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For Professional Use Only

# AmpliSens® *Parvovirus* B19-FRT

## PCR kit

## Instruction Manual

**AmpliSens®**

EC REP

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## 1. INTENDED USE

**AmpliSens® *Parvovirus* B19-FRT PCR kit** is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of *Parvovirus* B19 DNA in clinical material (peripheral or umbilical blood, amniotic fluid, oropharyngeal washes and swabs, and saliva) by using real-time hybridization-fluorescence detection of amplified products.

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

## 2. PRINCIPLE OF PCR DETECTION

*Parvovirus* B19 detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Parvovirus* B19 primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product during thermocycling. The real-time 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® *Parvovirus* B19-FRT** PCR kit is a qualitative test that contains the Internal Control (IC). It must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. **AmpliSens® *Parvovirus* B19-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). Chemically modified polymerase (TaqF) is activated by heating at 95 ºC for 15 min.

Detection of *Parvovirus* B19 DNA is based on:

– total DNA isolation from plasma of peripheral or umbilical blood, amniotic fluid, oropharyngeal washes and swabs, and saliva along with internal controls.
– simultaneous amplification (multiplex PCR) of DNA fragment of structural gene coding Parvovirus B19 VP1 protein and engineered DNA fragment cloned in Lambda phage DNA which is used as exogenous noncompetitive internal control with hybridization-fluorescence detection.

Exogenous internal control allows monitoring of main stages of PCR-analysis (DNA extraction, PCR amplification). The advantage of noncompetitive internal control application is an increase of analytical sensitivity of the assay.

## 3. CONTENT

**AmpliSens® *Parvovirus* B19-FRT PCR kit** is produced in 1 form:

AmpliSens® *Parvovirus* B19-FRT PCR kit variant FRT-50 F, **REF** R-V49(RG,iQ,Mx)-CE.

**AmpliSens® *Parvovirus* B19-FRT PCR kit** variant FRT-50 F includes:

| *Reagent* | | *Description* | *Volume (ml)* | *Quantity* |
|---|---|---|---|---|
| **PCR-mix-1-FRT *Parvovirus* B19** | | colorless clear liquid | 0.6 | 1 tube |
| **PCR-mix-2-FRT** | | colorless clear liquid | 0.3 | 1 tube |
| **Polymerase (TaqF)** | | colorless clear liquid | 0.03 | 1 tube |
| **DNA calibrators** | **KS1 B19** | colorless clear liquid | 0.2 | 1 tube |
| | **KS2 B19** | colorless clear liquid | 0.2 | 1 tube |
| **DNA-buffer** | | colorless clear liquid | 0.5 | 1 tube |
| **Positive Control DNA *Parvovirus* B19 / STI ($C+_{B19\ /\ STI}$)** | | colorless clear liquid | 0.1 | 1 tube |
| **Negative Control (C–)*** | | colorless clear liquid | 1.2 | 1 tube |
| **Internal Control STI-87 (IC)**** | | colorless clear liquid | 1.0 | 1 tube |

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

** add 10 µl of Internal Control during the DNA isolation procedure directly to the sample/lysis mixture (see DNA-sorb-AM, **REF** K1-12-100-CE, DNA-sorb-B, **REF** K1-2-100-CE or RIBO- prep, **REF** K2-1-Et-100-CE protocols.)

AmpliSens® *Parvovirus* B19-FRT PCR kit variant FRT-50 F is intended for 60 reactions (including controls).

## 4. ADDITIONAL REQUIREMENTS

- DNA isolation kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 200 µl).
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Refrigerator for 2–8 °C with a deep-freezer for ≤ –16°C.
- Waste bin for used tips.
- Personal thermocyclers: Rotor-Gene 3000/6000 (Corbett Research, Australia), iCycler iQ or iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), or equivalent.
- Rotor-Gene: 0.2-ml disposable flat-cap unstip polypropylene microtubes for PCR (for

instance, Axygen, USA) for a 36-well rotor or 0.1-ml microtubes (Corbett Research, Australia) for a 72-well rotor.

iCycler iQ or iQ5: 0.2-ml disposable domed polypropylene microtubes for PCR (for instance, Axygen, USA), strip domed tubes or a 96-well plate for PCR equipped with heat-proof optical transparent films (Bio-Rad, USA).

Mx3000P: 0.2-ml disposable polypropylene domed strip/unstrip microtubes for PCR (for instance, Axygen, USA) for a 36-well rotor or a plate for PCR equipped with heat-proof optical transparent films (Bio-Rad, USA).

- Automated nucleic acid extraction system NucliSENS easyMAG (bioMérieux, France)
- NucliSENS easyMAG automated system consumables(bioMérieux, France)

## 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 mucosa. If skin, eyes and mucosa 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 manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Parvovirus* B19-FRT PCR kit is intended to analyze DNA extracted with DNA isolation kits from:

- *peripheral or umbilical blood plasma;*
- *amniotic fluid;*
- *oropharyngeal washing fluids and swabs;*
- *saliva.*

6.1. *Peripheral or umbilical blood plasma* should be taken into a tube with 6% EDTA not earlier than 3 hours after ingestion. Then shake the tube to ensure proper mixing. Blood plasma should be collected and transferred to a new tube within 6 h after bleeding and centrifuged at 800–1600 rpm for 10 min.

AmpliSens® *Parvovirus* B19-FRT PCR kit can be used for analyses of individual and pooled samples. A minipool should consists of not more than 10 individual samples (100 µl of blood plasma obtained from each of 10 samples). DNA from 100 µl of an individual blood plasma sample can be isolated using the NucliSENS easyMAG automated system. DNA from a minipool can be isolated only using the NucliSENS easyMAG automated system.

6.2. *Amniotic fluid* should be obtained during amniocenteses by the standard procedure. Pretreatment of the sample is required. An amniotic fluid sample should be thoroughly resuspended. Remove 1.0 ml of the sample and transfer it to an Eppendorf tube using a automatic pipette with a tip with aerosol barrier. Centrifuge the tube at 8,000–9,000 g for 10 min. Carefully remove the supernatant using a tip with aerosol barrier leaving ~200 µl of the solution over the pellet. Resuspend the pellet by vortexing.

6.3. *Oropharyngeal swab specimen* is obtained using a dry sterile cotton swab. Before sampling, have the patient to rinse his mouth with water. Rotate the swab over the tonsillar area, palatine arches, and the posterior area of the pharynx. Place the effective part of the probe into a tube with 500 µl of transport medium. Break off the probe and tightly secure the cap.

6.4. *Saliva* sample (0.2–1.0 ml) is collected to a 1.5-ml sterile tube. Have the patient to rinse his mouth with water three times before taking the sample.

Only one freeze–thaw cycle of clinical material is allowed.

## 7. WORKING CONDITIONS

**AmpliSens® *Parvovirus* B19-FRT** PCR kit should be used at 18–25 °C.

## 8. PROTOCOL

### 8.1. DNA Isolation

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

- DNA-sorb-B, **REF** K1-2-100-CE (for all mentioned samples);
- RIBO-prep **REF** K2-1-Et-100-CE (for amniotic fluid, saliva, oropharyngeal washes and swabs);
- DNA-sorb-AM, **REF** K1-12-100-CE (for saliva and oropharyngeal washes and swabs);
- NucliSENS® easyMAG® automated system (for peripheral or umbilical blood plasma of individual or pooled sample).

Isolate DNA according to the manufacturer' protocol.
The volume of the clinical sample is 100 µl.
The volume of the Internal Control STI-rec (IC) is 10 µl.

When using the NucliSENS easyMAG automated system, set the sample volume as 0.1–1.0 ml and the eluate volume as 55 µl.
Select *On-board Lysis Buffer Dispensing* and *On-board Lysis Incubation* modes.

### 8.2. Preparing PCR

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

All reaction components should be mixed just before analysis.

#### 8.2.1 Preparing tubes for PCR

1. Prepare the **reaction mixture** taking the following volumes for one reaction:
   - **10 µl of PCR-mix-1-FRT Parvovirus B19,**
   - **5.0 µl of PCR-mix-2-FRT,**
   - **0.5 µl of polymerase (TaqF).**

When calculating the volume of the reaction mixture, take into account two controls (positive and negative controls of amplification) and one extra reaction (for possible losses). Refer to Table 1 for calculated reaction volumes. It is recommended to prepare the reaction mixture for an even number of reactions to ensure precise reagent dispensing.

Table 1

**Reaction mixture preparation scheme**

| Total reaction volume, 25 µl<br>Reagent volume for 1 reaction, 15 µl<br>DNA sample volume, 10 µl | | | |
|---|---|---|---|
| **Number of samples to be analyzed*** | **PCR-mix-1-FRT Parvovirus B19, µl** | **PCR-mix-2-FRT, µl** | **Polymerase (TaqF), µl** |
| **1** | **40** | **20** | **2.0** |
| **2** | **50** | **25** | **2.5** |
| **3** | **60** | **30** | **3.0** |
| **4** | **70** | **35** | **3.5** |
| **5** | **80** | **40** | **4.0** |
| **6** | **90** | **45** | **4.5** |
| **7** | **100** | **50** | **5.0** |
| **8** | **110** | **55** | **5.5** |
| **9** | **120** | **60** | **6.0** |
| **10** | **130** | **65** | **6.5** |
| **11** | **140** | **70** | **7.0** |
| **12** | **150** | **75** | **7.5** |
| **13** | **160** | **80** | **8.0** |
| **14** | **170** | **85** | **8.5** |
| **15** | **180** | **90** | **9.0** |
| **16** | **190** | **95** | **9.5** |
| **17** | **200** | **100** | **10.0** |
| **18** | **210** | **105** | **10.5** |
| **19** | **220** | **110** | **11.0** |
| **20** | **230** | **115** | **11.5** |
| **21** | **240** | **120** | **12.0** |
| **22** | **250** | **125** | **12.5** |
| **23** | **260** | **130** | **13.0** |
| **24** | **270** | **135** | **13.5** |
| **25** | **280** | **140** | **14.0** |
| **26** | **290** | **145** | **14.5** |
| **27** | **300** | **150** | **15.0** |
| **28** | **310** | **155** | **15.5** |
| **29** | **320** | **160** | **16.0** |
| **30** | **330** | **165** | **16.5** |
| **31** | **340** | **170** | **17.0** |
| **32** | **350** | **175** | **17.5** |
| **33** | **360** | **180** | **18.0** |
| **34** | **370** | **185** | **18.5** |

*** The number of clinical samples plus two controls (positive and negative controls of amplification) and one extra reaction (for possible losses).**

For quantitative determination of even one clinical sample of *Parvovirus* B19 DNA, it is necessary to carry out PCR amplification five more samples: 2 DNA calibrators KS1 and KS2 (repeating twice for each calibrator) and Negative

Control (DNA-buffer).

For qualitative determination of *Parvovirus* B19 DNA it's necessary to carry out the running of PCR amplification two points more: Positive Control DNA *Parvovirus* B19 and STI, DNA-buffer.

2. Prepare required number of tubes or stripes for amplification of DNA from clinical and control samples.
3. Transfer **15 µl** of prepared reaction mix into each tube.
4. Using tips with aerosol filter **add 10 µl of DNA samples**, obtained from clinical or control samples at the stage of DNA extraction.
5. Carry out the **control amplification reactions:**

   For quantitative detection of *Parvovirus* B19 DNA:

**NCA** - Add **10 µl** of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).

**KS1, KS2** - Add **10 µl** of DNA calibrator **KS1** to two tubes labeled KS1 and **10 µl** of DNA calibrator **KS2** to two tubes labeled KS2.

For qualitative detection of *Parvovirus* B19 DNA:

**NCA** - Add **10 µl** of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).

**$C+_{B19\ /\ STI}$** - Add **10 µl** of **Positive Control DNA *Parvovirus* B19 / STI** to the tube labeled **$C+_{B19\ /\ STI}$** (Positive Control of Amplification).

### 8.2.2. Amplification

1. Program the PCR instrument according to manufacturer's manual and Guidelines in case of **Rotor-Gene 3000** and **Rotor-Gene 6000**, **iCycler iQ** and **iQ5, Mx3000P** and **Mx3005P.**
2. Create a temperature profile in your instrument as follows:

Table 2

**AmliSens-1 RG amplification program (for Rotor-Gene 3000 and Rotor-Gene 6000)**

| ***Step*** | ***Temperature, °C*** | ***Time*** | ***Fluorescence detection*** | ***Cycles*** |
|---|---|---|---|---|
| Hold | 95 | 15 min | – | 1 |
| Cycling 1 | 95 | 5 s | – | 5 |
| | 60 | 20 s | – | |
| | 72 | 15 s | – | |
| Cycling 2 | 95 | 5 s | – | 40 |
| | 60 | 20 s | FAM/Green, JOE/Yellow | |
| | 72 | 15 s | – | |

Table 3

**AmliSens-1 iQ amplification program (for iQ5 and iQ iCycler)**

| ***Step*** | ***Temperature, °C*** | ***Time*** | ***Fluorescence detection*** | ***Cycles*** |
|---|---|---|---|---|
| Hold | 95 | 15 min | – | 1 |
| Cycling 1 | 95 | 5 s | – | 5 |
| | 60 | 20 s | – | |
| | 72 | 15 s | – | |
| Cycling 2 | 95 | 5 s | – | 40 |
| | 60 | 30 s | FAM, HEX | |
| | 72 | 15 s | – | |

Table 4

**AmliSens-1 Mx amplification program (for Mx3000P and Mx3005P)**

| ***Step*** | ***Temperature, °C*** | ***Time*** | ***Fluorescence detection*** | ***Cycles*** |
|---|---|---|---|---|
| Hold | 95 | 15 min | – | 1 |
| Cycling 1 | 95 | 5 s | – | 5 |
| | 60 | 20 s | – | |
| | 72 | 15 s | – | |
| Cycling 2 | 95 | 5 s | – | 40 |
| | 60 | 20 s | FAM, HEX | |
| | 72 | 15 s | – | |

AmpliSens-1 RG, AmpliSens-1 iQ, and AmpliSens-1 Mx universal amplification programs allow running any combination of tests in one Instrument with the same program (for example, along with the tests for detecting DNA of pathogens causing sexually transmitted infections). Analytical performances of this detection kit remain the same when the universal program is used.

3. Fluorescence is detected on the 2nd step **(60 °C)** of stage Cycling 2 in appropriate fluorometer channels (see Tables 2, 3, and 4).
4. Adjust the fluorescence channel sensitivity.

## 9. DATA ANALYSIS

The results are interpreted with the software of the instrument used by the crossing (or not crossing) of the fluorescence curve with the threshold line, which corresponds to the presence (absence) of a Ct value in appropriate columns of the result grid. The Internal Control is detected in the FAM/Green fluorescence channel, Parvovirus B19 DNA is detected in the JOE/Yellow/HEX fluorescence channel.

For data analysis settings and Ct values, see Guidelines in case of **Rotor-Gene 3000 and Rotor-Gene 6000**, **iCycler iQ** and **iQ5, Mx3000P** and **Mx3005P**.

**Interpretation of results**

**Results for controls**

| Control | Controlled stage | Results | | Interpretation |
|---|---|---|---|---|
| | | FAM/ Green (IC) | JOE/Yellow/HEX (*Parvovirus* B19) | |
| **C–** | DNA isolation | Pos (not more than indicated in bulletin) | Neg | OK |
| **NCA** | Amplification | Neg | Neg | OK |
| **C+$_{B19\ /\ STI}$** | Amplification | Pos (not more than indicated in bulletin) | Pos (not more than indicated in bulletin) | OK |

1. The sample is considered to be positive if its Ct value does not exceed value indicated in bulletin on JOE/Yellow/HEX channel.
2. The sample is considered to be negative if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) on JOE/Yellow/HEX channel and in the results grid on the FAM/Green channel the Ct value doesn't exceed the value indicated in bulletin.

The concentration of *Parvovirus* B19 DNA for plasma and amniotic fluid is calculated using the formula:

**КК *Parvovirus B19* DNA = К *Parvovirus B19* DNA/ К$_{STI-87}$ x IC coefficient**

**К $_{Parvovirus\ B19\ DNA}$ is the number of copies of Parvovirus B19 DNA in DNA sample;**

**К$_{STI-87}$ is the number of copies of STI-87 DNA in DNA sample;**

**IC coefficient corresponds to the number of copies of IC STI-87 DNA in DNA sample. It is indicated in bulletin and specified to each lot of the reagent kit.**

## 10. TROUBLESHOOTING

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

- If any Ct value appears in the result grid for Negative Controls (NCA, C–) in the JOE/Yellow/HEX (*Parvovirus* B19) and/or FAM/Green (IC STI-87) channel, this indicates contamination of reagents or samples. The results of analysis are considered irrelevant. Test analysis for all samples must be repeated and measures to detect the contamination source must be taken.
- If the Ct value is absent in results grid for the positive control C+$_{B19\ /\ STI}$, the results of analysis are irrelevant. PCR should be repeated for all samples.
- If Ct values are absent for the analyzed samples in the FAM/Green channel (Internal Control) in the results grid, this indicates the extraction stage failure. Analysis should be repeated for these samples starting from the extraction stage. If the Ct value of the Internal Control for the

analyzed sample exceeds the value indicated in the bulletin whereas the Ct value of *Parvovirus* B19 DNA is greater than the value indicated in the bulletin, analysis should be repeated for these samples starting from the extraction stage. High Ct values may occur as a result of DNA losses during DNA extraction or the presence of inhibitors.

If you have any questions or encounter problems, please contact our Authorized representative in the European Community.

## 11. TRANSPORTATION

**AmpliSens® *Parvovirus* B19-FRT** PCR kit should be transported at 2–8 ºC for no longer than 5 days.

## 12. STABILITY AND STORAGE

All components of the **AmpliSens® *Parvovirus* B19-FRT** PCR kit (except for PCR-mix-1-FRT *Parvovirus* B19, PCR-mix-2-FRT, and polymerase (TaqF)) are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *Parvovirus* B19-FRT** PCR kit are to be stable until labeled expiration date. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

PCR-mix-1-FRT *Parvovirus* B19, 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 *Parvovirus* B19 is to be stored away from light.

## 13. SPECIFICATIONS

### 13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Parvovirus* B19-FRT** PCR kit is 400 copies per 1 ml.

The linear range is 800 to 10 000 000 copies per 1 ml of sample (copies/ml).

The claimed analytical features of **AmpliSens® *Parvovirus* B19-FRT** PCR kit are guaranteed only when additional reagents kits DNA-sorb-B, RIBO-prep, or DNA-sorb-AM (manufactured by FBIS CRIE) or the NucliSENS easyMAG automated system (manufactured by bioMérieux, France) are additionally used.

### 13.2. Specificity

The analytical specificity of **AmpliSens® *Parvovirus* B19-FRT** PCR kit is ensured by selection of specific primers and stringent reaction conditions. The clinical specificity 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 Total Quality Management System, each lot of **AmpliSens® *Parvovirus* B19-FRT** PCR kit is tested against predetermined specifications to ensure consistent product quality.

## 16. KEY TO SYMBOLS USED

| | | | |
|---|---|---|---|
| REF | Catalogue number | | Caution |
| LOT | Batch code | Σ | Sufficient for |
| IVD | *In vitro* diagnostic medical device | | 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 |
| EC REP | Authorised representative in the European Community | **C+** | Positive control of Amplification |
| **FBIS CRIE** | Federal Budget Institute of Science "Central Research Institute for Epidemiology" | **IC** | Internal control |
| **KS1 B19, KS2 B19** | DNA calibrators | | |

## List of Changes Made in the Instruction Manual

| VER | Location of changes | Essence of changes |
|---|---|---|
| 30.06.11 LA | Cover page, text | The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology” |