GeneProof
Molecular diagnostics for your routine

GeneProof HIV type 1 (HIV-1) PCR Kit

CE 1023 IVD

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

	ISEX Version IS detected from sample Nucleic acid isolation and PCR inhibition control		
REF	**HIV1/ISEX/025** 25 rxn	**HIV1/ISEX/050** 50 rxn	**HIV1/ISEX/100** 100 rxn
MasterMix HIV-1	1x750 µl	2x750 µl	4x750 µl
CALIBRATOR HIV-1 10^5 IU/µl	1x200 µl	1x200 µl	1x200 µl
CALIBRATOR HIV-1 10^4 IU/µl	1x200 µl	1x200 µl	1x200 µl
CALIBRATOR HIV-1 10^3 IU/µl	1x200 µl	1x200 µl	1x200 µl
CALIBRATOR HIV-1 10^2 IU/µl	1x200 µl	1x200 µl	1x200 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	LTR sequence and *GaG* gene
Specificity	Human immunodeficiency virus type 1 (HIV-1)
Sensitivity (LOD)	reaches 20.045 IU/ml with the probability of 95 %
Accuracy of measurement	within the range of 10^7-10^3 IU/ml the detection accuracy is 0.5 log
Linear range of measurement	10^8-20.045 IU/ml
Sample types	plasma
Reporting units	IU/ml (1 IU/ml = 0.52 copies/ml)
Quality Control	regularly tested by QCMD and Instand e.V. External Quality Assessment Panels

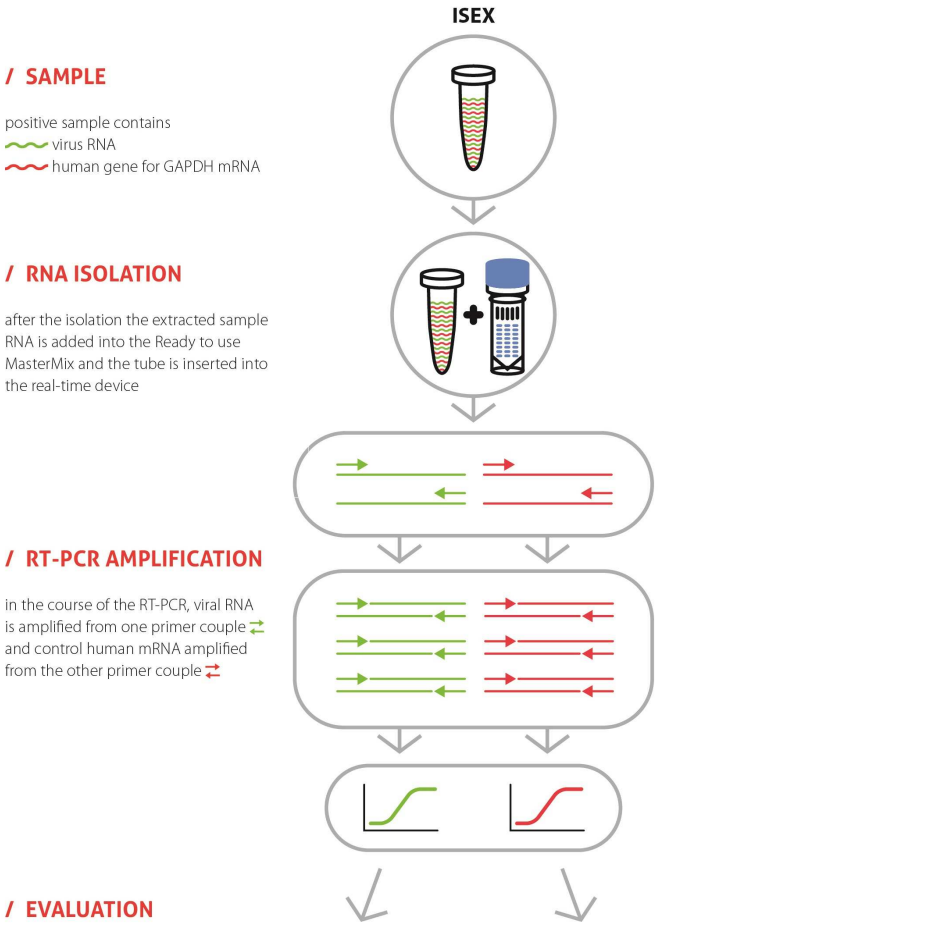
METHOD PRINCIPLES

The PCR kit is designed for HIV-1 virus RNA detection by the real-time Polymerase Chain Reaction (PCR) method. The HIV-1 detection is based on the duplex detection of a specific section of the LTR sequence and a section of the *GaG* gene sequence. Duplex targeting provides maximum sensitivity and specificity for all variants of the HIV-1 virus from the M group (including groups N and O) and for the virus CRF variants. The detection is performed by the Reverse Transcription Polymerase Chain Reaction (RT-PCR) and the measuring of the amplification product concentration growth using fluorescence labelled probes. HIV presence is indicated by FAM fluorophore fluorescence growth. For internal control (IS) the kit uses the mRNA detection of the human gene for GAPDH, which is isolated from the sample together with the RNA virus. Amplification of this control mRNA is visualized in the HEX channel. This type of the PCR kit therefore doesn't contain an independent tube with the IS (see the chart of Microbiological RNA Diagnostic Technology). This detection technology of the naturally occurring human mRNA provides control of the whole diagnostic process, specifically: sample quality (sample RNA degradation), efficiency of the RNA extraction from the sample, efficiency of the reverse-transcription step (transcription of RNA into cDNA) and efficiency of the subsequent PCR amplification (PCR inhibition). The detection kit takes advantage of the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. The kit performs very sensitive HIV detection in clinical samples (plasma). The kit is designed for *in vitro* diagnostics and provides qualitative and quantitative detection.

ISEX version

Internal Standard is detected from the sample. This PCR kit version enables both PCR inhibition control and nucleic acid purificaion process efficiency control.

MICROBIOLOGICAL RNA DETECTION TECHNOLOGY



USER MANUAL

SAMPLING AND SAMPLE STORAGE

It is possible to use plasma samples for the HIV virus RNA detection. Keep the samples frozen at the temperature between -85 °C and -10 °C for transport or long-term storage.

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

PCR SETUP

1. Add 30 µl of MasterMix into PCR tubes.

2. Add 20 µl of the isolated nucleic acid sample or 20 µl of Positive Control into the individual PCR tubes. The final reaction mix volume will be 50 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	50 °C	5 min		1
3. Hold	95 °C	20 s		1
4. Cycling	55 °C	20 s		3
	72 °C	5 s		
	95 °C	5 s		
5. Cycling	95 °C	5 s		45
	58 °C	35 s	FAM+HEX	

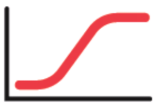
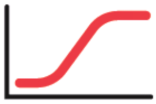
VALIDATED INSTRUMENTS

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 7300/7500 Real-Time PCR System
AriaMx Real-Time PCR System
Dx/CFX96™/CFX Connect™ Real-Time PCR Detection System
LightCycler ® 2.0, LightCycler ® 480
LineGene 9600
Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q
SLAN® Real-Time PCR System

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 HEX	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 while taking into account the volume of material entering the isolation:

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

SC - Sample Concentration (IU/µl)
EV - Elution Volume (µl)
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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