



For *in Vitro* Diagnostic Use

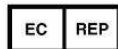


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AmpliSens® *Parvovirus B 19-FRT* PCR kit

Instruction Manual



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1. INTENDED USE.

AmpliSens® Parvovirus B 19-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of *Parvovirus B 19* DNA in the clinical material (peripheral or umbilical blood; amniotic fluid; throat washes and swabs; saliva) by using real-time hybridization-fluorescence detection of amplified products.

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 usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time PCR monitoring of the 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 B 19-FRT PCR kit** is a qualitative test, which contain 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 B 19-FRT PCR kit** uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95°C for 15 min.

Detection of Parvovirus B19 DNA is based on:

- total DNA isolation from plasma of peripheral or umbilical blood, amniotic fluid, throat washes and swabs, 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 B 19-FRT PCR kit is produced in 1 form:

AmpliSens® *Parvovirus B 19-FRT PCR kit* variant FRT-50 F, **REF** R-V49(RG,iQ,Mx)-CE.

AmpliSens® Parvovirus B 19-FRT PCR kit variant FRT-50 F includes:

<i>Reagent</i>	<i>Description</i>	<i>Volume (ml)</i>	<i>Quantity</i>	
PCR-mix-1-FRT Parvovirus B 19	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 and STI (C+)	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 deep-freezer with temperature no less than –16°C.
- Reservoir for disposed tips.
- Personal thermocyclers: “Rotor-Gene” 3000/6000 (Corbett Research, Australia), “iQ iCycler”, “iQ5” (Bio-Rad, USA), “Mx3000P” (Stratagene, USA) or equivalent.
- Rotor-Gene: disposable polypropylene undomed and unstripte 0.2 ml microtubes for PCR (for

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

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

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

- Automated nucleic acid extraction system “NucliSENS® easyMAG®” (bioMerieux, France)
- “NucliSENS® easyMAG®” automated system consumables(bioMerieux, 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 other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa 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 move to the Amplification and Detection Area. 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* B 19-FRT PCR kit is intended to analyze DNA extracted with DNA isolation kits from:

- *peripheral or umbilical blood plasma*
- *amniotic fluid*
- *throat washes and swabs*
- *saliva*

6.1. *Peripheral or umbilical blood plasma* is to be sampled into a tube with 6% EDTA solution not earlier than 3 hours after ingestion. Then shake to ensure proper mixing. During 6 hours from sampling the plasma is to be collected and transferred into the new tube. Centrifuge the tube with blood at 800 – 1600 rpm for 10 min.

AmpliSens® *Parvovirus* B19-FRT PCR kit can be used for both analyses of individual and pooled samples. Mini-pool should consists of not more than 10 individual samples (100 µl of blood plasma obtained from each of 10 samples). DNA isolation from 100 µl of individual sample blood plasma can be done by NucliSENS® easyMAG® automated system. DNA isolation from mini-pool can be done only by NucliSENS® easyMAG® automated system.

6.2. *Amniotic fluid* should be obtained during amniocenteses in accordance with standard procedure. Preprocessing is required for analyses. Amniotic fluid sample is to be thoroughly resuspended. Remove 1.0 ml of the sample and transfer in an Eppendorf tube using automatic pipette with barrier tip. Centrifuge the tube at 8,000 -9,000 g for 10 min. carefully remove supernatant using tip with barrier, leave 200 µl of the fluid. Resuspend the material on vortex.

6.3. *Throat swab specimen* is obtained by dry sterile cotton swab. Before sampling make patient rinse the mouth with water. Rotate the swab over tonsillar area, palatine arches, and posterior area of the pharynx. Place effective part of the probe into the 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 in a 1.5 ml sterile tube. Have the patient to rinse his mouth with water three times before the sample is taken.



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

7. PROTOCOL.

7.1. DNA Isolation

It's recommended to use the following nucleic acid extraction kits:

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



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



Using the NucliSENS® easyMAG® automated system set the sample volume as 0.1-1.0 ml and eluate volume as 55 µl.
Select *On-board Lysis Buffer Dispensing* and *On-board Lysis Incubation*.

7.2. Preparing the PCR.

Total reaction volume - **25 µl**, volume of DNA sample - **10 µl**.

All reaction components should be mixed straight before analysis starts.

7.2.1 Preparing tubes for PCR.

1. Prepare **reaction mix** calculating per one reaction:

- **10 µl PCR-mix-1-FRT Parvovirus B19**
- **5.0 µl PCR-mix-2-FRT**
- **0.5 µl polymerase (TaqF)**

When calculating amount of reaction mix, 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 reaction mix for even number of reactions to ensure precise reagents dispensing.

Table 1

Reaction mix preparation scheme

Total reaction volume - 25 µl Reagents volume per 1 reaction - 15 µl Volume of DNA sample - 10 µl			
Number of samples to be analysed*	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

* Values include two controls (positive and negative controls of amplification) and one extra reaction (for possible losses).



For quantitative determination of even one studied sample of *Parvovirus* B19 DNA it's necessary to carry out the running of PCR amplification five points more: 2 DNA calibrators KS1, 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 determination of *Parvovirus* B19 DNA:

- NCA - Add **10 µl** of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).
 C+ - Add **10 µl** of DNA calibrator **KS1** in two tubes and **10 µl** of DNA calibrator **KS2** in other two tubes for Positive Control of Amplification.

For qualitative determination of *Parvovirus* B19 DNA:

- NCA - Add **10 µl** of **DNA-buffer** to the tube for Negative Control of Amplification (NCA).
 C+ - Add **10 µl** of **Positive Control DNA *Parvovirus* B19 and STI** into the tube for Positive Control of Amplification.

7.2.2. Amplification

1. Program the applied instrument according to manufacturer's manual and Appendix 1 in case of **Rotor-Gene™ 3000 and Rotor-Gene™ 6000** using, Appendix 2 in case of **iQ5 and iQ iCycler** or Appendix 3 in case of **Mx3000P and Mx3005P**.
2. Create a temperature profile on your instrument as follows:

Table 2

AmpliSens-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 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Cycling 2	95	5 sec	–	40
	60	20 sec	FAM/Green, JOE/Yellow	
	72	15 sec	–	

Table 3

AmpliSens-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 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Cycling 2	95	5 sec	–	40
	60	30 sec	FAM, HEX	
	72	15 sec	–	

Table 4

AmpliSens-1 Mx amplification program (for Mx3000P and Mx3005P)

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



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

3. Fluorescence detection is on the 2-nd pass (**60 °C**) in appropriate fluorometer channels (see tables 2, 3, 4).
4. Make the adjustment of the fluorescence channel sensitivity.

8. DATA ANALYSIS.

The results are interpreted with the software of applied instrument through the presence (or absence) of fluorescence curve intersection with the threshold line, that corresponds to presence (absence) of Ct value in appropriate column of the result grid. Internal Control is detected in the FAM/Green fluorescence channel, *Parvovirus* B 19 DNA is detected in the JOE/Yellow/HEX fluorescence channel.

For data analysis settings and Ct values see Appendix 1 in case of **Rotor-Gene™ 3000 and Rotor-Gene™ 6000** using, Appendix 2 in case of **iQ5 and iQ iCycler** or Appendix 3 in case of **Mx3000P and Mx3005P**.

Results interpretation

Results for controls

Control	Controlled stage	Results		Interpretation
		FAM/ Green (IC)	JOE/Yellow/HEX (<i>Parvovirus B19</i>)	
C-	DNA isolation	Pos (not more than indicated in bulletin)	Neg	OK
NCA	Amplification	Neg	Neg	OK
C+	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 calculating of *Parvovirus B19* DNA concentration for plasma and amniotic fluid is carried out by formula:

$KK \text{ Parvovirus B19 DNA} = K \text{ Parvovirus B19 DNA} / K_{STI-87} \times IC \text{ coefficient}$

$K \text{ Parvovirus B19 DNA}$ – copies number *Parvovirus B19* DNA in DNA-sample;

K_{STI-87} - copies number of DNA STI-87 in DNA-sample;

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

9. TROUBLESHOOTING.

Results of analysis are not being registered in the following cases:

- If any Ct value appears in result grid for Negative Controls (NCA, C-) in JOE/Yellow/HEX (*Parvovirus B19*) and/or FAM/Green (IC STI-87) channel it indicates reagents or samples contamination. Results of analysis are considered irrelevant. Test analysis for all samples must be repeated and measures for detecting of contamination source must be undertaken.
- If Ct value is absent in result grid for positive control C+ (Positive Control DNA *Parvovirus B19* and STI), results of analysis are irrelevant. PCR should be repeated for all samples.
- If Ct values are absent for analyzed samples in FAM/Green channel (Internal Control) in result grid, it indicates extraction stage failure. Analysis should be repeated for these samples starting from the extraction stage. If Ct of Internal Control for analyzed sample exceeds the value indicated in bulletin, while Ct of DNA *Parvovirus B19* is more than value indicated in bulletin, analysis should be

repeated for these samples starting from the extraction stage. High Ct values can occur due to DNA losses during DNA isolating or presence of inhibitors.

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

10. 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 the temperature between 2 °C and 8 °C, when not in use. All components of the **AmpliSens® *Parvovirus B19-FRT*** PCR kit are to be stable until labeled expiration date.



PCR-mix-1-FRT *Parvovirus B19*, PCR-mix-2-FRT and polymerase (TaqF) are to be stored at the temperature not more than minus 16°C

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® *Parvovirus B19-FRT*** PCR kit is 400 copies per 1 ml.

Linear range is from 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 Federal State Institution of Science Central Research Institute of Epidemiology) or NucliSENS® easyMAG® (manufactured by Biomerieux, France) automated system are additionally used.

11.2. Specificity.

Specificity of **AmpliSens® *Parvovirus B19-FRT*** PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.















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.

13. QUALITY CONTROL.

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

14. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Authorised representative in the European Community.
	Contains sufficient for <n> tests		Caution, consult accompanying documents
	Consult instructions for use		Negative control of amplification
	Positive control		Negative control