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For Professional Use Only

AmpliSens® *HIV*-Monitor-FRT

PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE

AmpliSens® *HIV*-Monitor-FRT PCR kit is an *in vitro* nucleic acid amplification test for quantitative detection of *human immunodeficiency virus* type 1 (*HIV*-1) RNA in the biological material (blood plasma) using real-time hybridization-fluorescence detection of amplified products.

For research use only. Not for diagnostic procedures.

2. PRINCIPLE OF PCR DETECTION

Detection of *human immunodeficiency virus* type 1 (*HIV*-1) RNA by the polymerase chain reaction (PCR) is based on the amplification of a pathogen genome specific region using specific primers. In real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® *HIV*-Monitor-FRT PCR kit is a quantitative test that contains the Internal Control (Internal Control *HIV*-M-FRT (IC)). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

AmpliSens® *HIV*-Monitor-FRT PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase using a chemically modified polymerase (TaqF), which is activated by heating at 95 °C for 15 min.

HIV-1 RNA detection includes: RNA extraction and reverse transcription with real-time PCR of *HIV*-1 cDNA.

3. CONTENT

AmpliSens® *HIV*-Monitor-FRT PCR kit is produced in 3 forms:

Form 1: RIBO-prep variant 50, PCR kit variant FRT, **REF** TR-V0-P-M(RG,iQ,Mx,Dt)-CE.

Form 2: PCR kit variant FRT, *HIV*-Q calibration kit, **REF** R-V0-MC(RG,iQ,Mx,Dt)-CE.

Form 3: PCR kit variant FRT, *HIV*-Q calibration kit in bulk[1], **REF** R-V0-MC(RG,iQ,Mx,Dt)-CE-B.

[1] In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

RIBO-prep variant 50 includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
Solution for Lysis	clear liquid of blue color[2]	15	1 vial
Solution for Precipitation	colorless clear liquid	20	1 vial
Washing Solution 3	colorless clear liquid	25	1 vial
Washing Solution 4	colorless clear liquid	10	1 vial
RNA-buffer	colorless clear liquid	1.2	4 tubes

RIBO-prep variant 50 is intended for RNA/DNA extraction of 50 samples (including controls).

PCR kit variant FRT includes:

Reagent		*Description*	*Volume, ml*	*Quantity*
DTT frozen-dried		white powder	---	4 tubes
RT-PCR-mix-1-FRT *HIV*		colorless clear liquid	0.3	4 tubes
RT-PCR-mix-2-FEP/FRT		colorless clear liquid	0.2	4 tubes
Polymerase (TaqF)		colorless clear liquid	0.02	4 tubes
TM-Revertase (MMlv)		colorless clear liquid	0.01	4 tubes
DNA calibrator	**PIC1 *HIV****	colorless clear liquid	0.1	4 tubes
	PIC2 *HIV**	colorless clear liquid	0.1	4 tubes
Buffer for elution		colorless clear liquid	1.2	2 tubes
Negative Control (C–)**		straw-colored clear liquid	1.2	4 tubes
Positive Control-1-*HIV****		colorless clear liquid	0.06	4 tubes
Positive Control-2-*HIV****		colorless clear liquid	0.06	4 tubes
Internal Control *HIV*-M-FRT (IC)****		colorless clear liquid	0.28	4 tubes

* must be used in the amplification procedure as Positive Control of Amplification ($C+_1$, $C+_2$).

** must be used in the extraction procedure as the Negative Control of Extraction (C-).

*** must be used in the extraction procedure as the Positive Control of Extraction (PCE-1, PCE-2).

****must be used in the extraction procedure as the Internal Control (see Section 8.1 for details).

PCR kit variant FRT is intended for 80 tests including control samples and calibrators.

***HIV*-Q calibration kit** includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
Calibratior *HIV*-Q	yellow powder	---	1 tube
Solvent Q	colorless clear liquid	1.2	3 tubes

***HIV*-Q calibration kit** is intended for 1 calibration.

[2] If Solution for Lysis is stored at 2-8 °C, a crystalline precipitate may form.

Disk with **AmpliSens Soft Monitor FRT** (Microsoft Excel format) software intended for data processing and result obtaining is provided with PCR kit.

4. ADDITIONAL REQUIREMENTS

- RNA extraction kit for the forms 2 and 3.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters up to 200 µl and 1000 µl.
- Tube racks.
- Vortex mixer.
- PCR box.
- Disposable polypropylene 1.5 ml tubes.
- Refrigerator with the temperature from 2 to 8 °C.
- Deep-freezer with the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Thermostat with working temperature 25 °C to 100 °C (suitable for Eppendorf tubes).
- Desktop centrifuge up to 12,000 g (suitable for Eppendorf tubes).
- Vacuum aspirator with flask for removing a supernatant.
- Disposable 10-20 ml vial.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany), iCycler iQ5 (Bio-Rad, USA) or Mx3000P (Stratagene, USA).
- Disposable polypropylene 0.2 ml tubes for PCR:
  a) 0.2-ml PCR tubes with domed caps if a plate-type instrument is used;
  b) 0.2-ml PCR tubes with flat caps if a rotor-type instrument is used.
- Refrigerator with the temperature from 2 to 8 °C.
- Deep-freezer with the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all

other reagents and add it to the reaction mix in a distantly separated facility.

- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

Solution for Lysis  Danger	Contains substance: guanidine thiocyanate. H302: Harmful if swallowed. H312: Harmful in contact with skin. H314: Causes severe skin burns and eye damage H332: Harmful if inhaled. H412: Harmful to aquatic life with long lasting effects EUH032: Contact with acids liberates very toxic gas. P260: Do not breathe vapours. P264: Wash your hands thoroughly after handling. P273: Avoid release to the environment. P302+P352: IF ON SKIN: Wash with plenty of water. P501: Dispose of contents in accordance with national regulation.
Solution for Precipitation, Washing Solution 4  Danger	Isopropanol EC No 200-661-7 CAS No 67-63-0 H225: Highly flammable liquid and vapour. H319: Causes serious eye irritation. H336: May cause drowsiness or dizziness P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P261: Avoid breathing vapours. P264: Wash your hand thoroughly after handling. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. P403+P233: Store in a well ventilated place. Keep container tightly closed. P501: Dispose of contents in accordance with national regulation.
Washing Solution 3  Warning	Contains substance: isopropyl alcohol H226: Flammable liquid and vapour. H319: Causes serious eye irritation. H336: May cause drowsiness or dizziness P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P261: Avoid breathing vapours. P264: Wash your hand thoroughly after handling. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. P403+P233: Store in a well ventilated place. Keep container tightly closed. P501: Dispose of contents in accordance with national regulation.

6. SAMPLING AND HANDLING

Obtaining samples of biological material for PCR-analysis, transportation, and storage are described in detail in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting the work.

AmpliSens® *HIV*-Monitor-FRT PCR kit is intended for the analysis of RNA extracted with nucleic acid extraction kits from:

— *Peripheral blood plasma*

Collect a blood sample in a tube with 3% EDTA solution in the ratio of 20:1 (20 parts of blood to 1 part of EDTA). Invert the closed tube several times to ensure adequate mixing. Remove and transfer the plasma specimen in a new tube within 6 h from the time of blood taking. To do this, centrifuge the tube with blood at 800 – 1600 g for 20 min.

In some cases, blood serum can be used. In this case, the analytical sensitivity of the reagent kit is retained; however, the clinical sensitivity may be significantly decreased as a result of precipitation of viral particles during blood clot retraction.

Storage of plasma and serum samples:

– at 2–8 °C for up to 3 days;

– at ≤–68 °C for a long time.

7. WORKING CONDITIONS

AmpliSens® *HIV*-Monitor-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA extraction

It's recommended that the following nucleic acid extraction kits are used:

- **RIBO-prep**, (included in form 1; the extraction procedure is described in Section 8.1.1);
- **MAGNO-sorb**, REF K2-16-1000-CE;
- **NucliSENS easyMAG** automated nucleic acid extraction system (bioMérieux, France) can also be used. (See Guidelines [2] for details);
- See Section 8.1.2 if extraction is carried out with nucleic acid extraction kits not included in this PCR kit.

If using the MAGNO-sorb kit extract the RNA/DNA according to the manufacturer's protocol taking into account following additions and improvements:

- In case of DNA extraction from blood plasma sample of 1000 µl, the volume of the **Internal Control STI-87 (IC)** required for 24-tube panel is **0.28 ml**. In case of other panels and DNA extraction from blood plasma sample of 200 µl see the MAGNO-sorb instruction manual.

- To prepare the Positive Control of Extraction 1 (**PCE-1**), add **90 µl** of the **Negative Control (C–)** sample and **10 µl** of the **Positive Control-1-*HIV*** sample to the new tube containing **Lysis Solution MAGNO-sorb**.
- To prepare the Positive Control of Extraction 2 (**PCE-2**), add **90 µl** of the **Negative Control (C–)** sample and **10 µl** of the **Positive Control-2-*HIV*** sample to the new tube containing **Lysis Solution MAGNO-sorb**.
- To prepare the Negative Control of Extraction (**C–**), add **100 µl** of the **Negative Control (C–)** sample to the new tube containing **Lysis Solution MAGNO-sorb**.
- The volume of **Buffer for elution** required for extraction from both 1000 and 200 µl of blood plasma samples is **70 µl**.

8.1.1 RNA extraction with RIBO-prep variant 50

For sensitivity enhancement, it is recommended to carry out an additional ultracentrifugation of 1 ml of plasma within 1 hour at 24,000 g at the temperature from 2 to 8 °C. Remove 900 µl of supernatant and work with the pellet (100 µl) as described below. Carry out the ultracentrifugation in the 1.5 ml screw-cap tubes.

1. Warm up **Solution for Lysis** (if stored at 2–8 °C) at 65 °C until the ice crystals disappear.
2. Prepare the required number of 1.5-ml tubes including the tubes for Negative and Positive Controls of Extraction. Label the tubes.
3. Add **10 µl** of **Internal Control *HIV*-M-FRT (IC)** to the bottom of each test tube.
4. Add **300 µl** of **Solution for Lysis** per each tube. Label the test tubes.

If a large number of samples is being tested, it is acceptable to mix the **Solution for Lysis** and the **Internal Control** in a separate sterile flask (based on addition of **300 µl** of **Solution for Lysis** and **10 µl** of **Internal Control** per one sample), followed by a transfer of **300 µl** of the prepared mix into each of the previously prepared **1.5 µl** tubes.

5. Add **100 µl** of **test samples** using tips with filters. Secure the tubes and vortex them. Centrifuge the tubes to sediment the drops from the caps.
6. For each panel it is necessary to carry out the control reactions as follows:

PCE-1 – Add **90 µl of Negative Control (C–)** and **10 µl of Positive Control-1-*HIV*** to the tube with lysis solution labelled PCE-1 (Positive control of Extraction);

PCE-2 – Add **90 µl of Negative Control (C–)** and **10 µl of Positive Control-2-*HIV*** to the tube with lysis solution labelled PCE-2 (Positive control of Extraction);

C– – Add **100 µl of Negative Control (C–)** to the tube with lysis solution labelled C- (Negative control of Extraction).

Vortex the control tubes and sediment the drops from the caps.

7. Incubate the tubes at **65 °C** for **5 min** and vortex them. Vortex the tubes and sediment the drops from the caps.
8. Add **400 µl** of **Solution for Precipitation** and mix with vortex.
9. Centrifuge all tubes at **12,000 g** (for example, 13,400 rpm for the centrifuge *MiniSpin, Eppendorf*) for **5 min**.
10. Carefully remove and discard the supernatant from the tubes using vacuum aspirator. Do not disturb the pellet. Use a new tip for each tube.
11. Add **500 µl of Washing Solution 3** per each tube. Tightly close the tubes and carefully invert them 3-5 times to ensure washing of the pellet.
12. Centrifuge the tubes at **12,000 g** for **1-2 min**.
13. Carefully remove and discard the supernatant from the tubes using vacuum aspirator. Do not disturb the pellet. Use a new tip for each tube.
14. Add **200 µl of Washing Solution 4** per each tube. Tightly close the tubes and carefully invert them 3-5 times to ensure washing of the pellet.
15. Centrifuge the tubes at **12,000 g** for **2 min**.
16. Carefully remove and discard the supernatant from the tubes using vacuum aspirator. Do not disturb the pellet. Use a new tip for each tube.
17. Incubate the tubes at **65 °C** for **5 min** to dry the sediment. Make sure the tubes are open.
18. Add **50 µl** of **RNA-buffer** to each tube and vortex. Incubate at **65 °C** for **5 min** periodically stirring with vortex.
19. Centrifuge the tubes at **12,000 g** for **1 min**.

The RNA-samples are ready for reverse transcription and amplification. It is recommended to carry out the reverse transcription and amplification immediately after obtaining the purified RNA.

It is not recommended to store the RNA-samples longer than 30 min at the temperature from 2 to 8 °C. For long-time storage transfer the supernatant without disturbing the sorbent into a sterile tube and store at the temperature from minus 24 to minus 16 °C for 1 month or at the temperature below minus 68 °C for 1 year.

8.1.2 Calibration and calculation of the coefficient B using *HIV-Q* calibration kit if extraction is carried out with nucleic acid extraction kits not included in this PCR kit (for the forms 2 and 3).

The claimed analytical features of the PCR kit forms 2 and 3 are guaranteed only then the extraction is performed using the reagents kits recommended by Federal Budget Institute of Science "Central Research Institute for Epidemiology".

If coefficient B is not specified in the *Important Product Information Bulletin* for the extraction kit/automatic platform, the calibration for calculation of coefficient B should be carried out by oneself with the aid of the ***HIV-Q* calibration kit** included in this PCR kit. See below for details.

The calibration procedure is necessary to define Coefficient B and it is performed during the first PCR run for the given lot. Calibration is performed only once for each new lot of the **AmpliSens® *HIV*-Monitor-FRT** PCR kit and is conducted with the RNA extraction kit/automatic station used in the PCR assay.

To carry out calibration, it is necessary to analyse 5 extra samples: the repeat of Positive Control-1-*HIV*, the repeat of Positive Control-2-*HIV*, and calibrator *HIV*-Q in triplicate.

Calibrator *HIV*-Q preparation

1. Vortex the tube with **calibrator *HIV*-Q**, gently open the tube, and add **400 µl of solvent Q** avoiding the contents spraying.
2. Close the tube and incubate it at room temperature for 20 min vortexing periodically.
3. Once the contents are fully dissolved, vortex the tube for 3-5 s to make sure that there are no drops on the caps of the tube.

Perform calibration with the same RNA extraction kit used in the PCR assay.

Extract the RNA according to the manufacturer's protocol.

Transfer 10 µl of **Internal Control *HIV*-M-FRT (IC)** (per one sample) to samples or **Lysis solution** before extraction.

In case of extracting from 100 µl of plasma, add dissolved **calibrator *HIV*-Q** to three tubes for RNA extraction (100 µl per each tube).

In case of extracting from any other plasma volume (100 – 1000 µl), transfer dissolved **calibrator *HIV*-Q** to three tubes for RNA extraction (100 µl per each tube) and add **solvent Q** up to the extraction volume (for example, if the extraction volume is 1 ml then add 100 µl of **calibrator *HIV*-Q** and 900 µl of **solvent Q**).

When extraction is completed, perform RT-PCR as described in this instruction manual.

Use the mean concentration values obtained in the channels for the FAM and JOE fluorophores for three repeats with **calibrator *HIV*-Q** for calculation of coefficient B using the following formula:

$$\textbf{Coefficient B} = \frac{\text{IC cDNA copies in calibrator } \textit{HIV}\text{-Q (FAM channel)}}{\textit{HIV} \text{ cDNA copies in calibrator } \textit{HIV}\text{-Q (JOE channel)}} \times \text{coefficient C}$$

Coefficient C is specified in the *Important Product Information Bulletin* enclosed in the **AmpliSens® *HIV*-Monitor-FRT** PCR kit.

The calculated value of coefficient B should be within range specified in the *Important Product Information Bulletin* enclosed in the applied PCR kit lot

Write down the coefficient B value in *the Important Product Information Bulletin* enclosed with the <u>given lot</u> of the PCR kit and use it for concentrations calculation of biological and control samples (See the Data Analysis section).

Also see the Guidelines [2] to **AmpliSens® *HIV*-Monitor-FRT** PCR kit.

Write down the calculated values for Positive Control-1-*HIV* and Positive Control-2-*HIV* in the *Important Product Information Bulletin* enclosed with the <u>given lot</u> of the PCR kit.

Determine the mean value for both Positive Contorl-1-*HIV* and for Positive Control-2-*HIV*. Set the acceptable value range for both Positive Contorl-1-*HIV* and for Positive Control-2-*HIV* as follows: from *"calculated mean value" / 3* to *"calculated mean value" x 3.*

For example,

<u>the calculated values</u> for Positive Contorl-1-*HIV* in two replicates are 500,000 copies/ml and 700,000 copies/ml;

<u>the calculated mean value</u> for Positive Contorl-1-*HIV* is 600,000 copies/ml;

the <u>acceptable value range</u> for Positive Contorl-1-*HIV* varies from 200,000 to 1,800,000 copies/ml.

Write down the calculated acceptable value range for Positive Control-1-*HIV* and for Positive Control-2-*HIV* in the *Important Product Information Bulletin,* and use it to verify further assays conducted using <u>this lot</u> of the PCR kit. (See Data Analysis section).

8.2 Preparing the reverse transcription and PCR

RNase-free and DNase-free disposable sterile plastic ware should be used only. The choice of the tubes for amplification depends on the used real-time instrument.

8.2.1 Preparing tubes

The total reaction volume is **50 µl**, the volume of RNA sample is **25 µl**.

Prepare the reaction mixture just before PCR analysis. Reaction mixture should be made for required number of reactions including test samples and controls.

1. Thaw the reagents, thoroughly vortex, and centrifuge shortly to remove drops from the caps of the tubes.
2. Take the required number of PCR tubes including test samples, controls and calibrators.
3. **To prepare reaction mixture**: add the entire content of the tube with **RT-PCR-mix-2-FEP/FRT** to the tube with **DTT frozen-dried.** Thoroughly vortex the tube then remove drops from the tube walls by short centrifuging. The prepared mixture can be stored at 2–8 °C for up to 1 week. Mix in a new tube the following components (given volumes are calculated for one reaction):
   - **15 µl of RT-PCR-mix-1-FRT *HIV***,
   - **10 µl of the mixture of RT-PCR-mix-2-FEP/FRT with DTT frozen-dried**,
   - **1.0 µl of polymerase (TaqF)**,
   - **0.5 µl of TM-Revertase (MMlv)**.

   Thoroughly vortex the tube and then remove drops from the tube walls by short centrifuging. Reaction mixture for 20 reactions should be prepared in case of extraction from 16 samples (extraction with the use of two NucliSens easyMAG plates): to the tube with **DTT frozen-dried** add the entire content of the tube with **RT-PCR-mix-2-FEP/FRT**, the entire content of the tube with **RT-PCR-mix-1-FRT *HIV***, the entire content of the tube with **polymerase (TaqF)** and the entire content of the tube with **TM-Revertase (MMlv)**. Do not store the prepared mixture.
4. Add **25 µl** of the mixture to the tubes. Discard unused mixture.
5. Using filter tips add **25 µl of RNA samples** obtained at the RNA extraction stage. Thoroughly mix by pippeting. Avoid forming air bubbles.

Avoid transferring of the sorbent together with the RNA sample in case of extraction with NucliSENS easyMAG automated nucleic acid extraction system or MAGNO-sorb kit.

6. Carry out the control reactions:

PCE-1 – Add **25 µl of RNA sample extracted from Positive Control-1-*HIV*** to the tube labelled **PCE-1** (Positive Control of Extraction).

PCE-2 – Add **25 µl of RNA sample extracted from Positive Control-2-*HIV*** to the tube labelled **PCE-2** (Positive Control of Extraction).

C– – Add **25 µl of RNA sample extracted from Negative Control (C–) to** the tube labelled **C–** (Negative control of Extraction).;

$C+_1$ – Add **DNA calibrator PIC1 *HIV*** to the two tubes labelled **$C+_1$** (Positive Control of Amplification). (**25 µl per each tube)**;

$C+_2$ – Add **DNA calibrator PIC2 *HIV*** to the two tubes labelled **$C+_2$** (Positive Control of Amplification). (**25 µl per each tube)**.

Thoroughly mix by pippeting. Avoid forming air bubbles.

To rule out possible contamination, carry out an additional control reaction:

NCA – Add **25 µl** of **Buffer for elution** to the tubes labelled NCA (Negative Control of Amplification).

Remove drops from the tube's walls by short centrifuging (1-3 s) prior to placing into a plate-type instrument.

8.2.2. Amplification

1. Create a temperature profile on your real-time instrument as follows:

Table 1

HIV-Monitor-FRT amplification program for rotor-type instruments

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*
Hold	50	30 min	–	1
Hold	95	15 min	–	1
Cycling	95	20 s	–	5
	52	30 s	–	
	72	30 s	–	
Cycling 2	95	20 s	–	40
	55	30 s	FAM, JOE	
	72	30 s	–	

Table 2

HIV-Monitor-FRT amplification program for plate-type instruments

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*
1	50	30 min	–	1
2	95	15 min	–	1
3	95	20 s	–	5
	52	30 s	–	
	72	30 s	–	
4	95	20 s	–	42
	55	40 s	FAM, JOE	
	72	30 s	–	

2. Insert the tubes into the reaction module of the device.
3. Run the amplification program with fluorescence detection.
4. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels:

– IC cDNA amplification product is detected in the channel for the FAM fluorophore;

– *HIV* cDNA amplification product is detected in the channel for the JOE fluorophore.

Results are interpreted by the presence (or absence) of the intercept between the fluorescence curve and the threshold line set at a certain level (in the middle of linear fragment of the positive control fluorescence growth in log scale), which determines presence (or absence) of *Ct* (cycle threshold) value of a sample in the corresponding cell of the result table.

Based on the *Ct* values (the intercept of the fluorescence curve and the threshold line set at a certain level) and on the specified values for the calibrators, PIC1 *HIV* and PIC2 *HIV*, the calibration line will automatically plot and produce the values for the number of *HIV* cDNA copies (channel for the JOE fluorophore) and for the number of Internal Control cDNA copies (channel for the FAM fluorophore) in a PCR sample. The retrieved values are used for the *HIV* RNA concentration calculation in tested and control samples, using the formulae:

$$\frac{\textit{HIV}\text{ cDNA copies per PCR-sample}}{\text{IC cDNA copies per PCR-sample}} \times \text{coefficient A} \times \text{coefficient B} = \textit{HIV}\text{ RNA copies/ml of plasma}$$

$$\text{Coefficient A} = \frac{100}{\text{extraction volume, µl}}$$

Coefficient A = 1 when calculating PCE-1 and PCE- 2 concentrations

Coefficient B (number of copies of IC per ml of plasma) is specified in the *Important Product Information Bulletin* provided with the PCR kit and is specific for each lot. It cannot be used with PCR kits of different lots. If forms 4 and 5 are used then coefficient B is calculated as the result of calibration during the first PCR run of PCR kit of a specific lot (see section 8.1.4 for details).

If the result is greater than 10,000,000 copies/ml then it is interpreted as the **greater than 10,000,000 copies of *HIV* RNA/ml result.** If the obtained value is greater than the linear range, then the sample may be re-tested after 10x dilution; the produced result is multiplied by 10.

If the result is less than 500 copies/ml (extraction from 100 µl), or less than 250 copies/ml (extraction from 200 µl), or less than 50 copies/ml (extraction from 1 ml), than it is interpreted as the **less than 500,** or **less than 250,** or **less than 50 copies of *HIV* RNA/ml result**, respectively.

To convert results from copies/ml into International Units (IU/ml), multiply results obtained in copies/ml by 1.75 (1 copy= 1.75 IU, 1 IU=0.57 copy)

The boundary concentration values for IC sample are specified in the *Important Product Information Bulletin* enclosed in PCR kit.

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Positive and Negative Controls of extraction are correct (see Table 3).

Table 3

Results for controls

Control	Stage for control	Result of amplification in the channel for the fluorophore	
		FAM	**JOE**
C–	RNA extraction, PCR	**Positive** (IC concentration is greater than the boundary value)	Negative (*Ct* value is absent)
PCE-1	RNA extraction, PCR	**Positive** (IC concentration is greater than the boundary value)	**Positive** (concentration calculated with IC copies/ml should be within range)
PCE-2	RNA extraction, PCR	**Positive** (IC concentration is greater than the boundary value)	**Positive** (concentration calculated with IC copies/ml should be within range)
$C+_1$	PCR	Positive	Positive
$C+_2$	PCR	Positive	Positive
NCA	PCR	Negative *Ct* value is absent	Negative (*Ct* value is absent)

Boundary (minimum allowable) concentration values for IC and the range of values for **PCE-1 (Positive Control-1-*HIV)*** and **PCE-2 (Positive Control-2-*HIV)*** calculated with IC copies/ml are specified in the *Important Product Information Bulletin* enclosed to the PCR kit of specific lot.

10. TROUBLESHOOTING

Results of analysis are not taking into account in the following cases:

1. If *Ct* value is obtained for the Negative Control of extraction (C–) in the channel for the JOE fluorophore and/or Negative Control of amplification (NCA) in the channels for the FAM and JOE fluorophores, analysis (beginning with RNA extraction) should be repeated for all samples in which *HIV* RNA was detected.
2. If concentration value of IC in the results table is less than the boundary value specified in the Bulletin, the sample should be retested beginning with the first stage of the analysis.
3. If correlation coefficient R^2 is less than 0.98, PCR should be repeated for all samples
4. If calculated concentration values of Positive Control-1-*HIV* and Positive Control-2-*HIV* do not fall in the range specified in the *Important Product Information Bulletin*, analysis (beginning with RNA extraction) should be repeated for all samples.

11. TRANSPORTATION

AmpliSens® *HIV*-Monitor-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **RIBO-prep variant 50** are to be stored at 2–8 °C when not in use. All components of **PCR kit variant FRT** and ***HIV*-Q calibration kit** are to be stored at temperature from minus 24 to minus 16 °C when not in use. All components of the **AmpliSens® *HIV*-Monitor-FRT** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

RT-PCR-mix-1-FRT *HIV* is to be kept away from light.

Do not repeat freeze-thaw cycles more than twice for Positive Control-1-*HIV,* Positive Control-2-*HIV,* PIC1 *HIV,* PIC2 *HIV,* Internal Control *HIV*-M-FRT. Store the above-mentioned reagents at 2–8 °C for up to 6 months after thawing.

13. SPECIFICATIONS

13.1. Sensitivity

The linear measurement range of **AmpliSens® *HIV*-Monitor-FRT** PCR kit is specified in the table below.

Biological material	Volume of sample for extraction, µl	RNA extraction kit	Linear measurement range of *HIV*-1 RNA, copies/ml
Blood plasma	100	RIBO-prep NucliSENS easyMAG	500–10,000,000
Blood plasma *(ultracentrifuged)*	1,000	RIBO-prep	50–10,000,000
Blood plasma	1,000	NucliSENS easyMAG	50–10,000,000

13.2. Specificity

The analytical specificity of **AmpliSens® *HIV*-Monitor-FRT** PCR kit is ensured by selection of specific primers and probes as well as by selection of strict reaction conditions. The primers and probes were checked for possible homologies to all in sequences published gene banks by sequence comparison analysis as well as with genomic DNA/RNA of the following organisms and viruses: *hepatitis A virus; hepatitis B virus; hepatitis C virus; hepatitis D virus; cytomegalovirus; Epstein-Barr virus; herpes simplex virus* types 1 and 2; *varicella-zoster virus; human herpes virus* types 6 and 8; *parvovirus* B19; *tick-borne encephalitis virus; West Nile encephalitis; adenovirus* types 2, 3, and 7; *Escherichia coli*; *Staphylococcus aureus; Streptococcus pyogenes,*

Streptococcus agalactiae; and *Homo sapiens*. No cross-reaction was observed for the aforementioned organisms and viruses.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2010.
2. Guidelines to **AmpliSens® *HIV*-Monitor-FRT** PCR kit for quantitative detection of *human immunodeficiency virus* type 1 (*HIV*-1) RNA in the biological material by polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *HIV*-Monitor-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
RUO	Research use only		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation	**IC**	Internal control
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
	GHS02: Flame	**$C+_1$, $C+_2$**	Positive controls of amplification
	GHS05: Corrosion	**PCE-1, PCE-2**	Positive controls of extraction
	GHS07: Exclamation mark		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
29.01.15 ME	Footer	**REF** R-V0-MC(RG,iQ,Mx,Dt)-CE-B was added
	Content	The form in bulk was added
06.03.15 ME	Text	Text was corrected in accordance with the template
	4. Additional requirements	The section was completed with the additional requirements for RIBO-sorb-12, RIBO-prep and MAGNO-sorb kits
	8.1. RNA extraction	The following sections was added 8.1.1 RNA extraction with RIBO-sorb-12 nucleic acid extraction kit 8.1.2 RNA extraction with RIBO-prep nucleic acid extraction kit variant 50 8.1.3 RNA extraction with MAGNO-sorb nucleic acid extraction kit variant 100-1000 8.1.4 Calibration and calculation of the coefficient B using *HIV-Q* calibration kit if extraction is carried out with nucleic acid extraction kits not included in this PCR kit (for the forms 4 and 5)
31.03.15 PM	5. General precautions, 14. Key to symbols used	Information about hazards was corrected
08.05.15 ME	Text	Clinical material was changed to biological
	1. Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” was changed to “For research use only. Not for diagnostic procedures”
	13.2. Specificity	The phrase “The clinical specificity of AmpliSens® *HIV*-Monitor-FRT PCR kit was confirmed in laboratory clinical trials” was deleted
14.12.15 ME	12. Stability and storage	The storage temperature of Negative Control (C–) reagent was specified for different forms
30.06.16 PM	12. Stability and storage	The storage temperature of Negative Control (C–) reagent for different forms was deleted
07.11.17 PM	Content, through the text	The forms with RIBO-sorb-12 and MAGNO-sorb kits were deleted. Number of forms was changed from 5 to 3
	5. General precautions, 14. Key to symbols used	Information about hazards was rewritten according to the Regulation 1272/2008/EC.