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HIV-1 DNA Test PRO

MBK0087- 96 Tests
MBK0087-32T - 32 Tests

FOR RESEARCH USE ONLY

INTENDED USE

HIV-1 DNA Test PRO allows the detection and quantification of total HIV-1 DNA, M group, in whole blood samples and PBMC.

INTRODUCTION

HIV-1 DNA is the most widely used marker of HIV persistence in infected cells enabling an overall quantification of all viral forms of HIV DNA including stable integrated proviruses and unintegrated, including extrachromosomal 2-LTR, 1-LTR and linear forms. All these co-exist in infected cells during viral replication and their levels may vary among patients, according to the stages of HIV disease and the effectiveness of the anti-HIV therapy.
In addition, the molecular detection of HIV-1 proviral DNA is indicated for HIV diagnosis in infants born to infected mothers below 2-years of age, because serology may remain positive due to passive transfer of antibodies through placenta, while the viral DNA can be used to establish the actual status of the patients.

PRINCIPLE OF THE ASSAY

The HIV-1 DNA Test PRO is a qPCR assay to detect and quantify all forms of intracellular HIV-1 DNA by the amplification of a specific sequence with the use of fluorescent-labelled probe.
The kit provides a ready to use duplex real-time PCR mix specific for the amplification of HIV-1 DNA and the endogenous reference human Telomerase Reverse Transcriptase gene (hTERT), required for relative quantification of HIV-1 copy number. The hTERT target can be used as process control and to detect the potential inhibition factors in the sample.

The HIV-1 Master Mix_PRO offers robust and consistent performances, even in presence of PCR inhibitors. Moreover, the PCR mix provides a stringent automatic hot-start allowing reaction assembly and temporary storage at room temperature prior to PCR amplification.
The HIV-1 DNA Test PRO also contains a Standard Curve ready to use with 5 levels of HIV-1 copies and cell content.

KIT CONTENTS

	No. vial x Volume	
Reagent	**MBK0087**	**MBK0087-32T**
HIV-1 Master Mix_PRO	2 X 1650 µL	1 X 1650 µL
ROX_PRO	2 X 8 µL	1 X 8 µL
ROX Dilution Buffer_PRO	2 X 100 µL	1 X 100 µL
PCR Negative Control_PRO	2 X 100 µL	1 X 100 µL
HIV-1 Standard DNA 1_PRO	2 X 85 µL	1 X 85 µL
HIV-1 Standard DNA 2_PRO	2 X 85 µL	1 X 85 µL
HIV-1 Standard DNA 3_PRO	2 X 85 µL	1 X 85 µL
HIV-1 Standard DNA 4_PRO	2 X 85 µL	1 X 85 µL
HIV-1 Standard DNA 5_PRO	2 X 85 µL	1 X 85 µL

NOTE:
- If the box of the MBK0087 (96 Tests) has a BLU label, the kit is intended for the use with ELITe InGenius® (ELITechGroup S.p.A) and contains 2 vials of the HIV-1 Standard DNA 1_PRO (code: MBK0087-STD; quantity: 2 x 85µL), in addition to the materials indicated above.
- If the box of the MBK0087-32T (32 Tests) has a RED label, the kit is intended for the use with ELITe InGenius® (ELITechGroup S.p.A) and contains 1 vial of the HIV-1 Standard DNA 1_PRO (code: MBK0087-STD; quantity: 1 x 85µL), in addition to the materials indicated above.

OTHER SUPPLIES REQUIRED

- Disposable powder-free gloves
- DNA isolation kit or home-made DNA extraction procedures to obtain pure and PCR inhibitor-free DNA
- Pipettes
- Spectrophotometer or Nanospectrophotometer for purified nucleic acid quantification (optional)

- Sterile pipette tips with aerosol-preventive filters
- Vortex mixer
- Desktop Micro-centrifuge
- Real-time PCR instrument

STORAGE

Upon arrival, store at -20°C. If stored at the recommended temperature all reagents are stable until the expiration date.

GENERAL PRECAUTIONS

Before using the kit read the Product Information carefully and completely.
The operator should always pay attention to:

- Set up pre- and post-PCR areas. Do not share instruments or consumables (pipettes, tips, tubes etc) between those areas;
- Use pipette tips with filter;
- Store positive material separately from all other reagents and, if possible, add it to the reaction mix in a separated space;
- Do not use any reagent after the expiration date indicated on the label;
- Wear powder-free gloves during all procedures;
- Thaw all kit components and protect the HIV-1 Master Mix_PRO and ROX_PRO from light before starting the assay. After thawing, mix the components and centrifuge briefly;
- Minimize sample handling;
- Change gloves frequently;
- Wash the bench surfaces with 5% sodium hypochlorite.

PROCEDURE

1. SAMPLE PREPARATION

1.1 Sample collection

Blood samples should be collected in sterile tubes with EDTA as anticoagulant. Store whole blood up to 6 hours between 2 to 25°C, or at -20°C for longer period.

1.2 DNA extraction and purification

For DNA extraction Diatheva recommends the QIAamp DNA Blood Mini Kit (QIAGEN Cat. No. 51104/51106), processing a starting volume of 400 µL of blood according to the manufacturer's instructions. For a higher concentration, use an elution volume of 60 µL, reload the eluate onto the column and repeat the elution step. The kit is compatible with ELITe InGenius® (ELITechGroup S.p.A)
Alternative nucleic acid extraction systems and kits might also be appropriate.

1.3 Preparation of sample to be analysed

The suitable volume of DNA extract must be validated by the user (I.e.: when QIAamp DNA Blood Mini Kit is used for whole blood sample, volume from 3 to 20 µL of eluate DNA can be analysed, covering a range from 0.250 to 2 µg).

2. STANDARD CURVE (only for quantitative test)

The HIV-1 DNA Test PRO contains the calibration curve points ready to use. In a separate area, thaw, vortex 15" and centrifuge briefly HIV-1 Standard DNA 1,2,3,4 and 5_PRO vials.

Standard	HIV-1 DNA copies /reaction	Cells /reaction
HIV-1 Standard DNA 1_PRO	50,000	300,000
HIV-1 Standard DNA 2_PRO	5,000	30,000
HIV-1 Standard DNA 3_PRO	500	3,000
HIV-1 Standard DNA 4_PRO	50	300
HIV-1 Standard DNA 5_PRO	5	30

3. POSITIVE PCR CONTROL (only for qualitative test)

The HIV-1 Standard DNA 1_PRO must be used as Positive PCR Control. In a separate area, thaw, vortex 15" and centrifuge briefly HIV-1 Standard DNA 1_PRO.

4. PROGRAM SETUP

Program the PCR instrument before preparing the reaction mix.
The kit has been optimized to be used with:

- Applied Biosystems™ QuantStudio 3-5 and ABI 7500 thermal cyclers (Thermo Fisher Scientific),
- Rotor-Gene Q (Qiagen),
- CFX96,
- ELITe InGenius® (ELITechGroup S.p.A)
  For the compatibility with other instruments please contact Diatheva.

- Program the real-time PCR instrument with the following thermal profile:

Step	Temperature and times	Cycles
Initial denaturation	95°C 3 min	1 X
Denaturation	95°C 20 sec	50 X
Annealing-extension	60°C 60 sec	

Fluorescence is detected during annealing-extension step on green channel (FAM dye) for the target HIV-1 and yellow channel (VIC dye) for the cells (hTERT). Select the Non Fluorescent Quencher (NFQ) as quencher.

	For Rotor-Gene Q instrument that allows the gain optimization on the acquiring channels, set the gain optimization on NTC sample (tube position 1). • Select ROX as passive reference dye for instruments that require it (es. Applied Biosystems). The final reaction volume is 50 µL.
5. PCR MIX PREPARATION	Include in each amplification series a PCR Negative Control (NTC-No Template Control) and (Only for qualitative test) a Positive PCR Control. For the quantification, it is suggested to test the Standard Curve and samples in duplicate. • Thaw the HIV-1 Master Mix_PRO, ROX_PRO (protect from light) and PCR Negative Control_PRO. Vortex 15" and centrifuge briefly • For instruments that require a passive reference dye add ROX_PRO to HIV-1 Master Mix_PRO immediately before the use, according to the instructions below: -For Low ROX instruments (i.e.: ABI 7500, QuantStudio 3-5)→ add 24 µL of the ROX Dilution Buffer_PRO to the vial containing the 8 µL ROX, vortex for 30" and centrifuge briefly. Add 2.4 µL of Diluted ROX to the HIV-1 Master Mix_PRO vortex for 30" and centrifuge briefly. -For High ROX instruments (i.e.: ABI 7900)→ the ROX provided in the kit is ready-to-use (no dilution is required. Add 6.9 µL of ROX to the HIV-1 Master Mix_PRO vortex for 30" and centrifuge briefly. *Note: the kit MBK0087 provides separate vials of ROX, one for each HIV-1 Master Mix*_PRO *vial. It is recommended to dilute the ROX*_PRO *and to complete the HIV-1 Master Mix*_PRO *just before the use. The diluted ROX cannot be stored after the preparation.* • Aliquot 30 µL of HIV-1 Master Mix_PRO in the PCR tubes or in the plate prepared for the experiment. • Add 20 µL of PCR Negative Control_PRO into NTC. • In a separate area, add 20 µL of DNA samples to be tested, into the corresponding PCR tubes or wells containing amplification mixes. For qualitative test Add 20 µL of Positive PCR Control (prepared as indicated in section 3) into the corresponding PCR tubes or wells containing amplification mixes. For quantitative test Add 20 µL of calibration curve points ready to use (as indicated in section 2) into the corresponding PCR tubes or wells containing amplification mixes. • Seal hermetically the PCR tubes or plate and load into the real-time PCR instrument, following the manufacturer's instructions. Note: for PCR instrument with 96 wells block check that the reagents are at the bottom of each well, if not, briefly centrifuge at 800 x g for 1 minute.

6. DATA ANALYSIS

The analysis of the results should be done with the PCR instrument program, please refer to the manual for detailed information. Set the baseline and threshold values. Some software perform the data analysis automatically in this case it is advisable to check these settings. For a manual data analysis, analyse the PCR file for the two fluorophores separately. For a correct definition of the threshold it is necessary to select a value distinction from the background after the linear phase growth.
The analysis of results in Rotor-Gene Q Software must be done selecting only "Dynamic tube", without select the "Slope correct" function.

7. INTERPRETATION AND EXPRESSION OF RESULTS

The amplification signal must be characterised by a rapid and regular increase of the fluorescence values and not by peak events or gradual increase of the background signal.

7.1 QUALITATIVE TEST

a. Controls

Before proceeding with the analysis of samples, check the validity of controls. If results differ from those indicated in the tables below the PCR run is not valid and must be repeated.

QuantStudio, ABI 7500, CFX96

Control ID	Detection Channel	
	FAM (Green channel) Ct	*VIC (Yellow channel) Ct*
Positive PCR Control (HIV-1 Standard DNA 1_PRO)	21≤Ct≤25	22≤Ct≤26
PCR Negative Control_PRO (NTC)	No amplification signal	No amplification signal

Rotor-Gene Q

Control ID	Detection Channel	
	FAM (Green channel) Ct	*VIC (Yellow channel) Ct*
Positive PCR Control (HIV-1 Standard DNA 1_PRO)	21≤Ct≤25	19≤Ct≤24
PCR Negative Control_PRO (NTC)	No amplification signal	No amplification signal

b. Samples

Sample results shall be interpreted as shown in the table below:

HIV-1 (FAM-green channel)	Cells (hTERT) (VIC-yellow channel)	Interpretation
No amplification	Complying*	No HIV-1 DNA detected
	Not complying** or no amplification	*Partial or complete inhibition.* Sample DNA must be diluted. If the inhibition remains, a new extraction is recommended. *Unsuccess of the extraction step*. A new extraction is recommended.
Amplification	Non-significant	HIV-1 DNA present
*Cells (hTERT) is compliant if Ct ≤ 30 using QuantStudio, ABI 7500 and CFX96, Ct ≤ 26 using Rotor-Gene Q thermal cycler. **Cells (hTERT) is non-compliant if Ct > 30 using QuantStudio, ABI 7500 and CFX96, Ct > 26 using Rotor-Gene Q thermal cycler.		

7.2 QUANTITATIVE TEST

Controls and Standard

Before proceeding with the analysis of the samples, check the validity of PCR Negative Control and Standard Curve. If results differ from those indicated in the tables below the PCR run is not valid and must be repeated. If replicates of a standard dilution are not identical or one standard is significantly outside the dynamic range of the assay, it can be omitted to optimize the results and reach the parameters.

QuantStudio, ABI 7500, CFX96 and Rotor-Gene Q

Control ID	Detection Channel	
	FAM (Green channel) Ct	*VIC (Yellow channel) Ct*
PCR Negative Control_PRO (NTC)	No amplification signal	No amplification signal

Furthermore, for accurate quantification results, valid Standard Curves for the two target needs to be generated.

The Standard Curves must have the following control parameter values according to table below:

	Control parameters	Valid values
HIV-1 *FAM-green channel*	Slope	-3.74 / -3.00
	PCR efficiency	85% / 115%
	R^2	≥0.98
Cells (hTERT) *VIC-yellow channel*	Slope	-3.74 / -2.92
	PCR efficiency	85% / 120%
	R^2	≥0.98

The run is invalid if PCR Negative Control and Standard Curve parameters have not been met. In case of invalid run, repeat the PCR.

If the run is valid, continue with the interpretation of the sample results.

b. Samples
Sample results shall be interpreted as shown in the tables below:

HIV-1 (FAM-green channel)	Cells (hTERT) (VIC-yellow channel)	Interpretation
No amplification	Complying*	No HIV-1 DNA detected.
	Not complying** or no amplification	*Partial or complete inhibition.* Sample DNA must be diluted. If the inhibition remains, a new extraction is recommended. *Unsuccess of the extraction step*. A new extraction is recommended.
Amplification	Complying*	HIV-1 DNA present, possible quantification.
	Not complying** or no amplification	HIV-1 DNA present. *Partial inhibition.* Sample DNA must be diluted. If the inhibition remains, a new extraction is recommended. *Unsuccess of the extraction step.* A new extraction is recommended.
*Cells (hTERT) is compliant if Ct ≤ 30 using QuantStudio, ABI 7500 and CFX96, Ct ≤ 26 using Rotor-Gene Q thermal cycler. **Cells (hTERT) is non-compliant if Ct > 30 using QuantStudio, ABI 7500 and CFX96, Ct > 26 using Rotor-Gene Q thermal cycler.		

The instrument software automatically calculates the amount of HIV-1 DNA copies and cells content per reaction.
See the table below for correct interpretation:

Target	Quantification Result	Interpretation
HIV-1	N* ≥ 50,000 copies/reaction	HIV-1 DNA more than 50,000 copies
	5 ≤ N ≤ 50,000 copies/reaction	HIV-1 DNA quantitatively detected
	N ≤ 5 copies/reaction	HIV-1 DNA less than 5 copies
Cells (hTERT)	CN** ≥ 300,000 copies/reaction	Cells more than 300,000
	CN ≤ 300,000 copies/reaction	Cells quantitatively detected

*N is the HIV-1 copy number provided by the software
**CN is the cells content provided by the software

- The result could be reported as the number of HIV-1 DNA copies per 10^6 cells following the equation:

$$\mathbf{N}_{10(6)} = N \times 1{,}000{,}000 / CN$$

Where,
N: HIV-1 copies obtained by PCR reaction;
$\mathbf{N}_{10(6)}$: HIV-1 copy number in 10^6 cells;
CN: cell number obtained by PCR reaction, using hTERT target. Please consider that the obtained value is already converted in cell number (hTERT is present in two copies in a diploid genome).

- The result can be expressed as HIV-1 DNA copies/mL of blood following the equation:

$$\boldsymbol{N}1mL = \frac{\boldsymbol{N} \times [\boldsymbol{V}el \times (1000\mu L \div \boldsymbol{V}st)]}{\boldsymbol{V}DNAan}$$

Where,
N 1mL: HIV-1 copy number in 1 mL of blood;
Vel: volume in µL used in the elution step;
Vst: starting volume in µL of blood processed in the DNA extraction step;
VDNAan: volume in µL of extracted DNA (eluate) analysed in PCR (without dilution)

- If the DNA concentration is known, result could be expressed as HIV-1 DNA copies /µg DNA

Examples:
- 1 µg of extracted DNA is analysed: the quantification result is N/µg DNA
- 0.5 µg of extracted DNA is analysed: the quantification result is N x 2/µg DNA

- The result could finally be converted into copies of HIV-1 DNA/10^4 CD4+ cells, if the counts and the percentage of CD4+ lymphocytes in the analysed blood are known.