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For Professional Use Only

AmpliSens® RNA-*HIV*-FRT

PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE

AmpliSens® RNA-*HIV*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *human immunodeficiency virus (HIV-1)* RNA in the clinical material (blood plasma) by means of real-time hybridization-fluorescence detection.

The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Human immunodeficiency virus (HIV-1) detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product. The real-time PCR monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening of the reaction tubes after the PCR run. **AmpliSens® RNA-*HIV*-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 by using chemically modified polymerase (TaqF) that is activated by heating at 95ºC for 15 min.

HIV-1 RNA detection includes:

(a) total RNA extraction from blood plasma simultaneously with the Internal Control;

(b) reverse transcription of cDNA on RNA matrix followed by real-time PCR detection of cDNA.

3. CONTENT

AmpliSens® RNA-*HIV*-FRT PCR kit is produced in 1 form:

AmpliSens® RNA-*HIV*-FRT PCR kit variant FRT (for use with RG, iQ, Mx)

REF R-V0-R(RG,iQ,Mx)-CE.

AmpliSens® RNA-*HIV*-FRT 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

RNA-eluent		colorless clear liquid	0.07	4 tubes
DNA calibrator of Positive Control of Amplification	**C3 *HIV***	colorless clear liquid	0.025	4 tubes
DNA calibrator of Internal Control	**I3 *HIV***	colorless clear liquid	0.025	4 tubes
Positive Control-1-*HIV**		colorless clear liquid	0.01	4 tubes
Negative Control (C–)**		colorless clear liquid	0.5	4 tubes
Internal Control *HIV*-FRT (IC)***		colorless clear liquid	0.13	4 tubes

*must be used in the extraction procedure as Positive Control of Extraction (PCE)

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

*** must be used in the extraction as an Internal Control (see protocols for RIBO-sorb, **REF** K2-12-50-CE or NucliSENS easyMAG automated system (bioMérieux, France)).

AmpliSens® RNA-*HIV*-FRT PCR kit is intended for 76 reactions (including controls and calibrators).

4. ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia) Instrument; iCycler iQ or iQ5 (Bio-Rad, USA) Instrument; Mx3000P/Mx3005P (Stratagene, USA) Instrument.
- Disposable polypropylene microtubes for PCR (0.2-ml; for example, Axygen, USA)**.**
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.

- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes and mucose membranes. If any of these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- Workflow in the laboratory must proceed in a unidirectional manner, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

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

6. SAMPLING AND HANDLING

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

AmpliSens® RNA-*HIV*-FRT PCR kit is intended to analyze DNA extracted with DNA extraction kits from:

- *Blood plasma*

Take a blood sample in a tube with 3 % EDTA solution (1:20) after overnight fasting. Invert closed tube several times to ensure adequate mixing. Remove and transfer 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 rpm for 20 min.

Storage of plasma samples:

– at 2–8 °C for up to 3 days;

– at ≤ – 16 °C for a long time.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1 RNA extraction

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

- RIBO-sorb (adapted to the extraction of *HIV*-1 RNA), **REF** K2-12-50-CE;
- NucliSENS easyMAG automated system (bioMérieux, France).

Isolate RNA according to the manufacturer's protocol.

If the NucliSENS easyMAG automated system is used:

- set a sample volume as 0.1 ml or 1 ml;
- set an eluate volume as 55 µl;
- both *On-board* and *Off-board* Lysis Buffer Dispensing and Lysis Incubation are possible.

8.2 Preparing the PCR

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

8.2.1 Preparing tubes for PCR

1. Prepare required number of the 0.2 ml microtubes.
2. Prepare the **reaction mixture.** To do this, add successively **300 µl of RT-PCR-mix-1-FRT *HIV*, 200 µl of RT-PCR-mix-2-FEP/FRT, 20 µl of polymerase (TaqF), and 10 µl of TM-Revertase (MMlv)** to the tube with **DTT frozen-dried**. Thoroughly vortex. Make sure that there are no drops on the walls of the tubes; otherwise, centrifuge briefly Prepared mixture should be used immediately.
3. Transfer **25 µl of prepared mixture** per each tube. Discard the unused mixture.
4. Using tips with aerosol barrier **add 25 µl of RNA sample** obtained from clinical or control samples in RNA extraction. Carefully mix by pipetting. **Avoid sorbent transferring together with RNA samples.**
5. Carry out **control amplification reactions** (3 per one panel):

NCA - Add **25 µl of RNA-eluent** to the tube for Negative Control of Amplification (NCA);

C3 - Add **25 µl of C3 *HIV*** to the tube for Positive Control of Amplification of PC;

I3 - Add **25 µl of I3 *HIV*** to the tube for Positive Control of Amplification of IC.

8.2.2. Amplification

Program the thermocycler according to **Manufacturer's manual** and **Appendix 1, Appendix 2,** or **Appendix 3**.

9. DATA ANALYSIS

***HIV* RNA** amplification product is detected in the **JOE/Yellow/HEX** channel. **Internal Control** amplification product is detected in the **FAM/Green** channel.

The results are interpreted by the software of used instrument by the crossing (or no crossing) of fluorescence curve with the threshold line that corresponds with presence (or absence) of Ct value in the result grid.

Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed.

Results of controls

Control	Stage for control	Ct channel FAM (Green)	Ct channel JOE (Yellow)	Interpretation
C-	RNA extraction	<Ct* (Pos)	Neg	OK
PCE	RNA extraction	<Ct* (Pos)	<Ct* (Pos)	OK
NCA	Amplification	Neg	Neg	OK
C3	Amplification	Not applicable	<Ct* (Pos)	OK
I3	Amplification	<Ct* (Pos)	Not applicable	OK

*For Ct values, see **Appendix** enclosed in the instruction manual.

1. The sample is considered **positive** if its Ct detected in the JOE/Yellow/HEX channel is less than the value specified in the Appendix.
2. The sample is considered **negative** if its Ct detected in the JOE/HEX/Yellow channel is either absent or more than the value specified in the Appendix, while Ct obtained in the FAM channel is less than the value specified in the Appendix.

10. TROUBLESHOOTING

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

1. If positive result is not detected for Positive Control of Extraction (Positive Control-1-*HIV*). It indicates failures in RNA extraction. Repeat extraction for all samples with negative result.
2. If positive signal is not detected for Positive Control of Amplification (C+). It can indicate errors in PCR conducting. The PCR should be repeated.

3. If Ct value of a sample is not detected in both channels or the obtained Ct value is higher than the specified one. It indicates errors in clinical material processing that led to the loss of RNA or inhibition of RT and PCR. The analysis should be repeated starting from RNA extraction.
4. If positive signal is detected for Negative Controls (C- or NCA). It indicates the contamination of reagents or samples. In this case results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis and to take measures to detect and eliminate the source of contamination.

11. TRANSPORTATION

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

12. STABILITY AND STORAGE

All components of the **AmpliSens® RNA-*HIV*-FRT** PCR kit (except for DTT frozen-dried, RT-PCR-mix-1-FRT *HIV*, RT-PCR-mix-2-FEP/FRT, polymerase (TaqF), and TM-Revertase (MMlv)) are to be stored at 2–8 °C. All components of the **AmpliSens® RNA-*HIV*-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.

DTT frozen-dried, RT-PCR-mix-1-FRT *HIV*, RT-PCR-mix-2-FEP/FRT, polymerase (TaqF), and TM-Revertase (MMlv) are to be stored at temperature from minus 24 to minus 16 °C when not in use.

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

13. SPECIFICATIONS

13.1. Sensitivity

Analytical sensitivity of **AmpliSens® RNA-*HIV*-FRT** PCR kit is not less than 100 *HIV* RNA copies/ml.

The claimed analytical features of **AmpliSens® RNA-*HIV*-FRT** PCR kit are guaranteed only when RIBO-sorb reagent kits or NucliSENS easyMAG automated system are additionally used.

13.2. Specificity

Specificity of **AmpliSens® RNA-*HIV*-FRT** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all sequences published in the gene banks by sequence comparison analysis. Specificity of **AmpliSens® RNA-*HIV*-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, transportation, 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, 2008.

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 RNA-*HIV*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

	Catalogue number		Caution
	Batch code		Sufficient for
	Research use only		Expiration Date
	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**C+**	Positive control of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
20.01.11 RT	Content	The names of calibrators were corrected
	——	Corrections through the text
	Stability and storage	The phrase about keeping away from light of RT-PCR-mix-1-FRT *HIV* is added
09.07.11 LA	Cover page	The phrase “For Professional Use Only” was added
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of reagents before and after the first use was added
		Information that RT-PCR-mix-1-FRT *HIV* is to be kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
22.06.12 LA	Cover page	Symbol IVD was replaced by RUO symbol
	16. Key to symbols used	