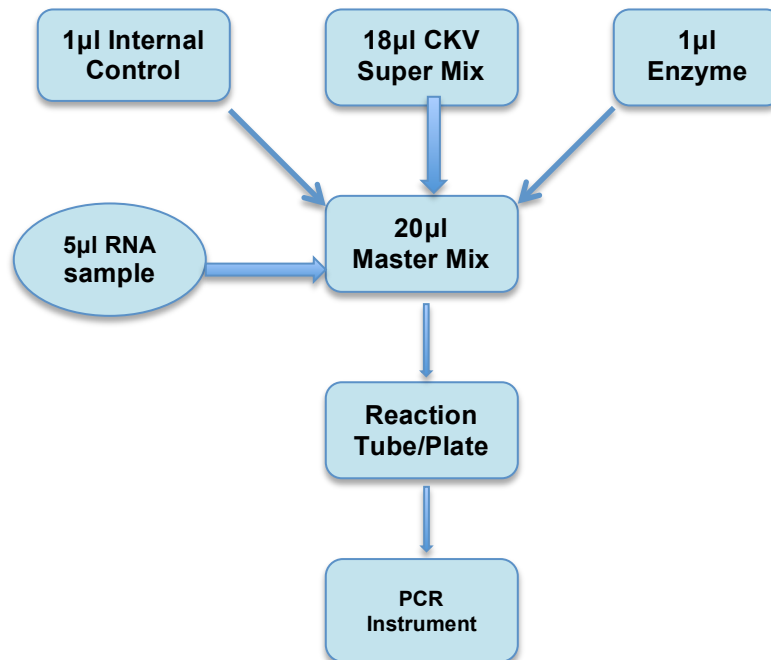
- The Biocan RT-PCR kit is designed for both Quantitative and Qualitative real time PCR.
- For Quantitative real time RT-PCR, standard dilution with Molecular grade water must be performed.
- Dilution is not needed for Qualitative real time RT-PCR.
- Take positive control ( $1 \times 10^7$  copies/ml) as the starting high standard in the first tube. Respectively pipette **36 $\mu$ l** of Molecular Grade Water into next three tubes. Do three dilutions as the following figure:



### 10.3 PCR Master Mix and Reaction Setup

*Note: All the reagents must be thawed before use and kept cool during the experiment.*

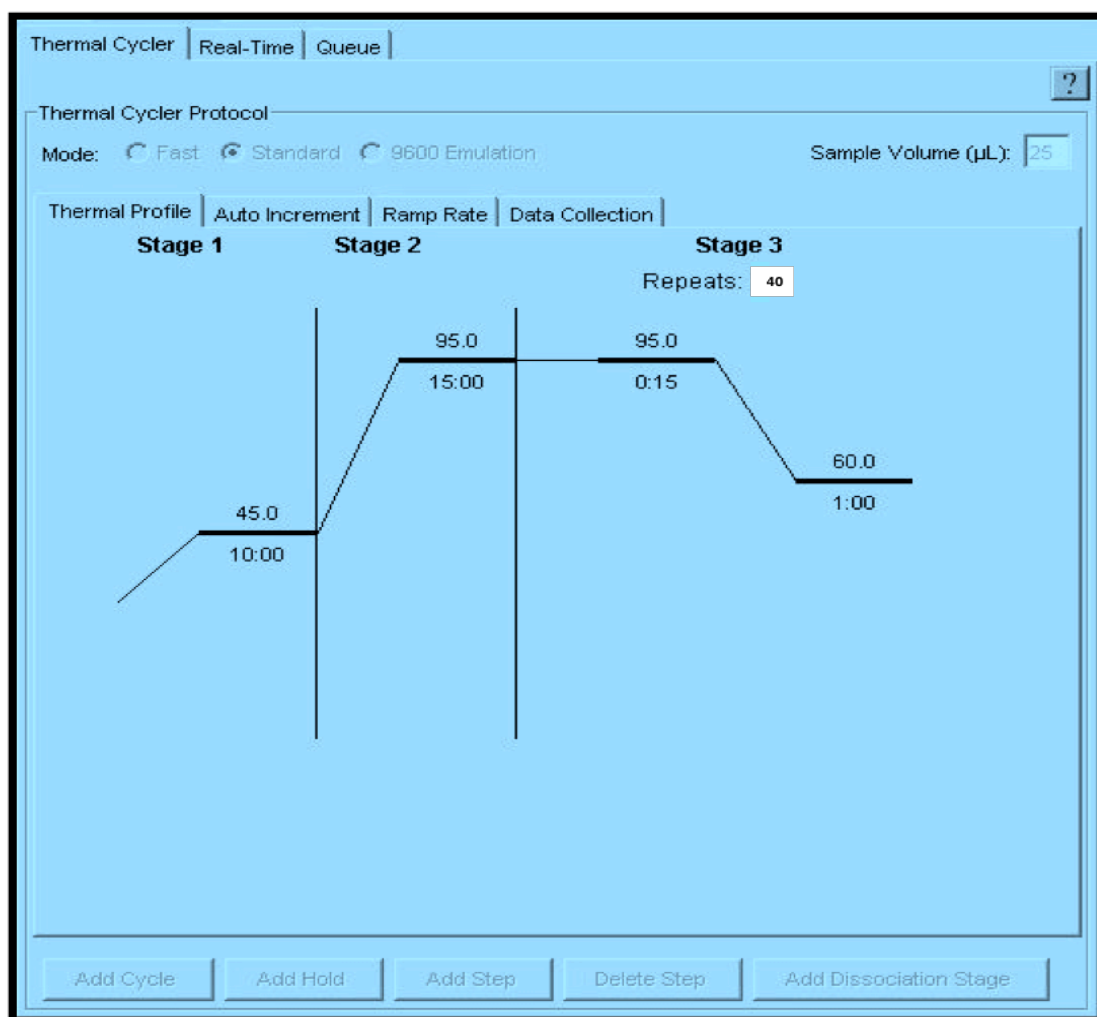
- It is necessary to add internal control (IC) in the reaction mix. Internal control (IC) allows the user to determine and control the possibility of PCR inhibition.
- Add the internal control (IC) 1 $\mu$ l/rxn and the result will be shown in the HEX/VIC/JOE.
- The volumes of Super Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of imprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
- Pipet 20 $\mu$ l master mix with micropipettes of sterile filter tips to each of the real time PCR reaction plate/tubes.
- Separately add 5 $\mu$ l RNA sample template, positive and negative controls to different plate/tubes.
- Transfer 25 $\mu$ l reaction mix to separate tubes/plates and immediately close avoid contamination.
- Centrifuge briefly in order to collect the Master Mix in the bottom of the reaction tubes.
- The Master Mix for each reaction must be prepared according to following figure:



*Note: PCR System without HEX/VIC/JOE channel may be treated with 1ul of Molecular grade water instead of 1ul of Internal control.*

### 10.4 PCR Instrument Set Up and Thermal Cycle Profile

Temperature	Duration	Cycles
45 C	10 minutes	1
95 C	15 minutes	1
95 C	15 seconds	40
60 C	1 minute	



Selection of Fluorescence Channels	
FAM	Target Nucleic Acid
HEX/VIC/JOE	Internal Control

*Note: Chose "NONE" as Passive reference and Quencher for ABI Prism System.*

**Threshold Setting:** Just above the maximum level of Molecular grade water,

**Calibration of Quantitative detection:** Input each concentration (Eg.  $1 \times 10^7$ ,  $1 \times 10^6$ ) of Standard controls at the end of the run, and Standard curve will be formed automatically.

### 10.5 Quality Control

Negative Control, Positive Control, Internal Control and QS curve must be performed correctly. Otherwise the results are invalid.

Channel/Control	Ct Value	
	FAM	HEX/VIC/JOE
Molecular Grade Water	UNDET	25-35
Positive Control (Qualitative Assay)	$\leq 35$	N/A
QS (Quantitative detection)	Correlation coefficient of QS curve $\leq -0.98$	

### 10.6 Data Analysis and Interpretation

	Ct Value		Results
	FAM	HEX/VIC/JOE	
1	UNDET	25-35	Below the detection limit or negative
2	$\leq 38$	N/A	Positive
3	38-40	25-35	Retest; if it is still 38-40, report as Below the detection limit or negative
4	UNDET	UNDET	PCR Inhibition; no diagnosis can be concluded

**10.7 Performance Characters:**

- Analysis Sensitivity:  $1 \times 10^3$  copies/ml
- Limit of Quantification:  $2 \times 10^3 - 1 \times 10^8$  copies/ml

**Reaction Set Up**

Reagent	Volume
CHKV Super Mix	18 $\mu$ l
Enzyme Mix	1 $\mu$ l
Internal Control	1 $\mu$ l
DNA	5 $\mu$ l
<b>Total</b>	<b>25 <math>\mu</math>l</b>

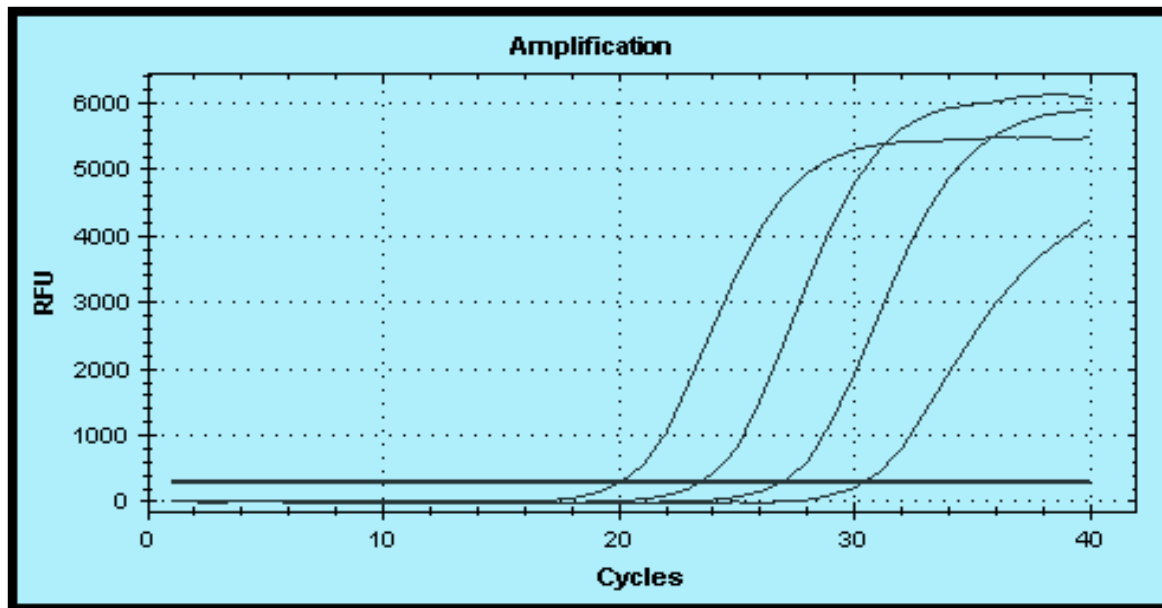
**Results**

Standards	Concentration	Ct Value	
		Standard	IC
<b>S1</b>	$1 \times 10^7$	20.09	29.14
<b>S2</b>	$1 \times 10^6$	23.45	29.15
<b>S3</b>	$1 \times 10^5$	26.97	29.25
<b>S4</b>	$1 \times 10^4$	30.42	29.25

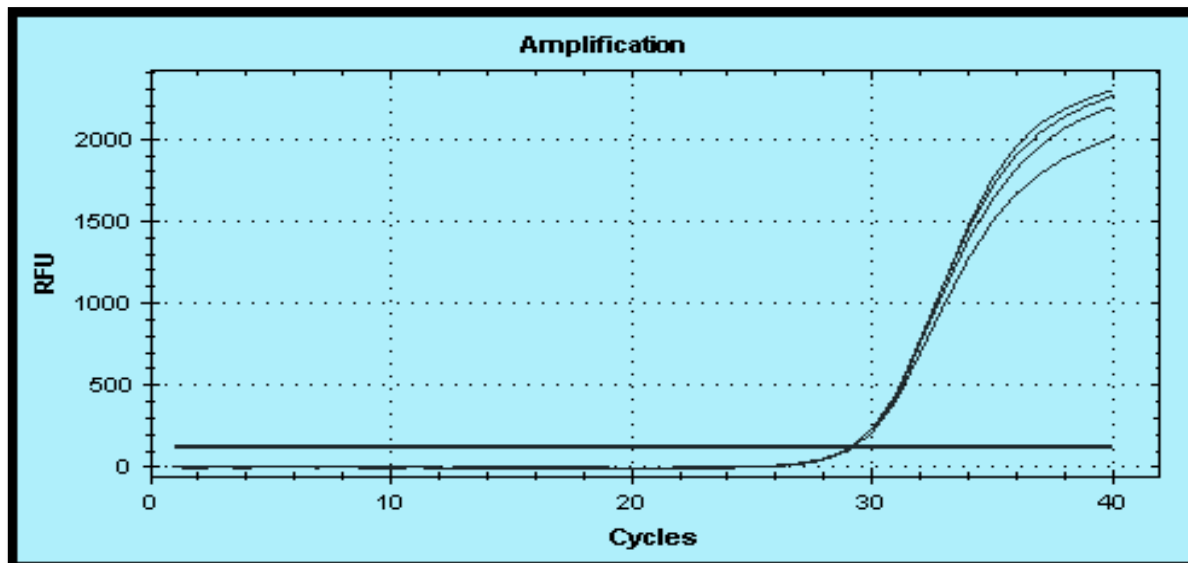




**Standards Amplification Plot**



**Internal Control Amplification Plot**



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