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For Professional Use Only

AmpliSens® DNA-*HIV*-FRT

PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE

AmpliSens® DNA-*HIV*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of human immunodeficiency virus type 1 (*HIV*-1) proviral DNA in clinical materials by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

HIV-1 proviral DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the viral genome specific region using specific *HIV*-1 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 during thermocycling. The real-time PCR 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® DNA-*HIV*-FRT** PCR kit is a qualitative test based on the use of an endogenous control, the β-globin gene. The DNA target selected as an endogenous internal control is a human genome fragment that is present in sample in a sufficient quantity equivalent to that of cells in the sample. **AmpliSens® DNA-*HIV*-FRT** PCR kit uses “hot-start,” which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® DNA-*HIV*-FRT PCR kit is produced in 1 variant:

Variant with sorption on silica gel:

AmpliSens® DNA-*HIV*-FRT includes **Gem-sorb** reagent kit, **AmpliSens** reagent kit variant FRT, **REF** TR-V0-G(RG,iQ)-CE.

Gem-sorb reagent kit includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
Hemolytic	colorless clear liquid	100	2 vials
Lysis Solution	colorless clear liquid	30	1 vial
Washing Solution 1	colorless clear liquid	30	1 vial
Washing Solution 2	colorless clear liquid	100	1 vial
Universal Sorbent	white suspension	1.25	2 tubes
TE-buffer for DNA elution	colorless clear liquid	5.0	2 tubes

Gem-sorb reagent kit is intended for 100 DNA extractions (including controls).

AmpliSens reagent kit variant FRT includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FRT *HIV*	colorless clear liquid	0.24	8 tubes
PCR-mix-2-FRT	colorless clear liquid	0.2	8 tubes
Polymerase (TaqF)	colorless clear liquid	0.02	8 tubes
TE-buffer	colorless clear liquid	0.07	8 tubes
Positive Control DNA *HIV*-1 (C+$_{HIV\text{-}1}$)*	colorless clear liquid	0.2	1 tube
Positive Control cellular DNA (C+$_{\text{cellular DNA}}$)**	colorless clear liquid	0.2	1 tube

***** must be used in the extraction procedure as Positive Control of Extraction (PCE) and in the PCR as Positive Control of Amplification (C+$_{HIV\text{-}1}$).

****** must be used in the PCR as Positive Control of Amplification (C+$_{\text{cellular DNA}}$).

AmpliSens reagent kit variant FRT is intended for 120 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA 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.
- Personal thermocyclers (for example, Rotor-Gene 2000/3000/6000 (Corbett Research, Australia); iCycler iQ or iQ5 (Bio-Rad, USA) or equivalent).
- Disposable polypropylene tubes for PCR (0.1- or 0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.
- Reagent kit for workplace processing.
- Thermostatic bath or dry block for tubes with controlled temperature and capable of incubating at 25°C and 100 °C.
- Vacuum aspirator with flask for removing supernatant.
- Disposable polypropylene 1.5-ml tubes with tightly sealing caps.
- Tube racks for 1.5 ml reaction tubes.

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 and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 samples and unused reagents in compliance with local authorities' requirements.
- Samples 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 another suitable disinfectant.
- Avoid contact with the skin, eyes, and mucous membranes. If skin, eyes, and mucous membranes contact, immediately flush with water, seek medical attention.
- 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.
- The laboratory process must be one-directional, 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 samples of biological materials for PCR-analysis, transportation and storage are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® DNA-*HIV*-FRT PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from whole blood samples. 2 ml of blood is taken into disposable tube with 0.2 ml of 3 % EDTA. After taking blood, tube is closed; the content of the tube is mixed by stirring 3-4 times. Store samples at 2–8 °C for no longer than 48 h.

6.1. Disinfection of test material.

Disinfection of biological material and reagents should be performed at the each stage separately. Place disposable plastic dish (tubes, tips), flasks of vacuum aspirators into special containers with 0.2 % DP-2T or another disinfectant for 20–24 h.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits:

- **Gem-sorb** reagent kit.

Extract DNA according to the manufacturer's instructions.

DNA extraction with Gem-sorb reagent kit.

Volume of clinical material is 0.25 µl.

Lysis of clinical material

1. Prepare the required number of 1.5-ml disposable polypropylene tubes with tightly

sealing caps for samples and controls and mark them. Add **1.0 ml of hemolytic** and **0.25 ml of whole blood** to the tubes intended for clinical samples according to labeling. Use a new tip for each tube. If blood from newborns is analyzed, the volume of blood is **0.1 ml**. Close the tubes and mix by vortexing.

2. Incubate tubes at room temperature for 3 min. Mix them by vortexing and incubate for 3 min once more.
3. Centrifuge the tubes at 8,000 rpm for 2 min. Carefully remove and discard the supernatant from each tube without disturbing the pellet using a vacuum aspirator. Use a new tip for each tube.
4. Add **0.5 ml of hemolytic** to the pellet, mix by vortexing and incubate for 3 min.
5. Centrifuge the tubes at 8,000 rpm for 2 min. Carefully remove and discard the supernatant from each tube without disturbing the pellet using vacuum aspirator. Use a new tip for each tube.
6. Repeat the washing leukocytes with **hemolytic**. After the last washing, the pellet should be white, a pink tint above the pellet (erythrocyte debris) is allowed.

   The pellet with leukocytes can be immediately lysed or frozen and stored at ≤ –68 °C for a long time.
7. **Lysis Solution** and **Washing Solution 1** (if stored at 2-8 °C) should be heated up to 65 °C until ice crystals disappear.
8. Add **300 µl** of **Lysis Solution** to leukocytes pellet to each tube. Mix by vortexing until the cells are fully resuspended.
9. **Preparing Positive Control (C+)**. Add **300 µl** of **Lysis Solution** and **5 µl of Positive Control DNA *HIV*-1 (C+$_{HIV\text{-}1}$).**
10. **Preparing Negative Control (NCA)**. Add **300 µl** of **Lysis Solution** and **5 µl of TE-buffer for DNA elution.**
11. Thoroughly resuspend **Universal Sorbent** on a vortex mixer. Add **25 µl** of **Universal Sorbent** to each tube using new tips. Carefully vortex the tubes. Leave the tubes in the tube rack for 10 min, vortexing them every 2 min.
12. Centrifuge the tubes with **Universal Sorbent** at 5,000 rpm for 30 s (for sorbent precipitation) and carefully remove the supernatant from each tube without disturbing the pellet using a vacuum aspirator. Use a new tip for every tube.
13. Add **300 µl** of **Washing Solution 1** to each tube. Vortex tubes until the Universal Sorbent is fully resuspended. Centrifuge at 5,000 rpm for 30 s. Carefully remove the supernatant from each tube without disturbing the pellet using a vacuum aspirator. Use a new tip for each tube.

14. Add **500 µl** of **Washing Solution 2** to each tube. Vortex the tubes until the sorbent is fully resuspended. Centrifuge at 10,000 rpm for 30 s. Carefully remove the supernatant from each tube using a vacuum aspirator. Use a new tip for every tube.
15. Repeat step 14.
16. Incubate all tubes with opened caps at 65 °C for 10 min (for drying the sorbent).
17. Add **50 µl** of **TE-buffer for DNA elution**. Vortex tubes. Incubate the tubes at 65 °C for 5 min.
18. Centrifuge the tubes at full speed for 1 min. The supernatant contains the purified DNA. Samples are ready for PCR amplification.

    The purified DNA can be stored:

    – at 2–8 °C for 1 week;
    – at ≤ –16 °C for 1 year.

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

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

1. Prepare the required number of 0.2-ml tubes for amplification of DNA from clinical and control samples.
2. To carry out 15 reactions, add **160 µl** of **PCR-mix-2-FRT** and **16 µl** of **Polymerase (TaqF)** to the tube with **PCR-mix-1-FRT *HIV*.** Vortex the tube, make sure there are no drops on the caps. Discard the rest of the reaction mixture.
3. Transfer **25 µl** of the prepared mixture to each tube for amplification.
4. Add **25 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage to the prepared tubes with reaction mixture using tips with aerosol barrier. Carefully mix by pipetting.

When adding DNA samples, avoid transferring the sorbent to the reaction mixture.

5. Carry out 3 control amplification reactions for each panel:

NCA - Add **25 µl** of **TE-buffer** instead of DNA sample to the tube labeled NCA (Negative Control of Amplification).

C+$_{HIV\text{-}1}$ - Add **25 µl** of **Positive Control DNA *HIV*-1 (C+$_{HIV\text{-}1}$)** diluted 10 times with TE-buffer to the tube labeled **C+$_{HIV\text{-}1}$** (Positive Control of Amplification).

C+$_{cellular\ DNA}$ - Add **25 µl** of **Positive Control cellular DNA** to the tube labeled **C+$_{cellular\ DNA}$** (Positive Control of Extraction).

8.2.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual.

1. Create a temperature profile on your instrument as follows (see Appendices 1 and 2):

Table 1

AmpliSens DNA-*HIV* amplification program

	Rotor-type instruments[1]			Plate-type instruments[2]		
Step	**Temperature, °C**	**Time**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
Hold	**95**	15 min	1	**95**	15 min	1
Cycling 1	**95**	20 s	5	**95**	20 s	5
	52	30 s		**52**	30 s	
	72	30 s		**72**	30 s	
Cycling 2	**95**	20 s	40	**95**	20 s	42
	55	30 s *fluorescent signal detection*		**55**	40 s *fluorescent signal detection*	
	72	30 s		**72**	30 s	

Fluorescent signal is detected in the channels designed for the FAM/Green and JOE/Yellow/HEX fluorophores on the 2nd step (55°C) of stage Cycling 2.

2. Insert 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

The fluorescent signal intensity is detected in two channels:

– The signal from the Internal Control amplification product is detected in the FAM fluorescence channel;

– The signal from the *HIV* DNA amplification product is detected in the JOE channel.

9.1. Interpretation of results

The results are interpreted by the Instrument software by the crossing (or not-crossing) of the fluorescence curve with the threshold line and are shown as the presence (or absence) of Ct (threshold cycle) in the result grid (see Appendices 1 and 2).

The result of the analysis is considered reliable only if the results for Positive and Negative Controls of Amplification as well as Negative Control of Extraction are correct.

1. The sample is considered to be **positive** if its Ct value detected in the result grid in the channel for Positive Control is less than the specified boundary Ct value.
2. The sample is considered to be **negative** if its Ct value is not detected in the result grid in the channel for Positive Control (the fluorescence curve does not cross the

[1] For example, Rotor-Gene 2000, Rotor-Gene 3000, Rotor-Gene 6000 or equivalent.
[2] For example, iCycler iQ and iQ5, or equivalent.

threshold line) and if the Ct value determined in the results grid in the channel for IC does not exceed the specified boundary Ct value.

10. TROUBLESHOOTING

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

- If the Ct value of negative control of extraction (C–) or amplification (NCA) is less than the specified boundary Ct value, this indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. Analysis must be repeated and measures to detect and eliminate the source of contamination must be taken.
- If no signal is detected for the positive controls of amplification (C+), this may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components did not comply with the manufacturer's instruction, or that the reagent kit expired. Programming of the used instrument, storage conditions, and the expiration date of the reagents should be checked, and then PCR should be repeated.
- If no signal is detected for the positive control of extraction (PCE) in any detection channel, this indicates incorrect extraction procedure. Repeat analysis starting from the DNA extraction stage.
- If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample that has a fluorescence curve without the typical exponential growth phase (the curve is linear), this may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be repeated (if iCycler iQ or iQ5 instruments are used).

11. TRANSPORTATION

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

12. STABILITY AND STORAGE

Components of the **Gem-sorb** and **AmpliSens** reagent kits (except for PCR-mix-1-FRT *HIV*, PCR-mix-2-FRT, and polymerase (TaqF)) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® DNA-*HIV*-FRT** PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of

unopened reagents, unless otherwise stated.

PCR-mix-1-FRT *HIV*, PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at temperature from minus 24 to minus 16 °C when not in use.

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

13. SPECIFICATIONS

13.1. Analytical sensitivity

The analytical sensitivity of **AmpliSens® DNA-*HIV*-FRT** PCR kit estimated in genome equivalents per 1 ml of sample (GE/ml) is specified in the table below.

Clinical material	Nucleic acid extraction kit	PCR kit	Volume of clinical material, µl	Analytical sensitivity, GE/ml DNA *HIV*-1
Whole blood	RIBO-prep, DNA-sorb-B	AmpliSens® DNA-*HIV*-FRT	250	100
			100	250
Whole blood	NucliSENS easyMAG	AmpliSens® DNA-*HIV*-FRT	100	$1x10^3$
Dried blood spot	RIBO-prep	AmpliSens® DNA-*HIV*-FRT	One spot, d = 12 mm	$1x10^3$

13.2. Specificity

The analytical specificity of **AmpliSens® DNA-*HIV*-FRT** PCR kit is ensured by selection of specific primers and probes as well as by selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all 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 D virus; hepatitis C virus; cytomegalovirus; Epstein-Barr virus; herpes simplex virus* types 1 and 2; *chicken pox virus; human herpes virus* types 6 and 8; *parvovirus B19*; *tick-borne encephalitis virus*; *West Nile encephalitis virus*; *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.

The clinical specificity of **AmpliSens® DNA-*HIV*-FRT** was confirmed in laboratory clinical trials.

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, 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® DNA-*HIV*-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		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
IC	Internal control	**C+**	Positive control of amplification

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
03.07.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
14.11.12 LA	Cover page	IVD symbol was replaced with RUO symbol
	16. Key to symbols used	