

For *in Vitro* Diagnostic Use

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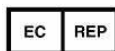
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AmpliSens[®] HCV-FRT

PCR kit

Instruction Manual

AmpliSens[®]



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1. INTENDED USE

AmpliSens® HCV-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of hepatitis C virus (*HCV*) RNA in the clinical materials (blood plasma) 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

Hepatitis C virus detection includes (1) RNA/DNA isolation from blood plasma together with the internal control sample (IC), which must be used in the isolation procedure to control the isolation of each individual sample and to detect possible reaction inhibition, (2) reverse transcription of RNA, and (3) real-time PCR amplification of cDNA. *HCV* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *HCV* 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 monitoring of fluorescence intensities during the real-time PCR allows detection of the amplified product without re-opening the reaction tubes after the PCR run. **AmpliSens® HCV-FRT** PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. The “hot-start” is guaranteed by separation of nucleotides and Taq polymerase by using a chemically modified polymerase (TaqF). The latter is activated by heating at 95 °C for 15 min.

The IC amplification product is detected in the FAM channel. The *HCV* cDNA amplification product is detected in the JOE/HEX channel.

The Positive Control of Extraction, Positive Control-1-*HCV*, is detected in FAM (IC) and JOE/HEX (*HCV*) channels.

DNA calibrator PIC2 *HCV* is a complex control for *HCV* and IC. It is detected in FAM (IC) and JOE/HEX (*HCV*) channels.

3. CONTENT

AmpliSens® HCV-FRT PCR kit is produced in one form:

AmpliSens® *HCV-FRT* PCR kit variant FRT (for use with RG, iQ, Mx, and Dt)

REF R-V1-Mod(RG,iQ,Mx,Dt)-CE.

AmpliSens® *HCV-FRT* PCR kit includes:

| Reagent | Description | Volume (ml) | Amount |
|-----------------------------------|-------------------------|-------------|---------|
| RT-G-mix-2 | colorless, clear liquid | 0.015 | 4 tubes |
| RT-PCR-mix-1-FL <i>HCV</i> | colorless, clear liquid | 0.3 | 4 tubes |
| RT-PCR-mix-2-FEP/FRT | colorless, clear liquid | 0.2 | 4 tubes |
| Polymerase (TaqF) | colorless, clear liquid | 0.02 | 4 tubes |
| TM-Revertase (MMLV) | colorless, clear liquid | 0.01 | 4 tubes |
| DNA calibrator PIC2 <i>HCV</i> * | colorless, clear liquid | 0.1 | 4 tubes |
| Buffer for elution | colorless, clear liquid | 1.2 | 2 tubes |
| Negative Control (C-)* | colorless, clear liquid | 1.2 | 4 tubes |
| Positive Control-1- <i>HCV</i> ** | colorless, clear liquid | 0.06 | 4 tubes |
| Internal Control ICZ-rec (IC)*** | colorless, clear liquid | 0.28 | 4 tubes |

* Serves as a Positive Control of Amplification (C+).

**Must be used in the isolation procedure as Negative Control of Extraction.

***Must be used in the isolation procedure as Positive Control of Extraction.

**** Must be added during the RNA/DNA extraction procedure directly to the sample/lysis mixture.

AmpliSens® *HCV-FRT* PCR kit is intended for 112 amplification reactions including controls.

4. ADDITIONAL REQUIREMENTS

- RNA/DNA isolation kit
- Disposable powder-free gloves and laboratory coat
- Pipettes (adjustable)
- Sterile RNase/DNase-free 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; or iQ5 (Bio-Rad, USA) instrument; or Mx3000P (Stratagene, USA) instrument
- Disposable 0.2-ml polypropylene microtubes for PCR (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 positive extracted 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, 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 all samples and unused reagents in compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in compliance 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 contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes contact, immediately flush with water and 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 detail in the manufacture's handbook [1]. It is recommended that this handbook is read before starting the work.

AmpliSens[®] HCV-FRT PCR kit is intended for analysis of RNA extracted with an RNA/DNA isolation kits from

- *Peripheral blood plasma.*

Blood samples are taken after overnight fasting into the tube with EDTA solution as anticoagulant. Closed tubes with blood are turned several times upside down and back again. Blood plasma should be taken and transferred to new tubes within 6 h after taking blood. For this purpose, tubes with blood are centrifuged at 800–1600 g for 20 min. Blood plasma can be stored unfrozen (at 2–8 °C) for at most 3 days or frozen (at or below 68 °C) for a long time.

In some cases, blood serum can be used. In this case, the analytical sensitivity of the reagent kit is retained; however, the clinical sensitivity may be significantly decreased as a result of precipitation of viral particles during blood clot retraction. Blood serum can be stored unfrozen (at 2–8 °C) for at most 3 days or frozen (at or below 68 °C) for a long time.

7. PROTOCOL

7.1. RNA/DNA Isolation

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

- RIBO-sorb, REF K2-1-Et-100-CE
- RIBO-prep, REF K2-9-Et-100-CE
- NucliSENS easyMAG automated system (BioMerieux) can also be used.



Extract RNA/DNA according to the manual provided by the manufacturer.



To prepare Positive Control of Extraction (PCE), mix **10 µl** of **Positive Control-1-HCV** and **90 µl** of **Negative Control**.



Volume of **Internal Control IC2-rec** that must be added at the extraction stage depends on the RNA/DNA isolation kit used:

- **10 µl** in the case of RIBO-sorb, REF K2-1-Et-100-CE
- **10 µl** in the case of RIBO-prep, REF K2-9-Et-100-CE



- If RNA/DNA is isolated using the RIBO-sorb REF K2-1-Et-100-CE extraction kit, after addition of clinical and control samples to lysis solution warm the mixture at 60 °C for 10 min prior to sorbent addition.

If RNA/DNA is isolated using the NucliSENS easyMAG automated system

- EM-plus kit REF K2-15-96-CE (manufactured by CRIE) must be used
- Add 30 ml (the whole content of the bottle) of the **RT-G component from the EM-Plus kit** to the bottle with the NucliSens lysis buffer, close tightly the cap and **carefully** mix by turning upside down 7-10 times (this procedure is performed once for each reagent kit).
- Mix 10 µl of the **Internal Control (IC) sample with 10 µl of NucliSens magnetic silica** and 10 µl of **Component A** from the **EM-plus kit** per one sample for RNA/DNA isolation in a new sterile tube using disposable tips with aerosol barriers.
- Set a sample volume as 0.1–1 ml
- Set the eluate volume as 50–60 µl (up to 100 µl)
- Both *On-board* and *Off-board* Lysis Buffer Dispensing and Lysis Incubation modes can be used
For details, see the Guidelines.



The purified RNA can be stored at 2–8 °C for at most 4 h, at temperatures not higher than minus 16 °C for 1 month, and at temperatures not higher than minus 68 °C for one year.

7.2. Preparing the reverse transcription and PCR

The total reaction volume is **25 µl**, the volume of RNA sample is **10 µl**.



All components of the reaction mixture should be mixed immediately before use. Mix reagents for the required number of reactions for experimental and control samples according to Appendix 1.

7.2.1 Preparing tubes for PCR

1. Before starting work, thaw and thoroughly vortex all reagents of the kit. Make sure that there are no drops on the caps of the tubes.
2. Take the required number of PCR tubes for amplification of clinical and control samples (including two controls of extraction and one control of amplification). The type of tubes depends on the real-time PCR instrument used for analysis.
3. **To prepare the reaction mixture**, mix the reagents (**10 µl of RT-PCR-mix-1-FL HCV**, **5 µl of RT-PCR-mix-2-FEP/FRT**, **0.25 µl of RT-G-mix-2**, **0.5 µl of Polymerase (TaqF)**, and **0.25 µl of TM-Revertase (MMIv)** per one reaction) in a new sterile tube (see also Appendix 1). Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.
4. Add **15 µl** of the prepared reaction mixture to each PCR tube.
5. Add **10 µl** of **RNA samples** isolated from the clinical samples to each PCR tube.



Avoid transferring sorbent beads together with the RNA sample in case of extraction using RIBO-sorb kit or the NucliSENS easyMAG automated system.

6. Run the control reactions:

- PCE** - Add **10 µl** of the **RNA sample** extracted from the Positive Control-1-HCV to the tube labeled PCE (Positive Control of Extraction)
- C-** - Add **10 µl** of the **RNA sample** extracted from the Negative Control to the tube labeled C- (Negative Control of Extraction)
- C+** - Add **10 µl** of **DNA calibrator PIC2 HCV** to the tube labeled C+ (Positive Control of Amplification).

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

- NCA** -Add **10 µl** of **Buffer for elution** to the tube labeled NCA (Negative Control of Amplification).

Make sure that there are no drops on the tube walls, otherwise vortex the tubes briefly.

7.2.2 Reverse transcription and amplification

7.2.2.1. RG

1. Program the Rotor-Gene instrument according to manufacturer's manual and the Guidelines.
2. Create a temperature profile on your Rotor-Gene instrument as follows:

AmpliSens-2 RG program for rotor-type instruments¹

| Step | Temperature, °C | Time | Fluorescence detection | Cycle repeats |
|---------------|-----------------|--------|---|---------------|
| 1 (Hold) | 50 | 15 min | – | 1 |
| 2 (Hold) | 95 | 15 min | – | 1 |
| 3 (Cycling 1) | 95 | 5 s | – | 5 |
| | 60 | 20 s | – | |
| | 72 | 15 s | – | |
| 4 (Cycling 2) | 95 | 5 s | – | 40 |
| | 60 | 20 s | FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red | |
| | 72 | 15 s | – | |

3. Adjust the fluorescence channel sensitivity as described in the Guidelines.



This program makes it possible to simultaneously carry out any combination of tests in the same instrument using the single amplification program.



Channels ROX/Orange and Cy5/Red are switched on when necessary (only in MULTIPRIME assays)

7.2.2.2. iQ

1. Program the iCycler iQ or iQ5 instrument according to manufacturer's manual and the Guidelines.
2. Create a temperature profile on your iQ5 instrument as follows:

AmpliSens-2 iQ program for plate-type instruments²

| Step | Temperature, °C | Step duration | Fluorescence detection | Cycle repeats |
|------|-----------------|---------------|------------------------|---------------|
| 1 | 50 | 15 min | – | 1 |
| 2 | 95 | 15 min | – | 1 |
| 3 | 95 | 5 s | – | 5 |
| | 60 | 20 c | – | |
| | 72 | 15 s | – | |
| 4 | 95 | 5 s | – | 40 |
| | 60 | 30 s | FAM, HEX, ROX, Cy5 | |
| | 72 | 15 s | – | |

3. Adjust the fluorescence channel sensitivity as described in the Guidelines.



This program makes it possible to simultaneously carry out any combination of tests in the same instrument using the single amplification program (e.g., together with tests for HBV, HCV genotyping, etc.).

¹ For example, Rotor-Gene 3000 or 6000 (Corbett Research, Australia)

² For example, iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), or equivalent



Channels ROX/Orange and Cy5/Red are switched on when necessary (only in MULTIPRIME assays)

8. DATA ANALYSIS

The signal from the Internal Control cDNA amplification product is detected in the FAM channel, the signal from the *HCV* cDNA amplification product is detected in the JOE/HEX channel.

For data analysis settings for Rotor-Gene 3000/6000, iQ5, and Mx3000 real-time PCR instruments, see the Guidelines.

Results interpretation

The results are interpreted by the real-time PCR instrument software by the crossing or not crossing of the threshold line by the fluorescence curve.

Results for controls

| Control | Stage for control | Ct in channel | | Interpretation |
|---------|-------------------|-------------------|-------------------|----------------|
| | | Green/FAM | JOE/HEX/ Yellow | |
| C- | RNA isolation | Pos (\leq Ct*) | Neg | OK |
| PCE | RNA isolation | Pos (\leq Ct*) | Pos (\leq Ct*) | OK |
| NCA | Amplification | Neg | Neg | OK |
| C+ | Amplification | Pos (\leq Ct*) | Pos (\leq Ct*) | OK |

* The boundary Ct values are summarized in the Important Product Information Bulletin.

1. The sample is considered **positive** for *HCV* RNA if the Ct value detected in the JOE/HEX/Yellow channel does not exceed the boundary value specified in the Important product information bulletin.
2. The sample is considered **negative** for *HCV* RNA if the Ct value in the JOE/HEX/Yellow channel is absent or if the Ct value detected in the JOE/HEX/Yellow is greater than the specified boundary value and the Ct value in the FAM channel does not exceed the boundary value specified in the Important Product Information Bulletin.
3. The sample is considered **equivocal** if an equivocal result is obtained in any of the channels. In this case, PCR analysis of this sample should be repeated once again.

For details, see the Guidelines.

Results are accepted as relevant if both positive and negative controls of amplification as well as negative and positive controls of extraction are passed properly (see the above table for controls).

9. TROUBLESHOOTING

- If the Ct value for PCE or C+ in the JOE/HEX/Yellow channel exceeds the specified boundary value, analysis of all samples in which *HCV* RNA was not detected should be repeated once again starting from the RNA extraction stage.

- If the Ct value for NCE and/or C- in the JOE/HEX/Yellow channel does not exceed the specified boundary value, analysis of all samples in which *HCV* RNA was detected should be repeated once again starting from the RNA extraction stage.

10. STABILITY AND STORAGE

All components of the **AmpliSens® HCV-FRT** PCR kit are to be stored at ≤ -16 °C. They are stable until the expiration date indicated on the label.



DNA calibrator PIC2 *HCV*, Positive Control-1-*HCV*, and the Internal Control ICZ-rec should not be frozen/thawed more than twice. After thawing, PIC2 *HCV*, Positive Control-1-*HCV*, and ICZ-rec should be stored at 2-8 °C for at most 6 months.

11. SPECIFICATIONS

11.1. Sensitivity

The analytical sensitivity of **AmpliSens® HCV-FRT** PCR kit is specified in the table below.

| Volume of sample for isolation, μ l | RNA/DNA isolation method | Analytical sensitivity, IU/ml |
|---|--|-------------------------------|
| 100 | RIBO-sorb RIBO-prep NucliSENS easyMAG | 100 |
| 1000 | NucliSENS easyMAG | 10 |



The claimed analytical features of **AmpliSens® HCV-FRT** PCR kit are guaranteed only when additional reagents kits RIBO-sorb or RIBO-prep (manufactured by Federal State Institution of Science "Central Research Institute of Epidemiology") are used.

11.2. Specificity

The analytical specificity of **AmpliSens® HCV-FRT** PCR kit is ensured by selection of specific primers and probes and stringent reaction conditions. The primers and probes were tested for possible homologies to all sequences deposited in gene banks by sequence comparison analysis as well as with genomic DNA/RNA of the following organisms and viruses: hepatitis A virus; hepatitis B virus; hepatitis D virus; human immunodeficiency 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*, *S.agalactiae*; and *Homo sapiens*. The clinical specificity of **AmpliSens® HCV-FRT** PCR kit was confirmed in laboratory clinical trials.

Cross-reactions for the above-mentioned organisms and viruses have not been detected.













12. REFERENCES

1. Handbook "Sampling, transportation, 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.
2. Guidelines to AmpliSens® HCV-FRT, AmpliSens® HDV-FRT, and AmpliSens® HCV-FRT PCR kits.

13. QUALITY CONTROL

In compliance with the Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485-Certified Quality Management System, each lot of **AmpliSens® HCV-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS

| | | | |
|---|---|---|--|
|  | Manufacturer |  | Temperature limitation |
|  | Use by |  | Batch code |
|  | For <i>in Vitro</i> Diagnostic Use |  | Version |
|  | Catalogue number |  | Internal Control |
|  | Contains sufficient for <n> tests |  | Authorised representative in the European Community. |
|  | Consult instructions for use |  | Caution, consult accompanying documents |
| NCA | Negative Control of Amplification | C+ | Positive control of amplification |
| PCE | Positive Control of Extraction | C- | Negative control of extraction |
| CRIE | Central Research Institute of Epidemiology (Moscow, Russia) | | |

List of Changes Made in the Instruction Manual

| VER | Location of changes | Essence of changes |
|----------|-----------------------|---|
| 01.06.10 | Page footer | Reference number is changed from R-V1-Mod(RG,iQ,Mx,Dt)-E to R-V1-Mod(RG,iQ,Mx,Dt)-CE |
| | Content, text | Name of Positive Control of amplification is changed from KB2 to PIC2 |
| 04.12.10 | Sampling and handling | Sentence «Blood samples are taken after overnight fasting into tubes with 3% EDTA solution (1 : 20)» is changed into «Blood samples are taken after overnight fasting into the tube with EDTA solution as anticoagulant». |
| | Data analysis | Mention about DT-96 device is deleted |
| | Through the text | MAGNO-sorb mention is deleted Corrections through the text |
| 13.12.10 | Through the text | Name of Positive Control of amplification is changed from Positive Control PIC2 HCV (C+) to DNA calibrator PIC2 HCV |