AmpliSens® F2/F5-SNP-FRT PCR kit

RUO

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	⚠	Caution
LOT	Batch code	Σ	Contains sufficient for <n> tests
RUO	Research Use Only		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer		Keep dry
	Date of manufacture	NCA	Negative control of amplification
C1+, C2+, C3+	Positive controls of amplification	C–	Negative control of extraction

1. INTENDED USE

AmpliSens® F2/F5-SNP-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative genotyping the 20210 G>A (rs1799963) polymorphism in prothrombin gene (F2) and R534Q G>A (rs6025) polymorphism in proaccelerin gene (F5) in biological material (whole venous blood) using real-time hybridization-fluorescence detection of amplified products in order to determine genetic predisposition to thrombosis. The material for PCR is DNA-samples extracted from test material.

Indications and contra-indications for use of the reagent kit

The reagent kit can be used to determine the predisposition to thrombosis develop (venous and arterial) in patients with thromboembolic diseases (especially recurrent and developing at a young age), in patients with cardiovascular diseases (including family history), in women with miscarriage and other obstetric pathologies in the period before surgery, before the appointment of oral contraceptives.

There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

There are no restrictions on the use of the PCR kit in various population and demographic groups.

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

2. PRINCIPLE OF PCR DETECTION

The principle of testing is based on the amplification of human genomic DNA fragments containing F2 gene 20210 G>A (rs1799963) polymorphism and F5 gene R534Q G>A (rs6025) polymorphism with real-time hybridization-fluorescence detection.

The detection by the polymerase chain reaction (PCR) is based on the amplification of the genome specific region using specific primers. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time 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® F2/F5-SNP-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

Variant FRT-50 F contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glicosylase (UDG) and deoxyuridine triphosphate (dUTP). The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because (dUTP) is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

Allele-specific fluorescently-labeled oligonucleotides allow determining the homozygous and heterozygous state of analyzed genetic polymorphisms. The determination of polymorphism alleles is carried out in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE	ROX	Cy5
DNA-target	rs1799963 G allele	rs1799963 A allele	rs6025 G allele	rs6025 A allele
Target gene	F2	F2	F5	F5

3. CONTENT

AmpliSens® F2/F5-SNP-FRT PCR kit is produced in 2 forms:

variant FRT-50 F, REF S-3451-1-CE

variant FRT-L, REF S-3452-1-4-CE

Variant FRT-50 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL F2/F5	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-buffer-C	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
C+ DNA 1	colorless clear liquid	0.2	1 tube
C+ DNA 2	colorless clear liquid	0.2	1 tube
C+ DNA 3	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube

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

Variant FRT-50 F is intended for 55 reactions (including controls).

Variant FRT-L includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix F2/F5-Lyo	white powder	–	48 tubes of 0.2 ml
C+ DNA 1	colorless clear liquid	0.2	1 tube
C+ DNA 2	colorless clear liquid	0.2	1 tube
C+ DNA 3	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.5	2 tubes
Negative Control (C–)*	colorless clear liquid	1.2	1 tube

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

Variant FRT-L is intended for 48 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- Vacuum tubes with coagulant (EDTA) for sampling, storage and transportation of blood samples for in vitro study.
- Sterile bilateral needles for vacuum tubes intended for venous blood collection for in vitro study.
- Reagent for pretreatment of whole peripheral and cord blood.
- RNA/DNA extraction kit.
- TE-buffer reagent.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 100, 200, 1000 µl).
- Tube racks.
- Microcentrifuge for Eppendorf tubes (RCF max. 12,000 x g).
- Vortex mixer.
- Vacuum aspirator with flask for removing supernatant.
- PCR box.
- Real-time instruments (for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes:
  a) screwed or tightly closed 1.5 and 2-ml tubes for pretreatment;
  b) screwed or tightly closed 1.5-ml tubes for reaction mixture preparation for variant FRT-50 F;
  c) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used for variant FRT-50 F;
  d) thin-walled 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used for variant FRT-50 F.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Disposable powder-free gloves and a laboratory coat.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section "Content").
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

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

6. SAMPLING AND HANDLING

AmpliSens® F2/F5-SNP-FRT PCR kit is intended for the analysis of DNA extracted with RNA/DNA extraction kits from the biological material (whole venous blood).

Sampling

Whole venous blood. Blood should be taken using disposable 0.8-1.1 mm diameter needle into the tube with EDTA (special vacuum system Vacuette®). After blood sampling the tube should be gently inverted several times for the thoroughly mixing with the anticoagulant. (Otherwise, blood will coagulate and DNA extraction will be impossible!) After mixing, place the tube in a rack.

The samples can be stored before the pretreatment:

– at the temperature from 20 to 25 °C – for 6 hours after material sampling,
– at the temperature from 2 to 8 °C – for 1 day.

Freezing of whole venous blood samples is unallowable.

Whole venous blood samples can be transported at the temperature from 2 to 8 °C for 1 day.

Pretreatment

The samples of whole venous blood while using **RIBO-prep** nucleic acid extraction kit are to be pretreated using **Hemolytic** reagent.

Add 1.0 ml of **Hemolytic** and 0.1 ml of blood to the disposable 1.5-ml tube. Gently vortex the tubes and leave them for 10 minutes, stirring occasionally. Centrifuge at 4,000 g (for example, 8,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 2 minutes. Remove the supernatant using vacuum aspirator not disturbing the pellet. After washing the cell pellet should be white, only a small pinkish bloom on the pellet is allowed (the remains of the destroyed erythrocytes). The washing using **Hemolytic** may be repeated if necessary. The obtained leucocytes pellet must be immediately lysed (in case of extraction using **RIBO-prep** add **300 µl of Solution for Lysis** and then extract DNA in accordance with the *Instruction Manual* enclosed to the **RIBO-prep** reagent kit without adding **Solution for Lysis** once again) or freezed.

The freezed pellet can be stored at the temperature from minus 24 to minus 16 °C for 2 weeks or at the temperature ≤ –68 °C for 1 year.

In case of using other reagent kits for DNA extraction the test material should be pretreated if necessary in accordance with the *Instruction Manual* to the using reagent kit.

Interfering substances and limitations of using test material samples

The next samples are inapplicable for analysis:

– the whole blood samples, collected in the tubes with heparin as anticoagulant,
– the whole blood samples, containing blood clot or which has been exposed to freezing.

Potential interfering substances

Endogenous and exogenous substances that may be present in the biological material (whole venous blood) used for the study were selected to assess potential interference. Samples of whole venous blood with known genotypes taken in tubes with EDTA as an anticoagulant without adding and with the addition of potentially interfering substances in concentrations exceeding the upper limit of the normal concentration of these substances in whole blood were tested to assess the effect of endogenous substances (Table 2).

Table 2

Potential interferent	Tested concentration	Interference presence
total bilirubin	210 µmol/l (upper limit of normal – 21 µmol/l)	Not detected
total cholesterol	78 mmol/l (upper limit of normal – 7,8 mmol/l)	Not detected
triglycerids	37,0 mmol/l (upper limit of normal – 3,7 mmol/l)	Not detected
hemoglobin	250 g/l (upper limit of normal – 170 g/l)	Not detected

Samples of whole venous blood with known genotypes, taken in test tubes with heparin and in test tubes with EDTA as anticoagulants were tested to assess the effect of exogenous substances (anticoagulants) (Table 3).

Table 3

Potential interferent	Tested concentration	Interference presence
potassium EDTA	to 2,0 mg/ml	Not detected
lithium heparin	from 12 IU/ml	Detected

Samples of three genotypes were tested for each of rs1799963 polymorphism in the prothrombin (F2) gene and rs6025 polymorphism in the proaccelerin (F5) gene: GG homozygote, AG heterozygote, and AA homozygote, each sample in three repeats. The genotype of whole blood samples was confirmed by the pyrosequencing method using the PyroMark genetic analysis system.

7. WORKING CONDITIONS

AmpliSens® F2/F5-SNP-FRT PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. DNA Extraction

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

- **RIBO-prep**.

Extract the DNA according to the manufacturer's protocol.

The volumes of reagents and samples when the DNA is extracted by the RIBO-prep reagent kit:

NOTE: The test sample is the pellet obtained after whole venous blood (**100 µl**) pretreatment using **Hemolytic.**

Add **100 µl of Negative Control (C–)** into the tube labeled C– (Negative Control of Extraction).

The volume of elution is **50 µl.**

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

It is recommended to define DNA concentration in obtained extracted samples and to dilute extracted DNA using TE-buffer to required concentration 10-30 ng per reaction (corresponds to concentration of extracted DNA $1.2*10^5$ – $3.6*10^5$ copies/ml) (it is recommended to use TE-buffer reagent manufactured by FBIS CRIE).

Variant FRT-50 F

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

1. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:
   – **10 µl** of **PCR-mix-FL F2/F5**,
   – **5 µl** of **PCR-buffer-C**,
   – **0.5 µl** of **polymerase (TaqF)**.

Prepare the reaction mixture for the total number of test and control samples plus several extra reactions. See the number of control samples in item 7.

NOTE: Prepare the reaction mixture just before use.

2. Thaw the tube with **PCR-mix-FL F2/F5**. Mix the content of the tubes with **PCR-mix-FL F2/F5, PCR-buffer-C** and **polymerase (TaqF)**. Sediment the drops by vortex.
3. To prepare the reaction mixture, mix the required quantity of **PCR-mix-FL F2/F5, PCR-buffer-C** and **polymerase (TaqF)** in a new sterile tube**.** Sediment the drops on vortex.

NOTE: The prepared mixture should be stored for no more than 2 hours.

4. Take the required number of tubes/strips for amplification of the DNA obtained from test and control samples.
5. Transfer **15 µl** of the prepared mixture to each tube. Utilize the rest of reaction mixture.

6 Add **10 µl** of **DNA samples** obtained at the DNA extraction stage from test samples to the prepared tubes.

7 Carry out the control amplification reactions:

- **C1+** – Add **10 µl** of **C+ DNA 1** to the tube with reaction mixture labeled **C1+** (Positive Control of Amplification)
- **C2+** – Add **10 µl** of **C+ DNA 2** to the tube with reaction mixture labeled **C2+** (Positive Control of Amplification)
- **C3+** – Add **10 µl** of **C+ DNA 3** to the tube with reaction mixture labeled **C3+** (Positive Control of Amplification)
- **NCA** – Add **10 µl** of **TE-buffer** to the tube with reaction mixture labeled **NCA** (Negative Control of Amplification)
- **C–** – Add **10 µl** of **the sample extracted from the Negative Control (C–) reagent** to the tube with reaction mixture labeled C– (Negative control of Extraction).

Variant FRT-L

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

Use disposable filter tips for adding reagents, DNA and control samples into tubes.

1. Take the required number of the tubes with ready-to-use lyophilized reaction mixture **PCR-mix-F2/F5-Lyo** for amplification of DNA from test and control samples (see the number of control samples in point 3).

2 Add **25 µl** of **DNA samples** extracted from test samples into the prepared tubes.

3 Carry out the control reactions:

- **C1+** – Add **10 µl** of **C+ DNA 1** and **15 µl** of **TE-buffer** to the tube with reaction mixture labeled **C1+** (Positive Control of Amplification)
- **C2+** – Add **10 µl** of **C+ DNA 2** and **15 µl** of **TE-buffer** to the tube with reaction mixture labeled **C2+** (Positive Control of Amplification)
- **C3+** – Add **10 µl** of **C+ DNA 3** and **15 µl** of **TE-buffer** to the tube with reaction mixture labeled **C3+** (Positive Control of Amplification)
- **NCA** – Add **25 µl** of **TE-buffer** to the tube with reaction mixture labeled **NCA** (Negative Control of Amplification)
- **C–** – Add **25 µl** of **the sample extracted from the Negative Control (C–) reagent** to the tube with reaction mixture labeled C– (Negative control of Extraction).

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

8.2.2. Amplification

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

Table 4

AmpliSens unified amplification and fluorescence detection program for rotor-[1] and plate-type instruments[2]

Step	Temperature, °C	Time	Fluorescence detection	Cycles
1	50	15 min	–	1
2	95	15 min	–	1
3	95	10 s	–	45
	60	20 s	FAM, JOE, ROX, Cy5	

NOTE: Any combination of the tests including test with reverse transcription and amplification can be performed in one instrument simultaneously with the use of the unified amplification program. If only the tests for DNA detection are performed in one instrument then the first step of reverse transcription (50 °C – 15 minutes) can be omitted for time saving

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin*.
3. Insert tubes into the reaction module of the instrument.

NOTE: It is recommended to sediment drops from walls of tubes by short centrifugation before placing them into the instrument.
Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument.

4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in four channels:

Table 5

Gene	rs	Channel for fluorophore			
		FAM	JOE	ROX	Cy5
F2	rs1799963	G allele	A allele	—	—
F5	rs6025	—	—	G allele	A allele

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid. Characteristics of threshold line settings for plate-type and rotor-type instruments are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.
Principle of interpretation is the following:

Genotyping according to 20210 G>A (rs1799963) polymorphism in F2gene

1. *Ct* values are analyzed for each sample in channels for FAM and JOE fluorophores.
2. The sample is **GG homozygote** if the *Ct* value determined for the test sample in the channel for FAM fluorophore is less or equal to the boundary value, whereas the *Ct* value in the channel for JOE fluorophore is absent or the difference between *Ct* values in channels for JOE and FAM fluorophores (Δ*Ct* (JOE/FAM)) is greater than the boundary value.
3. The sample is **AA homozygote** if the *Ct* value determined for the test sample in the channel for JOE fluorophore is less or equal to the boundary value, whereas the *Ct* value in the channel for FAM fluorophore is absent or the difference between *Ct* values in channels for FAM and JOE fluorophores (Δ*Ct* (FAM/JOE)) is greater than the boundary value.
4. The sample is **GA heterozygote** if the *Ct* value determined for the test sample in channels for FAM and JOE fluorophores is less or equal to the boundary value, whereas the difference between *Ct* values in absolute magnitude in channels for FAM and JOE fluorophores (Δ*Ct* (FAM/JOE)) is less than the boundary value.
5. The result is **invalid** for the test sample if the difference between *Ct* values in absolute magnitude in channels for FAM and JOE fluorophores (Δ*Ct* (FAM/JOE)) is greater than the boundary value for heterozygote and is less than the boundary value for homozygote.
6. The result is **invalid** for the test sample if the *Ct* value determined in both channels is greater than the boundary *Ct* value.

Genotyping according to R534Q G>A (rs6025) polymorphism in F5 gene

1. *Ct* values are analyzed for each sample in channels for ROX and Cy5 fluorophores.
2. The sample is **GG homozygote** if the *Ct* value determined for the test sample in the channel for the ROX fluorophore is less or equal to the boundary value, whereas the *Ct* value in the channel for Cy5 fluorophore is absent or the difference between *Ct* values in channels for Cy5 and ROX fluorophores (Δ*Ct* (Cy5/ROX)) is greater than the boundary value.
3. The sample is **AA homozygote** if the *Ct* value determined for the test sample in the channel for the Cy5 fluorophore is less or equal to the boundary value, whereas the *Ct* value in the channel for ROX fluorophore is absent or the difference between *Ct* values in channels for ROX and Cy5 fluorophores (Δ*Ct* (ROX/Cy5)) is greater than the boundary value.
4. The sample is **GA heterozygote** if the *Ct* value determined for the test sample in channels for ROX and Cy5 fluorophores is less or equal to the boundary value, whereas the difference between *Ct* values in absolute magnitude in channels for ROX and Cy5 fluorophores (Δ*Ct* (ROX/Cy5)) is less than the boundary value.
5. The result is **invalid** for the test sample if the difference between *Ct* values in absolute magnitude in channels for ROX and Cy5 fluorophores (Δ*Ct* (ROX/Cy5)) is greater than the boundary value for heterozygote and is less than the boundary value for homozygote**.**
6. The result is **invalid** for the test sample if the *Ct* value determined in both channels is greater than the boundary *Ct* value**.**

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

The result of the PCR analysis is considered reliable only if the results obtained for controls of extraction stages and DNA amplification are correct (according to Table 6 and the *Important Product Information Bulletin* enclosed to the PCR kit).

Table 6

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore			
		FAM	JOE	ROX	Cy5
C-	DNA extraction	Absent	Absent	Absent	Absent
NCA	PCR	Absent	Absent	Absent	Absent
C1+	PCR	< boundary value	Absent or difference between *Ct* values in channels for JOE and FAM fluorophores is more than the boundary value	< boundary value	Absent or difference between *Ct* values in channels for Cy5 and ROX fluorophores is more than the boundary value
C2+	PCR	Absent or difference between *Ct* values in channels for FAM and JOE fluorophores is more than the boundary value	< boundary value	Absent or difference between *Ct* values in channels for ROX and Cy5 fluorophores is more than the boundary value	< boundary value
C3+	PCR	< boundary value	< boundary value	< boundary value	< boundary value

10. TROUBLESHOOTING

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

1. The *Ct* value for Positive Control of Amplification (C1+ and/or C2+ and/or C3+) do not comply with criteria specified in the Table 6. The amplification and detection should be repeated for all samples.
2. *Ct* value is determined for Negative Control of Amplification (NCA) in channels for FAM and/or JOE and/or ROX and/or Cy5 fluorophores. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The amplification and detection should be repeated for all samples.
3. *Ct* value is determined for Negative Control of Extraction (C-) in channels for FAM and/or JOE and/or ROX and/or Cy5 fluorophores. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples.

11. TRANSPORTATION

AmpliSens® F2/F5-SNP-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days. PCR kit can be transported at 2–25 °C for no longer than 3 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® F2/F5-SNP-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-FL F2/F5, PCR-buffer-C and polymerase (TaqF)). All components of the **AmpliSens® F2/F5-SNP-FRT** PCR kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-FL F2/F5, PCR-buffer-C and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C

NOTE: PCR-mix-FL F2/F5 is to be kept away from light.

NOTE: PCR-mix F2/F5-Lyo is to be kept in packages with a desiccant away from light

13. SPECIFICATIONS

13.1. Analytical sensitivity (limit of detection)

Table 7

Test material	Sample volume for extraction, µl	Nucleic acid extraction kit	PCR kit	Analytical sensitivity (limit of detection), copies/ml
Whole venous blood	100	**RIBO-prep**	variant FRT-50 F, variant FRT-L	$1x10^4$

The claimed features are achieved while respecting the rules specified in the section *Sampling and Handling*

13.2. Analytical specificity

The analytical specificity of **AmpliSens® F2/F5-SNP-FRT** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.
AmpliSens® F2/F5-SNP-FRT PCR kit determines genotypes rs1799963 and rs6025 polymorphisms in F2 and F5 genes. The analytical specificity of the PCR kit was proved while testing 200 samples of human genomic DNA with known genotype by specific sequence method using PyroMark Q24.
Nonspecific reactions and incorrect genotyping rs1799963 and rs6025 polymorphisms in F2 and F5 genes were not observed while testing.
The information about known interfering substances is specified in the *Interfering substances and limitations of using test material samples*.

[1] For example, Rotor-Gene Q (QIAGEN, Germany).
[2] For example, CFX 96 (Bio-Rad).

14. REFERENCES

1. Simone B., De Stefano V., Leoncini E. Risk of venous thromboembolism associated with single and combined effects of Factor V Leiden, Prothrombin 20210A and Methylenetethraydrofolate reductase C677T: a meta-analysis involving over 11,000 cases and 21,000 controls. // Eur J Epidemiol. 2013. V. 28. №8. P. 621–647. doi:10.1007/s10654-013-9825-8

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® F2/F5-SNP-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

AmpliSens®

Federal Budget Institute of Science "Central Research Institute for Epidemiology"
3A Novogireevskaya Street
Moscow 111123 Russia