

IVD

For *in Vitro* Diagnostic Use

For Professional Use Only

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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 (whole blood and dried blood spots) 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 5 forms:

AmpliSens[®] DNA-HIV-FRT form 1 includes RIBO-prep variant 100, “Hemolytic” (1 vial), and PCR kit variant FRT.

AmpliSens[®] DNA-HIV-FRT form 2 includes RIBO-prep variant 100, Solution for Lysis (2 vials), Solution for Precipitation (1 vial), “Hemolytic” (1 vial), and PCR kit variant FRT.

AmpliSens[®] DNA-HIV-FRT form 3 includes DNA-sorb-B variant 100, “Hemolytic” (2 vials), and PCR kit variant FRT.

AmpliSens[®] DNA-HIV-FRT form 4 includes extraction kit for NucliSENS easyMAG (bioMérieux, France), “EM-plus”, “Hemolytic” (3 vials), and PCR kit variant FRT (3 kits).

AmpliSens[®] DNA-HIV-FRT form 5 includes PCR kit variant FRT.

ATTENTION! Forms 1 and 2 are intended for use with both whole blood and dried blood spots as a clinical material. Forms 3 and 4 are intended for use only with whole blood as a clinical material.

REF TR-V0-G-P1(RG,iQ,Mx,Dt)-CE; TR-V0-G-P2(RG,iQ,Mx,Dt)-CE; TR-V0-G-S(RG,iQ,Mx,Dt)-CE; R-V0-G(RG,iQ,Mx,Dt)-CE / **VER** 15.10.10-07.12.10 / Page 3 of 17

RIBO-prep nucleic acid extraction kit variant 100 includes:

Reagent	Description	Volume, ml	Quantity
Solution for Lysis	blue clear liquid ¹	30	1 vial
Solution for Precipitation	colorless clear liquid	40	1 vial
Washing Solution 3	colorless clear liquid	50	1 vial
Washing Solution 4	colorless clear liquid	20	1 vial
RNA-buffer	colorless clear liquid	1.2	8 tubes

RIBO-prep nucleic acid extraction kit variant 100 is intended for 100 RNA/DNA extractions (including controls).

RIBO-prep is a component of forms 1 and 2 of AmpliSens[®] DNA-HIV-FRT.

AmpliSens[®] DNA-HIV-FL form 2 is supplemented with the following reagents:

Reagent	Description	Volume, ml	Quantity
Solution for Lysis	blue clear liquid ¹	30	2 vials
Solution for Precipitation	colorless clear liquid	40	1 vial

AmpliSens[®] DNA-HIV-FRT form 1 is intended for 100 RNA/DNA extraction reactions (including controls) if whole blood is used and for 42 RNA/DNA extraction reactions (including controls) if dried blood spots are used as a clinical material .

AmpliSens[®] DNA-HIV-FRT form 2 is intended for 100 RNA/DNA extraction reactions (including controls) if whole blood is used and for 100 RNA/DNA extraction reactions (including controls) if dried blood spots are used as a clinical material.

DNA-sorb-B nucleic acid extraction kit variant 100 includes:

Reagent	Description	Volume, ml	Quantity
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

DNA-sorb-B nucleic acid extraction kit variant 100 is intended for 100 RNA/DNA extractions (including controls).

DNA-sorb-B is a part of form 3 of AmpliSens[®] DNA-HIV-FRT.

¹ If Solution for Lysis is stored at 2-8 °C, a crystalline precipitate may form.

REF TR-V0-G-P1(RG,iQ,Mx,Dt)-CE; TR-V0-G-P2(RG,iQ,Mx,Dt)-CE; TR-V0-G-S(RG,iQ,Mx,Dt)-CE; R-V0-G(RG,iQ,Mx,Dt)-CE / **VER** 15.10.10-07.12.10 / Page 4 of 17

Extraction kit for **NucliSENS easyMAG** (bioMérieux, France) includes:

Reagent	Description	Volume, ml	Quantity
NucliSens easyMAG Lysis Buffer	colorless clear liquid	1,000	1 vial
NucliSens easyMAG Extraction Buffer 1	colorless clear liquid	1,000	2 vials
NucliSens easyMAG Extraction Buffer 2	colorless clear liquid	1,000	2 vials
NucliSens easyMAG Extraction Buffer 3	colorless clear liquid	1,000	2 vials
NucliSens easyMAG Magnetic Silica	black suspension	0.6	24 tubes

Extraction kit for **NucliSENS easyMAG** is intended for 288 RNA/DNA extractions (including controls).

AmpliSens[®] DNA-HIV-FRT form 4 also includes **EM-plus** reagent kit:

Reagent	Description	Volume, ml	Quantity
RT-G	colorless clear liquid	30	1 vial
Component A	colorless clear liquid	3.0	1 tube

EM-plus is intended for 288 RNA/DNA extractions (including controls).

Hemolytic reagent for initial whole blood processing includes:

Reagent	Description	Volume, ml	Quantity
Hemolytic	colorless clear liquid	100	1 vial

Hemolytic reagent is intended for processing 100 clinical samples (including controls).

Hemolytic reagent is a part of forms 1 and 2 (1 vial), 3 (2 vials), and 4 (3 vials) of AmpliSens[®] DNA-HIV-FRT.

AmpliSens[®] DNA-HIV-FRT PCR kit variant FRT includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT HIV	colorless clear liquid	0.24	8 tubes
RT-PCR-mix-2-FEP/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 and human DNA (C+)*	colorless clear liquid	0.2	2 tubes

* must be used in the extraction procedure as positive control of extraction (PCE) and in the PCR as positive control of amplification (C+).

AmpliSens[®] DNA-HIV-FRT PCR kit variant FRT is intended for 120 reactions (including controls).

PCR kit variant FRT is a part of forms 1, 2, 3, and 5 (1 kit) and form 4 (2 kits) of AmpliSens[®] DNA-HIV-FRT.

4. ADDITIONAL REQUIREMENTS

- DNA/RNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 1000 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- 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.
- Tube racks for 1.5 ml reaction tubes.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); iCycler iQ or iQ5 (Bio-Rad, USA) or equivalent).
- Disposable polypropylene microtubes for PCR (0.1-ml or 0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ -16 °C.
- Waste bin for used tips.
- Permanent pen for labeling.

When dried blood spots are used as a clinical material:

- Hand-held puncher (1/4 inch or 6mm) (for example, Finnzymes, Finland).

When use NucliSENS easyMAG (bioMérieux, France):

1. Robotic station for RNA/DNA extraction NucliSENS easyMAG (bioMérieux, France).
2. Extraction kit and disposables for NucliSENS easyMAG (bioMérieux, France).
3. EM-plus reagent kit (CRIE, Russia).

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 is 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 or dried blood spots. Forms 1 and 2 are used to analyze both whole blood and dried blood spots; forms 3 and 4 are used to analyze whole blood only.

7. WORKING CONDITIONS

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

8. PROTOCOL

7.1. DNA extraction

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

- RIBO-prep, **REF** K2-9-Et-100-CE,
- DNA-sorb-B, **REF** K1-2-100-CE,
- Extraction kit for **NucliSENS easyMAG** (bioMérieux, France).



Extract DNA according to the manufacturer's instructions.

8.1.1. DNA extraction with RIBO-prep from whole blood

Volume of clinical material is 0.1 or 0.25 ml of whole blood

Initial processing of whole blood.

1. Prepare required number of 1.5 ml disposable polypropylene microcentrifuge tubes and mark them. Add **1.0 ml of «Hemolytic»** to each tube. Add **0.25 ml** of whole blood according labeling. Use new tip for each tube. If blood of newborns is analyzed, the volume of blood is **0.1 ml**. Close the tubes and mix by vortexing.
2. Incubate the tubes at room temperature for 5 min. Mix them by vortexing and incubate at room temperature for another 5 min.
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 and 200-µl tips. Use a new tip for each tube.

The pellet with leukocytes can be immediately lysed or frozen and stored at ≤ -16 °C for two weeks or ≤ -68 °C for a long time.

REF TR-V0-G-P1(RG,iQ,Mx,Dt)-CE; TR-V0-G-P2(RG,iQ,Mx,Dt)-CE; TR-V0-G-S(RG,iQ,Mx,Dt)-CE; R-V0-

(RG,iQ,Mx,Dt)-CE / **VER** 15.10.10-07.12.10 / Page 7 of 17

1. **Solution for Lysis** (if stored at 2–8 °C) should be heated at 60–65 °C until the ice crystals disappear.
2. Prepare one 1.5-ml disposable polypropylene microcentrifuge tube for Negative Control of Extraction (**Negative Control, C-**) and add **5 µl of RNA-buffer**.
3. Prepare one 1.5-ml disposable polypropylene microcentrifuge tube for Positive Control of Extraction (**Positive Control, PCE**) and add **5 µl of Positive Control DNA HIV-1 and human DNA**.
4. Add **300 µl** of **Solution for Lysis** to each tube.
5. Tightly close all tubes and incubate them at 65 °C for 5 min.
6. Add **400 µl** of **Solution for Precipitation** and mix by vortexing.
7. Centrifuge all tubes at 13,000 rpm for 5 min.
8. Carefully remove and discard the supernatant from each tube without disturbing the pellet using a vacuum aspirator and 200-µl tips. Use a new tip for each tube.
9. Add **500 µl of Washing Solution 3** to each tube, tightly close the tubes, and shake them carefully 3–5 times to ensure pellet washing.
10. Centrifuge all tubes at 13,000 rpm for 1–2 min.
11. Carefully remove and discard the supernatant from each tube without disturbing the pellet using a vacuum aspirator and 200-µl tips. Use a new tip for each tube.
12. Add **200 µl of Washing Solution 4** to each tube, tightly close the tubes, and shake them carefully 3–5 times to ensure pellet washing.
13. Centrifuge all tubes at 13,000 rpm for 1–2 min.
14. Carefully remove and discard supernatant from each tube without disturbing the pellet using a vacuum aspirator and 200-µl tips. Use a new tip for each tube.
15. Incubate all tubes with open caps at **65 °C for 5 min** (to partially dry the pellet).
16. Add **50 µl** of **RNA buffer** to each tube. Mix the contents of the tubes by vortexing. Then incubate them at 65 °C for 5 min under periodic stirring by vortexing.
17. Centrifuge the tubes at 13,000 rpm for 1 min.

The supernatant contains purified DNA and is ready for PCR amplification.

The purified RNA/DNA can be stored

- at 2-8 °C for 24 hours;
- at ≤ -16 °C for 1 year.

8.1.2. DNA extraction with RIBO-prep from dried blood spots

The volume of clinical material for DNA extraction is 1 dried blood spot.

1. **Solution for Lysis**, if stored at 2–8 °C, should be heated at 60–65 °C until the ice crystals disappear.
2. Prepare the required number of 1.5-ml disposable polypropylene microcentrifuge tubes and mark them.
3. Prepare the required number of sterilized punchers (6.0 mm).
4. Cut the whole dried blood spot using a new puncher and cutting mat for each sample and transfer it to the respectively labeled tube. Four or five serial cuttings for the whole spot may require.
5. Prepare one 1.5-ml disposable polypropylene microcentrifuge tube for Negative Control of Extraction (**Negative Control, C-**) and add **5 µl of RNA-buffer**.
6. Prepare one 1.5-ml disposable polypropylene microcentrifuge tube for Positive Control of Extraction (**Positive Control, PCE**) and add **5 µl of Positive Control DNA HIV-1 and human DNA**.
7. Add **700 µl** of **Solution for Lysis** to each tube. Tightly close all tubes and mix the contents of the tubes by vortexing.
8. Incubate all tubes at 65 °C for 30 min. While incubating, mix the contents of the tubes by vortexing every 8–10 min.
9. Prepare the required number of 1.5-ml disposable polypropylene microcentrifuge tubes for clinical samples and mark them.

REF TR-V0-G-P1(RG,iQ,Mx,Dt)-CE; TR-V0-G-P2(RG,iQ,Mx,Dt)-CE; TR-V0-G-S(RG,iQ,Mx,Dt)-CE; R-V0-

(RG,iQ,Mx,Dt)-CE / **VER** 15.10.10-07.12.10 / Page 8 of 17

10. Centrifuge all tubes at 10,000 rpm for 1 min.

11. For clinical samples, transfer the supernatant to new tubes according to labels.



It is important to transfer the maximal amount of the fluid. To do this, squeeze the piece of paper in the pellet with a tip when taking the fluid. It is allowed if a small piece of paper is transferred with the fluid.

12. Add **600 µl** of **Solution for Precipitation** and mix the contents of the tubes by vortexing.

13. Centrifuge all tubes at 13,000 rpm for 5 min.

14. Carefully remove and discard the supernatant from each tube without disturbing the pellet using a vacuum aspirator and 200-µl tips. Use a new tip for each tube.

15. Add **500 µl** of **Washing Solution 3** to each tube, tightly close the tubes, and shake them carefully 3–5 times to ensure pellet washing.

16. Centrifuge all tubes at 13,000 rpm for 1–2 min.

17. Carefully remove and discard supernatant from each tube without disturbing the pellet using a vacuum aspirator and 200-µl tips. Use a new tip for each tube.

18. Add **200 µl** of **Washing Solution 4** to each tube, tightly close the tubes, and shake them carefully 3–5 times to ensure pellet washing.

19. Centrifuge all tubes at 13,000 rpm for 1–2 min.

20. Carefully remove and discard the supernatant from each tube without disturbing the pellet using a vacuum aspirator and 200-µl tips. Use a new tip for each tube.

21. Incubate all tubes with open caps at **65 °C for 5 min** (to partially dry the pellet).

22. Add **50 µl** of **RNA buffer** to each tube. Mix the contents of the tubes by vortexing. Then incubate them at 65 °C for 5 min under periodic stirring by vortexing.

23. Centrifuge the tubes at 13,000 rpm for 1 min.

The supernatant contains purified DNA and is ready for PCR amplification.

The purified RNA/DNA can be stored

- at 2–8 °C for 24 h;
- at ≤ –16 °C for 1 year.

8.1.3. DNA extraction with DNA-sorb-B from whole blood

Volume of clinical material is 0.1 or 0.25 ml of whole blood

Initial processing of whole blood

1. Prepare required number of 1.5 ml disposable polypropylene microcentrifuge tubes and mark them. Add **1.0 ml** of «**Hemolytic**» to each tube. Add **0.25 ml** of whole blood according 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 vortex and incubate for 3 min 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 and 200-µl tips. Use a new tip for each tube.
4. Add **0.5 ml** of «**Hemolytic**» to each tube, mix them by vortex and incubate for 3 min.
5. Centrifuge the tubes at 8,000 rpm for 2 min. Carefully remove and discard supernatant from each tube without disturbing the pellet using vacuum aspirator and 200 µl tips. Use new tip for each tube.
6. Repeat the washing with «**Hemolytic**». After the last washing the pellet should be almost completely white color.

The pellet with leukocytes can be immediately lysed or frozen and stored at ≤ –16 °C for two weeks or

≤ –68 °C for a long time.

1. **Lysis Solution** and **Washing Solution 1** (if stored at 2–8 °C) should be heated to 65 °C until ice crystals disappear.
2. Prepare one 1.5-ml disposable polypropylene microcentrifuge tube for Negative Control of Extraction (**Negative Control, C–**) and add **5 µl** of **TE-buffer for DNA elution**.
3. Prepare one 1.5-ml disposable polypropylene microcentrifuge tube for Positive Control of Extraction (**Positive Control, PCE**) and add **5 µl** of **Positive Control DNA HIV-1 and human DNA**.
4. Add **300 µl** of **Lysis Solution** to each tube.
5. Thoroughly resuspend **Universal Sorbent** on a vortex mixer. Add **25 µl** of **Universal Sorbent** to each tube. Carefully vortex the tubes. Leave the tubes in the rack for 10 min vortexing them every 2 min.
6. Centrifuge all tubes 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.
7. Add **300 µl** of **Washing Solution 1** to each tube. Vortex vigorously until the 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.
8. Add **500 µl** of **Washing Solution 2** to each tube. Vortex vigorously 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.
9. Repeat step 8. Remove the supernatant completely.
10. Incubate all tubes with caps opened at 65 °C for 10 min (to partially dry the sorbent).
11. Add **50 µl** of **TE-buffer for DNA elution**. Vortex vigorously. Incubate the tubes at 65 °C for 5 min under periodic stirring by vortexing.
12. Centrifuge tubes at 12,000 rpm for 1 min. The supernatant contains purified DNA and is ready for PCR amplification. Be careful not to collect the sorbent while removing the DNA-containing solution. If solution is muddy, centrifuge the tube to precipitate the sorbent.

The purified DNA can be stored:

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

8.1.4. DNA extraction with NucliSENS easyMAG (BioMerieux, France) robotic station from whole blood

Volume of clinical material is 0.1 ml of whole blood.

Initial processing of whole blood

4. Prepare required number of 1.5 ml disposable polypropylene microcentrifuge tubes and mark them. Add **1.0 ml** of «**Hemolytic**» to each tube. Add **0.1 ml** of whole blood according to the labels on the tubes. Use a new tip for each tube. Close the tubes and mix by vortex.
5. Incubate tubes at room temperature for 5 min. Mix the contents of the tubes by vortexing and incubate for another 5 min.
6. 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 and 200-µl tips. Use a new tip for each tube.

The pellet with leukocytes can be immediately lysed or frozen and stored at ≤ –16 °C for two weeks or ≤ –68 °C for a long time.

A. easyMAG [OFF-BOARD LYSIS INCUBATION] option.

1. Add 30 ml of **RT-G** from **EM-plus** reagent kit to the **NucliSENS Lysis Buffer**, tightly close, and mix by inverting 7–10 times.
2. Turn on the **NucliSENS easyMAG** and prepare the station for extraction according to the manufacturer's instruction. Select the correct **HIV Protocol (Generic 2.0.1)**, the **Matrix (Other)**, the **Volume (0.1 ml)**, select the **Type (Lysed)**, **Elution Volume (50 mkl)**, **Off-board Lysis Incubation**, and **Off-board Silica Incubation**.
1. Add **450 µl NucliSENS Lysis Buffer** to the tubes with pellets. Tightly close and thoroughly mix by vortexing. Shortly centrifuge the tubes to spin down the drops.
2. Carefully transfer lysed samples to the sample vessel strips using a new tip for each tube.
3. Prepare one Negative Control of Extraction (**Negative Control, C-**). For this purpose, add **5 µl of TE-buffer for DNA elution** and **450 µl NucliSENS Lysis Buffer** to one well of the vessel strip. Thoroughly mix by pipetting.
4. Prepare one Positive Control of Extraction (**Positive Control, PCE**). For this purpose, add **5 µl of Positive Control DNA HIV-1 and human DNA** and **450 µl of NucliSENS Lysis Buffer** to one well of the vessel strip. Thoroughly mix by pipetting.
5. Incubate the vessel strip for lysis at room temperature for 10 min.
6. Prepare the mixture of **magnetic silica** and **component A** according to Table 1.

Table 1

Number of samples for extraction	Amount of magnetic silica NucliSens, mkl	Amount of component A EM-plus, mkl
1	50	10
8	450	90
16	850	170
24	1250	250

7. Add **60 µl of mixture** of magnetic silica and component A to each well of the vessel strip. Thoroughly mix liquid in wells by pipetting using a new tip for each well.
8. Incubate the vessel strip for sorption at room temperature for 10 min.
9. Install sample vessel strips in the NucliSENS easyMAG. Follow the instruction described in the operating procedure of the NucliSENS easyMAG and start the run.
10. Unload the sample vessel strips from the NucliSENS easyMAG after completion of the extraction run.
11. Transfer the nucleic acid extract, without silica, from the sample vessel strips to the PCR tubes within 30 minutes after completion of the extraction run using the new tip for each well. Take care not to transfer any silica particles.

The purified DNA can be stored:

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

B. easyMAG [ON-BOARD LYSIS INCUBATION] option

1. Add 30 ml of **RT-G** from **EM-plus** reagent kit to the **NucliSENS Lysis Buffer**, tightly close, and mix by inverting 7–10 times.
2. Turn on the **NucliSENS easyMAG** and prepare the station for extraction according to the manufacturer's instruction. Select the correct **HIV Protocol (Generic 2.0.1)**, the **Matrix (Other)**, the **Volume (0.1 ml)**, select the **Type (Primary)**, **Elution Volume (50 mkl)**, **On-board Lysis Incubation**, and **On-board Silica Incubation**.
3. Add **450 µl NucliSENS Lysis Buffer** to the tubes with pellets. Tightly close and thoroughly mix by vortexing. Shortly centrifuge tubes to spin down the drops.
4. Carefully transfer lysed samples to the sample vessel strips using a new tip for each tube.
5. Prepare one Negative Control of Extraction (**Negative Control, C-**). For this purpose, add **5 µl of TE-buffer for DNA elution** to one well of the vessel strip.
6. Prepare one Positive Control of Extraction (**Positive Control, PCE**). For this purpose, add **5 µl of Positive Control DNA HIV-1 and human DNA** to one well of the vessel strip.
7. Install sample vessel strips in the NucliSENS easyMAG. Follow the instruction described in the operating procedure of the NucliSENS easyMAG and start the run.
8. Wait until NucliSENS easyMAG do not stop (**Instrument State-Idle**). This will take about 15 min.
9. Prepare the mixture of **magnetic silica** and **component A** according to the table 1.
10. Add **60 µl of mixture** of magnetic silica and component A to each well of the vessel strip. Thoroughly mix liquid in wells by pipetting using the new tip for each well.
11. Continue the run following the instruction described in the operating procedure of the NucliSENS easyMAG.
12. Unload the sample vessel strips from the NucliSENS easyMAG after completion of the extraction run.
13. Transfer the nucleic acid extract, without silica, from the sample vessel strips to the PCR tubes within 30 minutes after completion of the extraction run using the new tip for each well. Take care not to transfer any silica particles.

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 tubes for amplification of DNA from clinical and control samples.

The choice of tube types depends on the type of instrument used for real-time amplification.

2. To carry out 15 reactions, add **160 µl** of **RT-PCR-mix-2-FEP/FRT** and **16 µl** of **polymerase (TaqF)** to the tube with **PCR-mix-1-FRT HIV**. Vortex the tube, then centrifuge shortly.



When the number of samples is not multiple of 16 (including controls), it is recommended that the reaction mixture is prepared as follows: **15 µl** of **PCR-mix-1-FRT HIV**, **10 µl** of **RT-PCR-mix-2-FEP/FRT**, and **1 µl** of **polymerase (TaqF)** per one sample. Use a new 1.5-ml tube to prepare the reaction mixture. Add 1 extra reaction when calculating reagent volumes. Vortex the tube, then centrifuge shortly.

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 using tips with aerosol barrier.



Avoid transferring silica beads together with the DNA sample in case of extraction by DNA-sorb-B kit or NucliSENS easyMAG robotic station.

5. Carry out the control amplification reactions:

- NCA** - Add **25 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification).
- C+** - Add **25 µl** of **Positive Control DNA HIV-1 and human DNA** to the tube labeled C+ (Positive Control of Amplification).

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:

AmpliSens DNA-HIV amplification program

Step	Rotor-type instruments ²			Plate-type instruments ³		
	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		55	40 s	
		<i>fluorescent signal detection</i>			<i>fluorescent signal detection</i>	
72	30 s	72	30 s			

Fluorescent signal is detected in the channels designed for the FAM/Green and JOE/Yellow/HEX

² For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.

³ For example, iCycler, iQ5, Mx3000P, Mx3000, or equivalent.

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

Internal Control is detected in the FAM/Green fluorescence channel, *HIV* DNA is detected in the JOE/Yellow/HEX fluorescence channel.

9.1. Interpretation of results

The results are interpreted by the software of instrument by the crossing (or not-crossing) of the fluorescence curve with the threshold line.

Table 2

Control	Stage for control	Ct value in channel		Interpretation
		JOE/Yellow/HEX	FAM/Green	
PCE	DNA extraction	Pos (< boundary value*)	Pos (< boundary value*)	OR
C-	DNA extraction	Neg	Neg (absent or > boundary value*)	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< boundary value*)	Pos (< boundary value*)	OK

*For boundary values, see the *Important Product Information Bulletin*.

1. The sample is considered to be positive for DNA *HIV-1* if its Ct value is detected in the results grid in the JOE/Yellow/HEX channel.
2. The sample is considered to be negative for DNA *HIV-1* if its Ct value is not determined in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/Yellow/HEX channel and if the Ct value determined in the results grid in the FAM/Green channel does not exceed the specified boundary value.

The result of the analysis is considered reliable only if the results obtained for both Positive and Negative Controls of amplification and extraction are correct (Table 2).

10. TROUBLESHOOTING

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

- If the Ct value of a sample is not detected in the FAM channel (IC detection) or is greater than the specified Ct boundary value, this indicates the loss of DNA or PCR inhibition due to improper clinical material processing.
- If the Ct value of negative control of extraction (C-) or amplification (NCA) is less than the specified Ct boundary 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 Extraction kit for **NucliSENS easyMAG** are to be stored according the manufacturer instruction. **DNA-sorb-B** nucleic acid extraction kit is to be stored at 2–25 °C when not in use. **RIBO-prep** nucleic acid extraction kit and **EM-plus** reagent kit are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® DNA-HIV-FRT** PCR kit are to be stored at ≤ –16 °C. All components of the **AmpliSens® DNA-HIV-FRT** 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.

Do not repeat freeze–thaw cycles more than twice for Positive Control DNA *HIV-1* and human DNA. After thawing, store the reagent at 2–8 °C for up to 6 months.



Do not store PCR-mix-1-FRT, RT-PCR-mix-2-FEP/FRT, and polymerase (TaqF) after thawing.

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 <i>HIV-1</i>
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 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 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*; *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 State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

15. QUALITY CONTROL

In compliance with Federal State Institution of Science “Central Research Institute of 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

	List Number		Caution!
	Lot Number		Contains sufficient for <n> tests
	For <i>in Vitro</i> Diagnostic Use		Version
	Store at	NCA	Negative Control of Amplification
	Manufacturer	C-	Negative control of Extraction
	Consult instructions for use	C+	Positive Control of Amplification
	Expiration Date	PCE	Positive Control of Extraction
	Authorised representative in the European Community	IC	Internal Control
RG	For working with Rotor-Gene 3000/6000 (Corbett Research)		
iQ	For working with iQ5, iCycler iQ (Bio-Rad)		
Mx	For working with Mx3000P or Mx3005P (Stratagene)		