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HIV-1 DNA Test

MBK0086- 96 Tests
MBK0086-32T - 32 Tests

FOR RESEARCH USE ONLY

INTENDED USE

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

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 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 PCR mix that offers robust and consistent performances, even in presence of PCR inhibitors found in the most challenging samples. 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 Master Mix contains an Internal Control (IC) to assess the efficiency of amplification reaction revealing the presence of inhibitory factors in the sample.
The HIV-1 DNA Test also contains a Standard Curve ready to use with 5 levels of HIV-1 copies: 50,000, 5,000, 500, 50 and 5 copies/20 µL.
The system is optimized to analyse high amounts of good quality purified genomic DNA (up to 1 µg, about 150,000 cells) in 50 µL/reaction.

KIT CONTENTS

	No. vial x Volume	
Reagent	**MBK0086**	**MBK0086-32T**
HIV-1 Master Mix	3 X 1100 µL	1 X 1380 µL
ROX	3 X 5 µL	1 X 5 µL
ROX Dilution Buffer	1 X 100 µL	1 X 100 µL
PCR Negative Control	1 X 100 µL	1 X 100 µL
HIV-1 Standard DNA 1	1 X 170 µL	1 X 85 µL
HIV-1 Standard DNA 2	1 X 170 µL	1 X 85 µL
HIV-1 Standard DNA 3	1 X 170 µL	1 X 85 µL
HIV-1 Standard DNA 4	1 X 170 µL	1 X 85 µL
HIV-1 Standard DNA 5	1 X 170 µL	1 X 85 µL

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

SHIPPING CONDITIONS

Shipping at room temperature has no dendrimental effect on the performances of this kit

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 and ROX 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.
Different home-made protocols or commercial kits can be used to isolate DNA free of PCR inhibitors from whole blood. For further information regarding the compatibility with different extraction methods please contact Diatheva.

1.3 Determination of DNA concentration

Measure the optical density of each DNA sample by reading at 260 nm using a Nanospectrophotometer or Spectrophotometer.

1.4 Preparation of sample to be analysed

The total DNA concentration of each sample should be 50 ng/µL. This concentration precisely corresponds to 1 µg of DNA tested/PCR reaction. If necessary, samples can be diluted in distilled water (molecular biology grade) in order to obtain 50 ng/µL.

2. STANDARD CURVE (only for quantitative test)

The HIV-1 DNA Test 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.

Standard	Concentration
HIV-1 Standard DNA 1	50,000 copies /20 µL
HIV-1 Standard DNA 2	5,000 copies /20 µL
HIV-1 Standard DNA 3	500 copies /20 µL
HIV-1 Standard DNA 4	50 copies /20 µL
HIV-1 Standard DNA 5	5 copies /20 µL

3. POSITIVE PCR CONTROL (only for qualitative test)

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

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, ABI 7500 and 7900 thermal cyclers (Thermo Fisher Scientific),
- CFX96 Touch™ Deepwell Detection System (Biorad),
- Rotor-Gene Q (Qiagen).
  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 IC. 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 please consider that Standard Curve and samples are tested in duplicated.

- Thaw the components protect from light. Vortex 15" and centrifuge briefly
- For instruments that require a passive reference dye add ROX to HIV-1 Master Mix immediately before the use, according to the instructions below:

-For Low ROX instruments (i.e.: ABI 7500, QuantStudio 3-5)→ add 15 µL of the ROX Dilution Buffer to the vial containing the 5 µL ROX, vortex for 30" and centrifuge briefly. Add the Diluted ROX to the HIV-1 Master Mix according to the following table:

	MBK0086	MBK0086-32T
Diluted ROX	1.6 µL	2 µL

-For High ROX instruments (i.e: ABI 7900) → the ROX provided in the kit is ready-to-use (no dilution is required). Add the Diluted ROX to the HIV-1 Master Mix according to the following table:

	MBK0086	MBK0086-32T
Diluted ROX	4.5 µL	5.6 µL

Note: the kit provides separate vials of ROX, one for each HIV-1 Master Mix vial. It is recommended to dilute the ROX and to complete the HIV-1 Master Mix just before the use. The diluted ROX cannot be stored after the preparation.

- Thaw the HIV-1 Master Mix and PCR Negative Control. Vortex 15" and briefly spin vials in a microcentrifuge before opening.
- Aliquot 30 µL of HIV-1 Master Mix in the PCR tubes or in the plate prepared for the experiment.

- Add 20 µL of PCR Negative Control 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 performs 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

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, 7900, and CFX96

Control ID	Detection Channel	
	FAM (Green channel) Ct	*VIC (Yellow channel) Ct*
Positive PCR Control (HIV-1 Standard DNA 1)	22≤Ct≥26	Non-significant
PCR Negative Control (NTC)	No amplification signal	28≤Ct≥35

Rotor-Gene Q

Control ID	Detection Channel	
	FAM (Green channel) Ct	*VIC (Yellow channel) Ct*
Positive PCR Control (HIV-1 Standard DNA 1)	21≤Ct≥25	Non-significant
PCR Negative Control (NTC)	No amplification signal	27≤Ct≥34

b. Samples

HIV-1 (FAM-green channel)	IC (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 (is suggested to dilute the sample to 25 ng/uL and repeat the PCR run analysing 0.5 µg per PCR). If the inhibition remains, a new extraction is recommended
Ct≥10	Complying	HIV-1 DNA present, possible quantification
	Not complying* or no amplification	HIV-1 DNA present. Partial inhibition. Sample DNA must be diluted (is suggested to dilute the sample to 25 ng/uL and repeat the PCR run analysing 0.5 µg per PCR). If the inhibition remains, a new extraction is recommended
*The IC of a sample is non-compliant if the Ct value is not included in the interval 28≤Ct≥35 using QuantStudio, ABI 7500, 7900, and CFX96 instruments, 27≤Ct≥34 using Rotor-Gene Q thermal cycler.		

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

7.2 QUANTITATIVE TEST

a. 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. Only one standard replicate can be eliminated.

QuantStudio, ABI 7500, 7900, and CFX96

Control ID	Detection Channel	
	FAM (Green channel) Ct	*VIC (Yellow channel) Ct*
PCR Negative Control (NTC)	No amplification signal	28≤Ct≥35

Rotor-Gene Q

Control ID	Detection Channel	
	FAM (Green channel) Ct	*VIC (Yellow channel) Ct*
PCR Negative Control (NTC)	No amplification signal	27≤Ct≥34

Furthermore, for accurate quantification results, a valid Standard Curve needs to be generated. The Standard Curve must have the following control parameter values according to table below

Control parameters	Valid value
Slope	-3.74 / -3.00
PCR efficiency	85% / 115%
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)	IC (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 (is suggested to dilute the sample to 25 ng/uL and repeat the PCR run analysing 0.5 µg per PCR). If the inhibition remains, a new extraction is recommended
Ct≥10	Complying	HIV-1 DNA present, possible quantification
	Not complying* or no amplification	HIV-1 DNA present. Partial inhibition. Sample DNA must be diluted (is suggested to dilute the sample to 25 ng/uL and repeat the PCR run analyzing 0.5 µg per PCR). If the inhibition remains, a new extraction is recommended
*The IC of a sample is non-compliant if the Ct value is not included in the interval 28≤Ct≥35 using QuantStudio, ABI 7500, 7900, and CFX96 instruments, 27≤Ct≥34 using Rotor-Gene Q thermal cycler.		

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

HIV-1 DNA Quantification Result	Interpretation (HIV-1 DNA copies/reaction)
N* ≥50,000 copies/reaction	HIV-1 DNA more than 50,000 copies HIV-1 DNA detected but not quantifiable because above the limit of quantification
5≤N≥50,000 copies/reaction	5≤HIV-1 DNA ≤50,000 copies HIV-1 quantitatively detected
N ≤5 copies/reaction	HIV-1 DNA less than 5 copies HIV-1 DNA detected but not quantifiable because below the limit of quantification

*N is the copy number provided by the software

<u>The result should be expressed as HIV-1 DNA copies /µg DNA.</u>

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*2/µg DNA

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

$$\text{HIV-1 DNA}_{\text{copies/ 10(6) cells}} = \text{HIV-1 DNA}_{\text{copies/µg DNA}} \times (1{,}000{,}000 / 150{,}000)$$

- 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) analyzed in PCR (without dilution).

- The result could finally be converted into copies of HIV-1 DNA/104 CD4+ cells, if the counts and the percentage of CD4+ lymphocytes in the analysed blood are known.