

GeneProof BK/JC Virus (BK/JC) PCR Kit



In vitro diagnostic medical device

The kit has been manufactured according to EC Directive 98/79/EC as an *in vitro* diagnostic medical device and it has been designed for professional use in specialized clinical and research laboratories.

KIT CONTENT

| REF | ISIN Version IS included in the MasterMix | | | ISEX Version IS supplied in a separate tube Nucleic acid isolation and PCR inhibition control | | |
|--|--|-------------------------|--------------------------|---|-------------------------|--------------------------|
| | BKJC/ISIN/025 25 rxn | BKJC/ISIN/050 50 rxn | BKJC/ISIN/100 100 rxn | BKJC/ISEX/025 25 rxn | BKJC/ISEX/050 50 rxn | BKJC/ISEX/100 100 rxn |
| MasterMix BKJCV | 1x750 µl | 2x750 µl | 4x750 µl | 1x750 µl | 2x750 µl | 4x750 µl |
| CALIBRATOR BKJC 10 ⁴ copy/µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl |
| CALIBRATOR BKJC 10 ³ copy/µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl |
| CALIBRATOR BKJC 10 ² copy/µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl |
| CALIBRATOR BKJC 10 ¹ copy/µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl | 1x200 µl |
| Internal Standard BKJC | - | - | - | 1x1000 µl | 1x1000 µl | 2x1000 µl |

STORAGE AND TRANSPORTATION CONDITIONS

The kits should be transported and stored at temperatures between -85 °C and -10 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept. Repeated freezing and thawing of the kit components may result in lower detection quality.

TECHNICAL SPECIFICATION

| | |
|------------------------------------|--|
| Target sequence | DNA conservative region of a single-copy gene overlapping the boundary between the gene for the VP1 and VP2 proteins |
| Specificity | BK virus (BKV) and JC virus (JCV) |
| Sensitivity (LOD) | for BK virus reaches 0.596 copies/µl with the probability of 95 % for JC virus reaches 0.528 copies/µl with the probability of 95 % |
| Accuracy of measurement | for BK virus within the range of 10 ⁴ -10 ¹ copies/µl the detection accuracy is 0.5 log for JC virus within the range of 10 ⁴ -10 ¹ copies/µl the detection accuracy is 0.5 log |
| Linear range of measurement | for BK virus within the range of 10 ⁷ -0.596 copies/µl for JC virus within the range of 10 ⁷ -0.528 copies/µl |
| Sample types | EDTA whole blood, urine, cerebrospinal fluid |
| Reporting units | copies/ml |
| Quality Control | regularly tested by QCMD and Instand e.V. External Quality Assessment Panels |



METHOD PRINCIPLES

The PCR kit is designed for BK and JC virus detection and differentiation by the real-time Polymerase Chain Reaction (multiplex PCR) method. The BKV/JCV detection and differentiation are based on the principle of amplifying the specific conservative DNA sequence overlapping the boundary between the gene for the VP1 and VP2 proteins and measuring the concentration increase of the amplification product during the PCR process using fluorescence labelled probes. BK virus presence is indicated by FAM fluorophore fluorescence growth and the JC virus presence is indicated by Cy5 fluorophore fluorescence growth. An Internal Standard (IS) is included in the reaction mix controlling the possible inhibition of the PCR reaction (ISIN version) and possibly also the quality of DNA extraction (ISEX version). IS positive amplification is detected in the HEX fluorophore fluorescence channel. The detection kit takes advantage of the “hot start” technology minimizing non-specific reactions and assuring maximum sensitivity. Ready to Use MasterMix contains uracil-DNA-glycosylase (UDG) eliminating possible contamination of the PCR reaction by amplification products. The kit performs very sensitive detection of BK and JC viruses in clinical material (EDTA whole blood, urine, cerebrospinal fluid). The kit is designed for *in vitro* diagnostics and provides qualitative and quantitative detection.

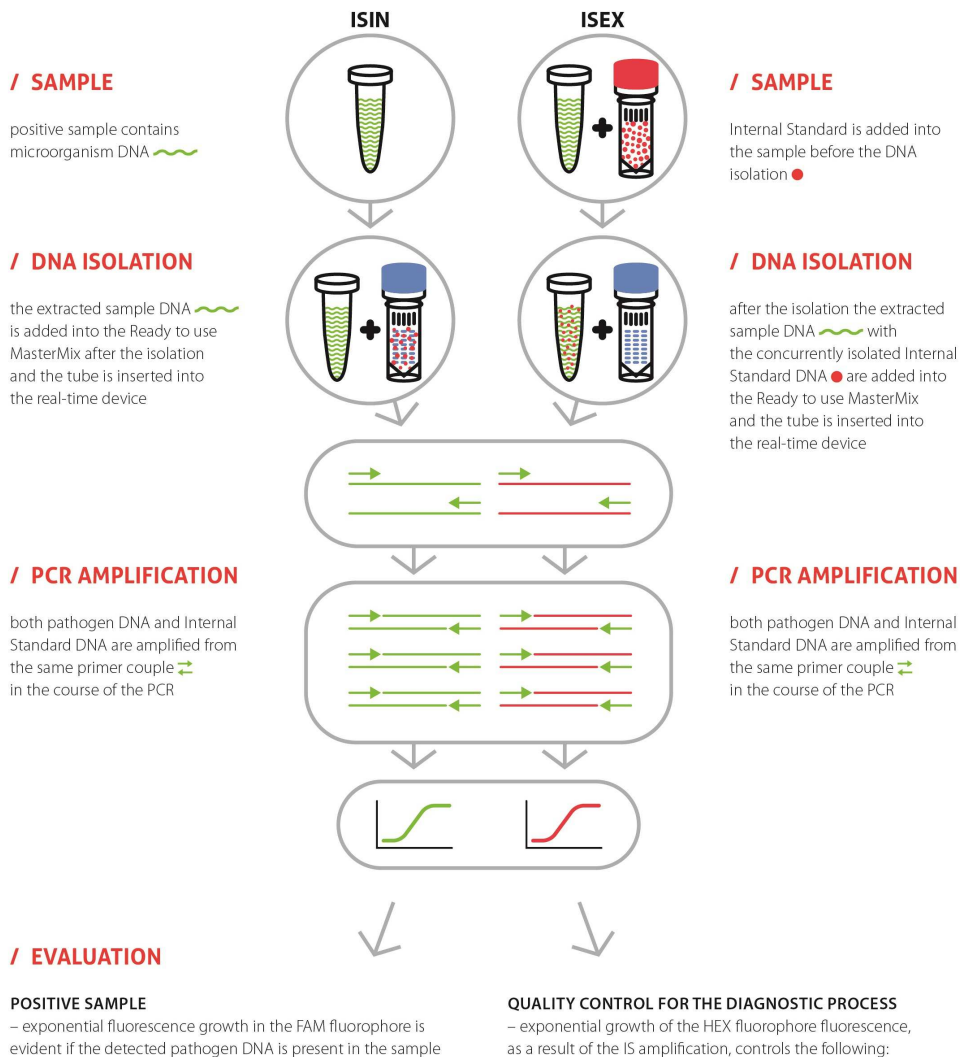
ISIN version

Internal Standard is included in the MasterMix tube. This PCR kit version enables PCR inhibition control.

ISEX version

Internal Standard is provided as independent item within the package. This PCR kit version enables both PCR inhibition control and nucleic acid purification process efficiency control.

MICROBIOLOGICAL DNA DETECTION TECHNOLOGY



1. Inhibition and efficiency of the PCR amplification – ISIN version
2. DNA extraction quality, inhibition and efficiency of the PCR amplification – ISEX version



USER MANUAL

SAMPLING AND SAMPLE STORAGE

Whole blood, urine and CSF are relevant materials for BKV and JCV detection. When working with urine it is recommended to perform centrifuging (min. 1000 g for 20 min) before the DNA isolation for NAT to assure sedimentation of the cells containing the virus. When isolating DNA from whole blood it is not suitable to freeze the samples; it is advisable to keep them at room temperature (a sample from non-coagulating peripheral blood should be sampled into EDTA and transported to the laboratory at the temperature between +2 and +8 °C within 24 hours). CSF samples can be frozen to the temperature between -85 °C and -10 °C and the testing can be performed by means of direct isolation of the nucleic acid.

NUCLEIC ACID PURIFICATION

Nucleic acid isolation should be performed by isolation kits available at the market according to protocols for the particular clinical material isolation. The manufacturer recommends the following isolation kits:

GeneProof PathogenFree DNA Isolation Kit
croBEE NA16 Nucleid Acid Extraction System

When using the ISEX versions of the PCR kits the IS should be added directly into the sample at the beginning of the isolation process so that in the end 1 µl of the resulting elution volume contains 0.1 µl of the IS:

| Elution volume | 25 µl | 50 µl | 100 µl | 200 µl |
|-------------------|--------|-------|--------|--------|
| Internal Standard | 2,5 µl | 5 µl | 10 µl | 20 µl |

PCR SETUP

1. Add 30 µl of MasterMix into PCR tubes.
2. Add 10 µl of the isolated nucleic acid sample or 10 µl of Positive Control into the individual PCR tubes. The final reaction mix volume will be 40 µl.
It is necessary to keep all components at +2°C to +8°C during the PCR preparation.

3. Close the tubes, centrifuge shortly, insert them into the device and let them amplify according to the following PCR profile.
Be very careful when handling the Positive Control or the clinical material, incorrect handling could result in contamination and the consequent impairment of the kit components or the MasterMix! The manufacturer is not responsible for the kit impairment due to incorrect handling.

AMPLIFICATION PROFILE

| Step | Temperature | Time | Data collection | Cycles |
|---------|-------------|--------|-----------------|--------|
| 1. Hold | 37 °C | 2 min | | 1 |
| 2. Hold | 95 °C | 10 min | | 1 |
| 3. PCR | 95 °C | 5 s | | |
| | 60 °C | 40 s | FAM+HEX+Cy5 | 45 |
| | 72 °C | 20 s | | |

VALIDATED INSTRUMENTS

GeneProof PCR kits are designed for use with real-time devices from various manufacturers. This PCR kit has been validated with the following devices:



















| | |
|--|---------------------------------|
| Applied Biosystems 7500 Real-Time PCR System | LineGene 9600* |
| AriaMx Real-Time PCR System | Mx3000P/3005P QPCR System |
| Dx/CFX96™ Real-Time PCR Detection System | Rotor-Gene 3000*, Rotor-Gene Q* |
| LightCycler ® 480 | SLAN® Real-Time PCR System |

* Validation applies to a device model providing detection in the following channels: FAM, HEX and Cy5.

GeneProof diagnostic kits are continually validated with various types of devices. Please request the current list at support@geneproof.com.



CLINICAL SAMPLE ANALYSIS EVALUATION

| Channel FAM | Channel Cy5 | Channel HEX | Result | Interpretation |
|--|---|---|---------|------------------|
|  |  |  | Valid | BKV positive |
|  |  |  | Valid | JCV positive |
|  |  |  | Valid | BKV+JCV positive |
|  |  |  | Valid | negative |
|  |  |  | Invalid | |
|  |  |  | Invalid | |

QUANTITATIVE DETECTION EVALUATION

Use the following formula to calculate the virus concentration in copies/ml while taking into account the volume of material entering the isolation:

$$\text{copy/ml} = \frac{\text{SC} \times \text{EV}}{\text{IV}}$$

SC - Sample Concentration (copy/ μ L)

EV - Elution Volume (μ l)

IV - Isolation Volume (ml)

You can use the calculator for pathogen concentration conversion at www.geneproof.com to make the calculation easier.

WARNING

A single valid package insert for a specific kit is included in the package or to be requested for the particular lot from the manufacturer. The kit should be disposed of after use according to the current legal regulations considering the fact that the kit doesn't contain any dangerous, infectious or toxic components that would be subject to special safety regulations, and the packaging materials are made of paper and polypropylene. If you have any questions please contact our Customer Service.

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