# GeneProof HIV type 1 (HIV-1) PCR Kit

CE 1023 IVD

*In vitro* diagnostic medical device

The kit has been manufactured according to the 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: | HIV1/ISEX/025<br>25 rxn | HIV1/ISEX/100<br>100 rxn |
|---|---|---|
| MasterMix<br>HIV-1 | 1x 750 µl | 4x 750 µl |
| **Calibrator A**<br>HIV-1<br>$10^5$ IU/µl | 1x 400 µl | 1x 400 µl |
| **Calibrator B**<br>HIV-1<br>$10^4$ IU/µl | 1x 400 µl | 1x 400 µl |
| **Calibrator C**<br>HIV-1<br>$10^3$ IU/µl | 1x 400 µl | 1x 400 µl |
| **Calibrator D**<br>HIV-1<br>$10^2$ IU/µl | 1x 400 µl | 1x 400 µl |

## STORAGE AND TRANSPORTATION CONDITIONS

The kit must be transported at temperature below -20 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept (-20 ± 5) °C. The components are stable for a maximum of 4 repeated freezing/thawing cycles after the first use of a particular vial. The component must be used before the expiry date or 25 days after the first use of a particular vial (whichever comes first).

## TECHNICAL SPECIFICATION

| | |
|---|---|
| **Target Sequence** | LTR sequence and *GaG* gene |
| **Analytical Specificity** | HIV genotypes A - D, AE, F, AG-GH, Group N, Group O, BF, H, K, CRF03_AB, 100 % |
| **Analytical Sensitivity** | 273.971 IU/ml i.e. 153.424 cp/ml (on HIV-1 NIBSC 16/194 using GeneProof PathogenFree RNA Isolation Kit) |
| (LoD with the probability of 95%) | 548.121 IU/ml i.e. 306.948 cp/ml (on HIV-1 NIBSC 16/194 using croBEE 201A Nucleic Acid Extraction Kit) |
| | 98.59 IU/ml i.e. 55.21 cp/ml (on Acrometrix HIV-1 Panel UI/ml using SpinStar Viral Nucleic Acid Kit 1.0 with SpinStar Pretreatment Solution) |
| **Diagnostic Specificity** | 100 % ($CI_{95\%}$: 99.10 % - 100 %) |
| **Diagnostic Sensitivity** | 93.66 % ($CI_{95\%}$: 87.96 % - 96.88 %) |
| **Linear Range** | $10^9$ - $10^{2.5}$ IU/ml with precision of ± 0.5 log (GeneProof PathogenFree RNA Isolation Kit) |
| | $10^9$ - $10^3$ IU/ml with precision of ± 0.5 log (croBEE 201A Nucleic Acid Extraction Kit) |
| **Dynamic Range** | $10^9$ - 273.971 IU/ml (GeneProof PathogenFree RNA Isolation Kit) |
| | $10^9$ - 548.121 IU/ml (croBEE 201A Nucleic Acid Extraction Kit) |
| **Reporting units** | IU/ml (1 IU = 0.56 cp) |
| **Validated Specimen** | plasma |
| **Quality Control** | regularly tested by QCMD and Instand e.V. External Quality Assessment Panels |
| **Regulatory Status** | $CE_{1023}$ IVD |

Quality management system is certified in compliance with the requirements of the standard ČSN EN ISO 13485 ed.2:2016

## INTERFERENCES

The interferences testing was performed using negative plasma with set level of biochemical markers, which can be potential endogenous interferences. The negative plasma was spiked with HIV positive material at 3x LoD. Elevated levels of bilirubin (342 µmol/l), albumin (60 g/l), haemoglobin (2 g/l), urea (42.9 mmol/l), uric acid (1.4 mmol/l) and D-glucose (55 mmol/l) in samples have been tested in the presence and absence of HIV DNA. The evaluation and settings of pathological values for interference testing were performed according to CLSI guidelines EP7-A2.

**PLASMA**

| **Tested substance** | **Tested level(s)** | **Observed interference** | **Tested substance** | **Tested level(s)** | **Observed interference** |
|---|---|---|---|---|---|
| Albumin | 60 g/l | None | Haemoglobin | 2 g/l | None |
| Bilirubin | 342 µmol/l | None | Urea | 42.9 mmol/l | None |
| Glucose | 55 mmol/l | None | Uric acid | 1.4 mmol/l | None |

The tested endogenous interferences were shown not to interfere with the GeneProof HIV type 1 (HIV-1) PCR Kit in plasma samples.

## METHOD PRINCIPLE

The HIV-1 detection is based on the amplification of a specific region of the LTR sequence and a region of the *GaG* gene sequence and the measurement of fluorescence increase. The kit provides maximum sensitivity and specificity for all variants of the HIV-1virus from the M group (including groups N and O) and for the virus CRF variants. The presence of HIV-1 is indicated by the increased FAM fluorophore fluorescence. The human GAPDH mRNA is used as an Internal control (IC), it serves as a control for the whole diagnostic process, i.e. RNA extraction efficiency, reverse-transcription step efficiency (transcription of RNA into cDNA) and PCR amplification efficiency (PCR inhibition). The IC positive amplification is detected in the HEX fluorophore fluorescence channel. The PCR kit is designed as Ready-to-Use Master Mix for *in vitro* diagnostics for both qualitative and quantitative detection and it utilizes the "hot start" technology minimizing non-specific reactions and ensuring maximum sensitivity.

# USER MANUAL

## SAMPLING AND SAMPLE STORAGE

Whole blood samples should be centrifuged and the plasma removed to secondary tube within 4 hours from blood collection. If there is not possibility to process the sample within 4 hours, it is recommended to centrifuge whole blood and freeze the plasma sample at -20 °C or -70 °C.

## NUCLEIC ACID PURIFICATION

Nucleic acid extraction should be performed by extraction kits available at the market according to protocols for the particular clinical material extraction. The manufacturer recommends the following products:
GeneProof PathogenFree RNA Isolation Kit
croBEE 201A Nucleic Acid Extraction Kit

## PCR SETUP

1. Gently vortex and briefly centrifuge the Master Mix and Calibrators' tubes.

2. Add 30 µl of Master Mix into PCR tubes.

3. Add 20 µl of the isolated nucleic acid sample or 20 µl of Calibrator into the individual PCR tubes and mix by pipetting. The total reaction mix volume will be 50 µl*. It is necessary to keep all components at (2 – 8) °C during the PCR preparation. The customer must use his own negative control in the form of isolate of negative clinical material in each test. All 4 Calibrators must be used for setting the standard curve for quantitative detection.*

4. 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 Calibrators or the clinical material; incorrect handling could result in contamination and the consequent impairment of the kit components! The manufacturer is not responsible for the kit impairment due to incorrect handling.*

## AMPLIFICATION PROFILE

| Step | Temperature | Time | Data Collection | Cycles |
|---|---|---|---|---|
| Hold | 37 °C | 2 min | | 1 |
| Hold | 50 °C | 5 min | | 1 |
| Hold | 95 °C | 20 s | | 1 |
| PCR | 55 °C | 20 s | | 3 |
| | 72 °C | 5 s | | |
| | 95 °C | 5 s | | |
| PCR | 95 °C | 5 s | | 45 |
| | 58 °C | 35 s | FAM + HEX | |

# INSTRUMENTS

GeneProof HIV type 1 (HIV-1) PCR Kit is designed for use with real-time devices from various manufacturers:

**croBEE Real-Time PCR System**
AMPLilab Real-Time PCR System
Applied Biosystems 7300 / 7500 Real-Time PCR System
AriaMx Real-Time PCR System
CFX Connect™ / CFX96™/ Dx Real-Time PCR Detection System
LightCycler® 480
LineGene 9600 Plus
Mic qPCR Cycler
QuantStudio™ 3 / 5 Real-Time PCR System
Rotor-Gene 6000 / Q*
SLAN® Real-Time PCR System

Required channels: FAM, HEX

GeneProof diagnostic kits are continually verified with various types of devices. Current list is available at *www.geneproof.com* or can be requested at *support@geneproof.com*

**NOTE: For Rotor-Gene instruments, the third channel must also be set to switch on.*

## CLINICAL SAMPLE ANALYSIS EVALUATION

| Channel FAM (HIV-1) | Channel HEX (IC) | Result | Interpretation |
|---|---|---|---|
| + | + | Valid | HIV-1 positive |
| + | - | Valid | HIV-1 positive |
| - | + | Valid | HIV-1 negative |
| - | - | Invalid | - |

## QUANTITATIVE DETECTION EVALUATION

Use the following formula to calculate the virus concentration in IU/ml for **manual** extraction (using GeneProof PathogenFree RNA Isolation Kit):

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

SC - Sample concentration (IU/µl)

EV - Elution volume (µl)

IV - Isolation volume (ml)

To easily calculate pathogen concentrations using manual or automated extraction, you can use the calculator at *www.geneproof.com*

## WARNING

A single valid Instruction for Use for a specific kit is included in the package or to be requested for the particular lot from the manufacturer. Use only combination of components from the specific PCR kit. 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 care.

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