# RTA®

# BKV Real-Time PCR Kit Handbook

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**IVD**

Quantitative detection of BK Virus DNA

For professional use only.

**REF** 09046025 – 25tests
09046100 – 100 tests

CE

### Kit Contents

| | CapColor | Reagents | 25 Tests | 100 Tests |
|---|---|---|---|---|
| 1 | BROWN | BKV Mix A | 350 µl | 662,5 µl X 2 |
| 2 | YELLOW | BKV Mix B | 126 µl | 477 µl |
| 3 | BLUE | BKV Internal Control | 70 µl | 265 µl |
| 4 | RED | BKV Quantification Standart 1 ($10^7$ IU/ml) | 100 µl | 100 µl |
| 5 | RED | BKV Quantification Standart 2 (106 IU/ml) | 100 µl | 100 µl |
| 6 | RED | BKV Quantification Standart 3 (105 IU/ml) | 100 µl | 100 µl |
| 7 | RED | BKV Quantification Standart 4 (104 IU/ml | 100 µl | 100 µl |
| 8 | WHITE | PCR Grade Water | 100 µl | 100 µl |

### Storage

All reagents of RTA BKV Real-Time PCR Kit should be stored at -20°C. Storage at higher temperatures should be avoided (e.g. +4°C). Under these conditions, kit contents should be stable through the expiration date printed on the label. The reagents should not be freeze-thawed more than 2 times; otherwise the shelf of the kit will reduce. During the working steps all reagents should be kept on ice.

### Intended Use

RTA BKV Real-Time PCR Kit is an *in vitro* nucleic acid amplification assay for quantification of BK Virus (BKV) DNA in human serum, using RTA Viral DNA Isolation Kit and BIO-RAD CFX96-IVD System for amplification, detection and analysis. RTA BKV Real-Time PCR Kit is intended for use as an aid in the management of patients with chronic BKV infection undergoing anti-viral therapy to assess response to treatment in conjunction with all relevant clinical and laboratory findings. RTA BKV Real-Time PCR Kit is not intended for screening of blood and blood products for the presence of BKV DNA or confirmation of the diagnosis of infection with BKV.

### Product Use Limitations

- All reagents of the kit is for in vitro diagnostic use only.
- RTA BKV Real-Time PCR Kit is not intended for screening of blood and blood products for the presence of BKV DNA or confirmation of the diagnosis of infection with BKV.
- This kit has been validated for use with human serum or human plasma collected in EDTA anticoagulant. Test with other sample types may result in inaccurate results.
- This kit has been validated for use with RTA Viral DNA Isolation Kit. Using other isolation kits may adversely affect the performance characteristics of the kit.
- This kit has been validated for use with BIO-RAD CFX96-IVD and Real-Time PCR Detection Systems. Using other instruments may adversely affect the performance characteristics of the kit.
- This kit has been optimized for use with specific PCR plastic consumables listed under Additional Materials Required section of the Handbook. Using other PCR plastic consumables may adversely affect the performance characteristics of the kit.
- Trustworthy results depends on proper sample collection, transport, storage and processing methods.
- It is intended for professional use by properly trained personnel.
- RTA BKV Real-Time PCR Kit is intended for use as an aid in the management of patients with BKV infection in conjunction with all relevant clinical and laboratory findings.
- The instructions in user manual should be followed strictly for optimum PCR results.
- The expired kits should not be used. Kit components from different lots should not be mixed.

### Product Description

RTA BKV Real-Time PCR assay is a fluorogenic probe-based PCR assay in which, situated between two PCR primers, there is an internal oligonucleotide probe with a fluorescent label attached at the 5'-end and a quenching molecule that suppresses the fluorescent reporter at the 3'-end. During DNA replication in the PCR process, the internal oligonucleotide hybridizes to the template and is digested by the 5'-3' endonuclease activity of the Thermus aquaticus (Taq) DNA polymerase as the PCR primer is extended. The internal oligonucleotide is digested only if DNA replication occurs, separating the fluorescent and quencher molecules. PCR products are detected within minutes by monitoring the increase in fluorescence that occurs exponentially with successive PCR amplification cycles. The parameter Ct (threshold cycle) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. A plot of the log of initial target copy number for a set of standards versus Ct is a straight line. Quantification of the amount of target in unknown samples is accomplished by measuring Ct and using the standard curve to determine starting copy number. RTA BKV real-time PCR assay utilizes external standards to gather quantitative results and includes an internal control, which controls for target isolation and amplification. RTA BKV Real-Time PCR assay is a real-time PCR assay in which DNA templates amplified by DNA polymerase-mediated DNA amplification. During DNA replication in the PCR process, the internal oligonucleotide hybridizes to the template and is digested by the 5'-3' endonuclease activity of the *Thermus aquaticus* (*Taq*) DNA polymerase as the PCR primer is extended. The internal oligonucleotide is digested only if DNA replication occurs, separating the fluorescent and quencher molecules. PCR products are detected within minutes by monitoring the increase in fluorescence that occurs exponentially with successive PCR amplification cycles. The parameter Ct (threshold cycle) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold.
RTA BKV real-time PCR assay utilizes external standards to gather quantitative results and includes an internal control, which controls for target isolation and amplification. The target region is situated in different sequences of Glycoprotein G region of BKV genome. For BKV, the target is 169-bases long. BKV DNA concentrations are noted in IU/ml.
BKV DNA concentration is noted in International Units/ml (IU/ml).

### Pathogen Information

BKV is a Polyomavirus of the family Polyomaviridae, which is characterized by a run of the mill morphology of nonenveloped virions with icosahedral capsids with a 40-nm distance across that encase the little roundabout twofold stranded DNA genome of 5 kb. The genome is consist of the noncoding area that contains origin of replication and bidirectional promoters, the early coding region that has small tumor antigen (STag) and the Large tumor antigen (LTag), and other coding region encoding the viral capsid proteins (VP1– VP3) and the agnoprotein. LTag is a conserved multifunctional controller of polyomavirus translation and replication. LTag communicates with the cell proteins, including the tumor-silencers p53 and retinoblastoma pRb, for viral DNA replication. On contrary to the replication of herpesviruses, polyomavirus replication is dependent to cell factors and, except LTag DNA helicase, does not encode antiviral medication targets, for example, thymidine kinases or viral DNA polymerase. BKV replication is cytopathic. The release of viral and cellular particles leada to a nonspecific inflammatory reaction, followed by specific immune responses in immunocompetent hosts.

### Warnings and Precautions

- All clinical specimens and all resulting waste materials should be treated as potentially infectious; the samples should be prepared in Bio-safety Level 2 area.
- Before and after work all surfaces should be disinfected with a freshly prepared solution of 10% bleach or antiviral agents. Dispose of unused reagents, waste and specimens in accordance with country or local regulations.
- Do not pipette by mouth. Do not eat, drink or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats and eye-wear when handling clinical specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents. Avoid contact of reagents with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water,
- The procedures should preferably be performed in four separated areas (i.e. for DNA extraction, PCR setup, sample addition, amplification) to aid in preventing contamination. All supplies for a particular procedure should be stored in the area where that procedure is performed and should not be moved between areas Gloves should be removed and disposed of before leaving one area to proceed to the next. Lab coats should be specific to an area and never worn outside of that area.
- The work should flow in one direction, beginning in the extraction area, moving to the PCR setup area in which PCR Master Mix is prepared, then moving to the third area in which samples, negative control and quantification standards are added, finally moving to amplification area in which real-time PCR equipment is run.
- Use all pipetting devices and instruments with care and follow the manufacturer's instructions for calibration and quality control; to prevent sample contamination, use new, sterile aerosol barrier or positive displacement Rnase/DNase-free pipette tips and sterile pipettes.
- Handle all materials containing specimens or controls according to Good Laboratory Practices in order to prevent cross-contamination of specimens or controls. Store the kit away from any source of contaminating DNA, especially amplified nucleic acid.
- Do not mix reagents with different lot numbers or substitute reagents from other manufacturers.
- A single type of BKV DNA assay should be used for monitoring a patient. If RTA BKV Real-Time PCR Kit substitutes another BKV DNA assay, both tests should be used in parallel for at least two subsequent samples.
- Do not use a kit after its expiration date.

### Performance Characteristics

**Analytical Sensitivity** Analytical sensitivity was analyzed by use of a dilution series of WHO standard, and the cutoff value of the kit was determined by probit analysis. A dilution series of a WHO International BKV standard (NIBSC code: 14/212) was prepared to give the final concentrations of $10^4$, $10^3$ , $10^2$ and 10 IU/ml. Dilutions were extracted by RTA Viral DNA Isolation Kit (Cat No: 09030100) according to RTA Viral DNA Isolation Kit Handbook. Starting sample volumes were 200 µl and elution volumes were 50 µl. Each dilution was tested in 24 replicates for each instrument. Lower limit was calculated by probit analysis done by PASW Statistics 18 program. The 95 % cutoff concentration of RTA BKV Real-Time PCR Kit is 0,373 IU/µl for BIO-RAD CFX96.

**Linear Range** To determine the upper limit, a dilution series of WHO 1st BKV International standard (Cat. No: 14/212) ranging from 1 x $10^1$ IU/ml to 1 x $10^{10}$ IU/ml were prepared. Viral DNA was extracted from standards by RTA Viral DNA Isolation Kit (Cat No: 09030100) according to RTA Viral DNA Isolation Kit Handbook. Starting sample volumes were 250 µl and elution volumes were 50 µl. High concentrationsamples (1 x $10^7$, 1 x $10^8$, 1 x $10^9$ and 1 x $10^{10}$ IU/ml) were prepared by using calibrated plasmid DNA bearing BKV external standard. Within the range of 314 - $10^9$ IU/ml the relationship between log of target DNA and Ct values is linear.
Lower limit was calculated by probit analysis done by PASW Statistics 18 program according to the quantification results of BKV Analytical Sensitivity Studies.

**Precision** For each experiment, 24 replicates of $10^4$ IU/ml WHO International Standard for BKV DNA assays were used.

**Diagnostic Specificity** BKV negative clinical specimens were analyzed to determine the diagnostic specificity of RTA BKV Real-Time PCR Kit. BKV negative clinical specimens were analyzed to determine the diagnostic specificity of RTA BKV Real-Time PCR Kit. 50 BKV DNA negative clinical **serum** specimens were used.
None of the BKV negative clinical specimens gave positive test result for BKV DNA. Diagnostic specificity of RTA BKV Real-Time PCR Kit is 100 %. All of the Internal Controls of tests gave positive result.

**Cross Reactivity** To examine the specificity of an assay, cross-reactivity studies should be performed for potential cross-reactive markers. In this study, the specificity of the assay was evaluated by testing 5 reference organisms which were positive. RTA BKV Real-Time PCR Kit do not show any cross-reactivity with other potential cross-reactive markers given in the table below:

| Organism | Source | Test Result |
|---|---|---|
| Herpes Simplex Virus (Type 1) Working Reagent for Nucleic Acid Amplification Testing (NAT) | NIBSC Code: 08/224 | Negative |
| Herpes Simplex Virus (Type 2) Working Reagent for Nucleic Acid Amplification Testing (NAT) | NIBSC Code: 08/226 | Negative |
| Varicella Zoster Virus (Type B) Working Reagent for Nucleic Acid Ampli-fication Testing (NAT) | NIBSC Code: 08/310 | Negative |
| 3rd WHO International Standard for HBV for NAT | NIBSC Code: 10/264 | Negative |
| Human Immunodeficiency Virus type 1 for Nucleic Acid Amplification Techniques | NIBSC Code: 10/152 | Negative |

### Additional Materials Required

- RTA Viral DNA Isolation Kit (Cat No: 09030100; RTA Laboratories, Turkey)
- Real-Time PCR system,
- Disposable powder-free gloves
- Micropipettes (0.5 µl – 1000 µl),
- Sterile micropipette tips with filters,
- Microcentrifuge tubes,

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- Vortex mixer,
- Desktop microcentrifuge for 2.0 ml tubes and for PCR strip tubes,
- PCR Workstation,
  Real-Time PCR reaction tubes/plates/capillaries For BIO-RAD CFX96-IVD or :
- Hard-Shell Thin-Wall 96-Well Skirted PCR Plates with Bar Codes (BIO-RAD, Cat#: HSP-9955)
- Hard-Shell Thin-Wall 96-Well Skirted PCR Plates (BIO-RAD, Cat#: HSP-9655)
- Microseal 'B' Adhesive Seals, optically clear (BIO-RAD, Cat#: MSB-1001),
- Individual PCR Tubes, Low Tube Strips, 8-tubes strip, 0.2 ml Low Profile, White (BIO-RAD, Cat#: TLS0851)
- Flat Cap Strips, Optically Clear, 8-cap strip, 0.2 ml (BIO-RAD, Cat#: TCS0803)

**Sample Preparation**

This kit has been validated for use with human serum.
Aseptic techniques must be employed during collection to prevent the introduction of micro-organisms into the patient's anatomical space, and to prevent the sample from being contaminated during the process of collection.
All samples should be regarded as potentially infectious and standard precautions guidelines should be followed by all healthcare workers during sample collection and handling.
Samples must be collected into appropriate containers before despatch to the laboratory.
Be careful to check for cracks in the containers and to ensure that the lids of containers are properly tightened to prevent leakage of samples during handling and transportation. This can pose infection hazards to transport and laboratory staff.
Ensure that the outer surfaces of the containers are not contaminated by the patients' samples.
Store whole blood at room temperature for no longer than 4 hours. Centrifuge blood within 4 hours of collection. Transfer serum or plasma to a screw cap cryovial tube.
Transportation of whole blood, serum or plasma must conform to country or local regulations for the transport of etiologic agents.
Serum or plasma samples may be stored at 2-8°C for up to 3 days or frozen at -70°C or colder for long-term storage.
Avoid multiple freeze/thaw cycles of specimens.

## Protocol

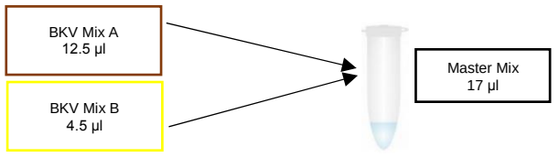
**Viral DNA Isolation** RTA Viral DNA Isolation Kit (Cat No: 09030100; RTA Laboratories, Turkey) should be used for Viral DNA extraction from clinical samples. Please follow the manufacturer's instructions as stated in the kit manual.

**Internal Control** During DNA isolation, addition of the supplied internal control (IC) is necessary. IC allows the user to monitor DNA extraction step as well as to determine any PCR inhibition. For each sample, add 2.5 µl IC together with Solution DL of the Viral DNA Isolation Kit for a 50 µl elution. Depending on your final elution vol- ume, the volume of IC to be added can be calculated (0.05 µl IC/1 µl Elution Buffer). The Ct value of internal control of a negative sample should be equal to 33 ± 5, other- wise, it denotes a problem during purification.

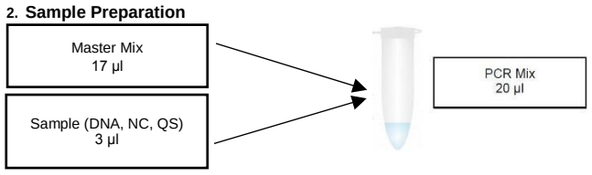
**Quantification Standards** For generating a standard curve to obtain accurate quantification data on the Real-Time system, four quantification standards should be used. For each standard the corresponding concentration should be defined properly to the Real-Time PCR system before each run and the standard curve will be generated accordingly at the end of the reaction. Work with BKV Quantification Standards after preparation of clinical samples and negative control in a separate area. Caps of the tubes or capillaries of Clinical Samples SHOULD be closed in that area.

**PCR Protocol**
**1.** Thaw all components, except BKV Mix B, at room temperature. Thaw BKV Mix B on ice. Thaw BKV Mix A at 37°C for 5 min if there is a precipitate. Mix each component thoroughly, then centrifuge briefly before use. Transfer all the reagents onto ice or cooling block.
**2.** The final volume of Master Mix is calculated by multiplying single reaction volumes of Mix A and Mix B by the total sample size. The number of negative controls, quantification standards and the clinical samples should be included when calculating total sample size. Against possible pipetting errors, addition of an extra sample to the total sample size is recommended. PCR Grade Water should be used as the negative control.
**3.** To prepare master mix, add 12,5 µl of BKV Mix A (brown tube) and 4,5 µl of BKV Mix B (yellow cap) for each sample to the master mix tube. Vortex the tube and spin down briefly in a microcentrifuge. Add 17 µl of Master Mix into Real-Time PCR reaction tubes or capillaries for each sample. Add 3 µl DNA of each sample, negative control and quantification standards into the tubes. Spin down briefly.
**4.** Perform the following protocol for BIORAD CFX96: 95°C for 10 min, 1 cycle; 95°C for 30 sec, 54°C for 30 sec, 72°C for 30 sec, 45 cycles.
**5.** Fluorescence is measured at 72°C for BIO-RAD CFX96; FAM and Texas Red channels should be chosen
**6.** Refer to the Operator's Manual of the related instruments to program and analyze the results.

**1. Preparation of Master Mix**




**2. Sample Preparation**




**3. PCR Protocol**

| Program Name | Cycles | Program for BIO-RAD CFX96-IVD* |
|---|---|---|
| Hot Start | 1 | 95ºC, 10 min |
| Amplification | 45 | 95ºC, 30 sec |
| | | 54ºC, 30 sec |
| | | 72ºC, 30 sec |

*** For BIO-RAD instrument, fluorescence is measured at 72°C; FAM and Tx Red channels should be chosen.**

**Data Analysis**

To be able to evaluate the experiment, PCR efficiency of the Standard Curve must be between 90%-110% and $R^2$ value must be more than 0.98. Otherwise, the experiment should be repeated.
During analysis on BIO-RAD CFX96-IVD softwares, adjust threshold fluorescence value automatically.
Concentration of each positive sample will be calculated by the software according to the standard curve as International Unit per milliliter (IU/ml)
Due to different starting sample volumes and elution volumes during Viral DNA isolation, the following formula **SHOULD** be used to calculate the concentration of the original clinical sample:

The interpretation on the calculated results can be done as follows:

| Signal detected in FAM/ Green channel | Signal detected in Texas Red/Orange channel | Calculated concentration of the original clinical sample | Conclusion |
|---|---|---|---|
| + | + | <0,373 IU/µl for BIO-RAD | The result is valid. Quantitation is not possible since the quantitative result is below the analytical sensitivity value of the assay. Reproducibility of the positive result is not guaranteed. |
| + | +/- | ≥0,373 IU/µl for BIO-RAD and ≤1 x $10^9$ IU/ml | The result is valid. BKV DNA is detected at the concentration calculated by the software since the quantitative result is within the linear range of the assay. |
| + | +/- | >1 x $10^9$ IU/ml | The result is valid. BKV DNA is detected at a concentration >1 x $10^9$ IU/ml. Quantita- tion is not possible since the quantitative result is above the linear range of the assay. |
| - | + | N/A | The result is valid. Target (BKV DNA) is not detected. |
| - | - | N/A | The result is invalid. No diagnostic interpretation can be done, |

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