AmpliSens® DNA-*HIV*-FRT PCR kit

RUO

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Contains sufficient for <n> tests
RUO	Research use only		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C+	Positive control of amplification
	GHS07: Exclamation mark	C–	Negative control of extraction
	GHS02: Flame	PCE	Positive control of extraction
	GHS05: Corrosion	IC	Internal control
	GHS08: Health hazard		

1. INTENDED USE

AmpliSens® DNA-*HIV*-FRT PCR kit is not a medical device. PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *human immunodeficiency virus* type 1 (*HIV*-1) proviral DNA in the biological material (whole blood) using real-time hybridization-fluorescence detection of amplified products.

NOTE: For research use only. Not for diagnostic procedures.

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 the 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 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 a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	IC DNA	*HIV*-1 DNA
Target gene	DNA region of the β-globin gene	*Rev*/5'LTR

3. CONTENT

AmpliSens® DNA-*HIV*-FRT PCR kit is produced in 2 forms:

Form 1: Gem-sorb, **PCR kit variant FRT**, REF TR-V0-G(RG,iQ)-CE,

Form 2: Gem-sorb, **PCR kit variant FRT in bulk**[1], REF TR-V0-G(RG,iQ)-CE-B.

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	suspension from white to dark beige colour	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).

PCR kit variant FRT includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT *HIV*	clear liquid from colorless to light lilac colour	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 ($C+_{cellular\ DNA}$)**	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 ($C+_{cellular\ DNA}$).

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

4. ADDITIONAL REQUIREMENTS

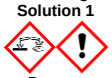
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- Thermostatic bath or dry block for tubes with controlled temperature and capable of incubating at 25°C and 100 °C.
- Vacuum aspirator with flask for removing supernatant.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 2000/3000/6000 (Corbett Research, Australia); iCycler iQ or iCycler iQ5 (Bio-Rad, USA) or equivalent).
- Disposable polypropylene 0.2-ml PCR tubes.
- Disposable polypropylene 1.5-ml tubes with tightly sealing caps.
- Refrigerator at 2-8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

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

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters 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 inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- 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.

Lysis Solution, Washing Solution 1  **Danger**	Contains substance: guanidine thiocyanate. H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H318: Causes serious 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. P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. P501: Dispose of contents in accordance with national regulation.
Washing Solution 2  **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 hands 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.
Universal Sorbent  **Danger**	Contains substance: Celite® H350: May cause cancer. H372: Causes damage to organs through prolonged or repeated exposure. P260: Do not breathe dust. P281: Use personal protective equipment as required. P314: Get Medical advice/attention if you feel unwell. P405: Store locked up. P501: Dispose of contents in accordance with national regulations.

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

6. SAMPLING AND HANDLING

NOTE: 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 biological material (whole blood).

Sampling

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.

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

NOTE: Extract DNA according to the manufacturer's instructions.

DNA extraction with Gem-sorb reagent kit.
Volume of biological material is 0.25 ml.
Lysis of biological 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** into the tubes intended for clinical samples according to labeling. Use a new tip for each tube. If newborns' blood 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 the temperature not more than minus 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 of extraction (PCE).** Add **300 µl** of **Lysis Solution** and **5 µl of Positive Control DNA *HIV*-1 (C+$_{HIV\text{-}1}$).**
10. **Preparing Negative Control of extraction (C–).** 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 the temperature not more than minus 16 °C for 1 year.

8.2. Preparing the PCR

8.2.1 Preparing tubes for PCR

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

1. Take 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)** into 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 into 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

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

5. Carry out the 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 10-fold diluted **Positive Control DNA *HIV*-1 (C+$_{HIV\text{-}1}$)** with TE-buffer to the tube labeled **C+$_{HIV\text{-}1}$** (Positive Control of Amplification).

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

C– — Add **25 µl** of **the sample extracted from the TE-buffer for DNA elution reagent** to the tube labeled C– (Negative control of Extraction).

PCE — Add **25 µl** of **the sample extracted from the Positive Control DNA *HIV*-1 (C+$_{HIV\text{-}1}$) reagent** to the tube labeled PCE (Positive control of Extraction).

8.2.2 Amplification

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

Table 2

AmpliSens DNA-*HIV* amplification program

	Rotor-type instruments[2]			Plate-type instruments[3]		
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 for the FAM and JOE fluorophores

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

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

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:

- The signal from the Internal Control amplification product is detected in the channel for the FAM fluorophore;
- The signal from the *HIV* DNA amplification product is detected in the channel for the JOE fluorophore.

The results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value in the corresponding column of the in the result grid.

Principle of interpretation is the following:

- The sample is considered to be **positive** if its *Ct* value detected in the result grid in the channel for the JOE fluorophore is less than the specified boundary *Ct* value.
- The sample is considered to be **negative** if its *Ct* value is not detected in the result grid in the channel for the JOE fluorophore (the fluorescence curve does not cross the threshold line) and if the *Ct* value determined in the results grid in the channel for the FAM fluorophore does not exceed the specified boundary *Ct* value.

NOTE: Boundary *Ct* values are specified in the Guidelines [2]

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

Table 3

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		JOE	FAM
C–	DNA extraction	Absent	Absent
PCE	DNA extraction	<boundary value	Absent
NCA	PCR	Absent	Absent
C+$_{HIV\text{-}1}$	PCR	<boundary value	not evaluated
C+$_{cellular\ DNA}$	PCR	not evaluated	<boundary value

10. TROUBLESHOOTING

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

1. If no signal is detected for the positive control of extraction (PCE), this indicates incorrect extraction procedure. Repeat analysis starting from the DNA extraction stage.
2. If no signal is detected for the positive controls of amplification (C+), this indicates mistakes at the PCR stage (for example, an incorrect amplification program). The PCR should be repeated.
3. If the *Ct* value is not determined in the channel for the JOE fluorophore as well as in the channel for the FAM fluorophore or the determined *Ct* value exceed the specified boundary value, this indicates incorrect pretreatment of biological material which led to the DNA loss or PCR inhibition. The PCR analysis should be repeated from the extraction stage.
4. If any *Ct* value is determined for the negative control of extraction (C–) in the channel for the JOE fluorophore or for the negative control of amplification (NCA) in any channel, 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.

11. TRANSPORTATION

AmpliSens® DNA-*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 **Gem-sorb** are to be stored at 2–8 °C when not in use. All components of the **PCR kit variant FRT** (except for TE-buffer, Positive Control DNA *HIV*-1 (C+$_{HIV\text{-}1}$) and Positive Control cellular DNA (C+$_{cellular\ DNA}$)) are to be stored at the temperature from minus 24 to minus 16 °C when not in use. All components of the **AmpliSens® DNA-*HIV*-FRT** PCR kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

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

NOTE: TE-buffer, Positive Control DNA *HIV*-1 (C+$_{HIV\text{-}1}$) and Positive Control cellular DNA (C+$_{cellular\ DNA}$) are to be stored at 2–8 °C when not in use.
When received the PCR kit should be dekitted in accordance with the stated temperature limits

13. SPECIFICATIONS

13.1. Analytical sensitivity

The analytical sensitivity of **AmpliSens® DNA-*HIV*-FRT** PCR kit is 500 genome equivalents per 1 ml of sample (GE/ml).

13.2. Specificity

The analytical specificity of **AmpliSens® DNA-*HIV*-FRT** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

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.
2. Guidelines to the **AmpliSens® DNA-*HIV*-FRT** PCR kit for qualitative detection of human immunodeficiency virus type 1 (*HIV*-1) proviral DNA 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® DNA-*HIV*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

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	
12.03.15 PM	Footer	REF TR-V0-G(RG,iQ)-CE-B was added
	Content	The form in bulk was added
18.03.15 ME	Text	Corrections according to the template
	9. Data analysis	The table "Results for controls" was added
	10. Troubleshooting	The section was rewritten
	13.1. Analytical sensitivity	Analytical sensitivity was corrected to 500 GE/ml
	13.2. Specificity	The list of organisms and viruses was deleted
	14. References	Reference to Guidelines was added
	16. Key to symbols used	"PCE" designation was added
31.03.15 ME	5. General precautions, 14. Key to symbols used	Information about hazards was added
14.05.15 PM	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® DNA-*HIV*-FRT PCR kit was confirmed in laboratory clinical trials" was deleted
10.07.15 ME	8.2.1. Preparing tubes for PCR	The volume of the reagent for PCE carrying out was corrected
08.11.17 PM	Through the text	Correction according to the template
	5. General precautions, 14. Key to symbols used	Information about hazards was rewritten according to the Regulation 1272/2008/EC.
08.12.17 ME	Content	Descriptions of Universal Sorbent and PCR-mix-1-FRT *HIV* was specified
27.12.17 PM	12. Stability and storage	The storage temperature of PCR kit components was specified
05.02.18 PM	8.2.1. Preparing tubes for PCR	The name of control was specified
15.07.20 KK	Through the text	The text formatting was changed
	Footer	The phrase "For research use only. Not for diagnostic procedures" was added
14.08.20 KK	2. Principle of PCR detection	The table with targets was added
16.11.20 MM	Content	The second form was deleted – PCR kit variant FRT in bulk
	Footer	REF TR-V0-G(RG,iQ)-CE-B was deleted
04.08.23 BA	Footer	The REF TR-V0-G(RG,iQ)-CE-B was added
	3. Content	The form in bulk was added
14.12.23 EM	5. General precautions	Information about hazards was rewritten according to the Regulation (EU) 2020/878

AmpliSens®

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