



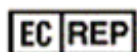
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**For professional use only**

## **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit**

### **INSTRUCTION FOR USE**



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## 1. INTENDED USE

The **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** is intended for research and diagnostic applications. The **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** is an *in vitro* Nucleic Acid Test (NAT) – human genotyping-based product. The **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** is designed to detect polymorphisms of genes associated with the risk of dyscrasia (bleeding, thrombosis): F2 (prothrombin, coagulation factor II, 20210 G>A); F5 (coagulation factor V Leiden, 1691 G>A) by real-time PCR in human biological material (whole peripheral blood, dry blood spots, buccal epithelium).

**Indications:** Diagnosis of genetic predisposition to dyscrasia; screening of pregnant women.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit**.

The **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: personnel qualified in molecular diagnostics methods and working in the clinical and diagnostic laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

## 2. METHOD

The implemented PCR method is based on amplification of a target DNA sequence. The detection is based on melting curve analysis.

The **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** employs fluorescent probes each of one specific to one of two alleles of a gene. The PCR-mix contains two distinguishably labelled allele-specific probes bearing reporter fluorescent dyes (Fam and Hex) for each variant of polymorphism. After amplification melting of amplicon-signal probe complexes is performed. It results in changing fluorescence level and is detected by the real-time thermal cycler and is represented by the software as a graph. If the signal probe is partially complementary to the DNA-target the melting temperature will be less than in case when signal probe is absolute complementary to the DNA-target. The interpretation of results is made based on melting temperatures.

In PCR-mix for each polymorphism the system for human genomic DNA amplification is included. It allows to control quantity of human DNA in amplification tube to exclude mistakes in genotyping.

The system for human genomic DNA amplification includes DNA-probe with fluorescent tag (Cy5) and quencher molecule. While being hybridized to a target sequence, fluorescent probes are inactivated (quenched). When the amplicon is synthesized the probes denature and fluorescent tag is no more quenched and therefore provide fluorescent signal. The intensity of fluorescence is measured by Real-time PCR thermal cycler at every step and analyzed with the software provided.

The **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** includes amplification mixtures specific for each genetic polymorphism. A list of gene polymorphisms and possible genotypes detected by the kit is given in Table 1.

Table 1. List of gene polymorphisms and possible genotypes determined by the **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit**

Polymorphism	Genotype		
	Frequent homozygous	Rare homozygous	Heterozygote
F2: 20210 G>A	GG	AA	GA
F5: 1691 G>A (Arg506Gln)	GG	AA	GA

The application of three fluorescent dyes makes it possible to determine two alleles and estimate the amount of genomic DNA simultaneously in one tube. Table 2 shows the detection channels of PCR-mix.

Table 2. Detection channels of amplification products

PCR-mix	Fam	Hex	Rox	Cy5	Cy5.5
F2: 20210 G>A	G	A	-	IC	-
F5: 1691 G>A (Arg506Gln)	G	A	-	IC	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** (see the catalogue at <https://www.dna-technology.com> to see available supply options). The current version of the software is available for download at <https://www.dna-technology.com/software>.

To control the detection of frequent and rare homozygous, control samples (CS) for the **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** are used in the assay.

Control samples are mixtures of cloned gene regions detected using the kit that are in frequent or rare homozygous states and are intended for quality control (determination of frequent and rare homozygous) by the end user of the kit.

**ATTENTION!** Control samples are compatible with **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** only.

Table 3. **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** control samples

Control sample	Controlled amplification mix	Expected genotyping result
frequent homozygous	F2: 20210 G>A	GG
	F5: 1691 G>A (Arg506Gln)	GG
rare homozygous	F2: 20210 G>A	AA
	F5: 1691 G>A (Arg506Gln)	AA

### 3. CONTENT

The **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** contains is represented in Table 4, 5.

Table 4. The **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** content, package N, Set № 1 for R1-H958-N3/4EU

Reagent	Description	Total volume	Amount
PCR-mix F2: 20210 G>A	Colorless transparent liquid	960 µL	1 tube
PCR-mix F5: 1691 G>A (Arg506Gln)	Colorless transparent liquid	960 µL	1 tube
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	80 µL	1 tube
PCR-buffer	Colorless transparent liquid	2.0 mL (1.0 mL in each tube)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each tube)	2 tubes

Table 5. The **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** content, package N, Set № 2 for R1-H959-N3/4EU

Reagent	Description	Total volume	Amount
PCR-mix F2: 20210 G>A	Colorless transparent liquid	960 µL	1 tube
PCR-mix F5: 1691 G>A (Arg506Gln)	Colorless transparent liquid	960 µL	1 tube
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	80 µL	1 tube
PCR-buffer	Colorless transparent liquid	2.0 mL (1.0 mL in each tube)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each tube)	2 tubes
Control sample (frequent homozygous)	Colorless transparent liquid	120 µL	1 tube
Control sample (rare homozygous)	Colorless transparent liquid	120 µL	1 tube

All components are ready to use and do not require additional preparation for operation.

The kit is intended for single use and designed for 48 tests for **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit**.

#### 4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

##### 4.1. Specimen collection

- For blood collection: 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant.

Please use only salt of EDTA or sodium citrate as an anticoagulant, since other substances can provide PCR inhibition.

##### 4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 16000);
- Thermostat (temperature range 50-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-RAPID Genetics** ([REF](#) P-021/4EU), **PREP-GS Genetics** ([REF](#) P-023/4EU), **PREP-CITO DBS** ([REF](#) P-029-N/2EU), **PREP-MB MAX** ([REF](#) P-103-A/8EU), **PREP-OPTIMA** ([REF](#) P-016-1/2EU, [REF](#) P-016-N/2EU), and **PREP-OPTIMA MAX** ([REF](#) P-015-N/2EU) extraction kits are recommended);
- Physiological saline solution 0.9% NaCl (Sterile);

- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for 0.2 mL strips;
- PCR tube 0.2 mL or strips;
- PCR tube rack for 0.2 mL tubes or strips;
- 1.5 mL tubes;
- Tube rack for 1.5 mL tubes;
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

Real-time PCR thermal cycler.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from <https://www.dna-technology.com/software>.

The OS supported: all versions of Windows starting from 7.

## 5. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

All components of the **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** except TechnoTaq MAX polymerase must be stored at temperatures from 2 °C to 8 °C during the storage period. The Taq-AT-polymerase must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period. PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period.

The excessive temperature and light can be detrimental to product performance.

The kit has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions of the kit components.

Transportation of the kit, except TechnoTaq MAX polymerase, is allowed in thermobox with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but no more than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

It is allowed to transport the TechnoTaq MAX polymerase in thermobox with ice packs by all types of roofed transport at temperatures up to 25 °C but no more than 5 days and should be stored at temperatures from minus 18 °C to minus 22 °C immediately on receipt.

Shelf-life of the kit following the first opening of the primary container:

- components of the kit should be stored at temperatures from 2 °C to 8 °C during the storage period;
- PCR-mix for amplification should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period;
- TechnoTaq MAX polymerase should be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

The kit stored in under undue regime should not be used.

An expired the **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** should not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit**.

## 6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial DNA synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the

extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose. Remove PCR waste only in a closed form. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Do not open the tubes after amplification. Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

### **Emergency actions**

**Inhalation:** Inhalation of the PCR-mix contained within this kit is unlikely, however care should be taken.

**Eye Contact:** If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

**Skin Contact:** If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

**Ingestion:** If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

## **7. SAMPLES**

The **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** is designed to detect DNA extracted from the peripheral blood, dried blood spots and buccal epithelium.

**ATTENTION!** Intravenous injections of heparin, infusions of parenteral nutrition are not allowed if done less than 6 hours before the test.

Sampling, sample processing procedures and storage are carried out in accordance with the instructions to the DNA extraction kit from biological material.



### **Interfering substances**

The maximum concentration of interfering substances, which do not affect the amplification of the laboratory control sample and internal control: hemoglobin – 0,35 mg/mL DNA sample, isopropyl alcohol – 100 µL/mL DNA sample, methyl acetate – 100 µL/mL DNA sample.

### **Blood sampling**

Peripheral blood sampling is carried out in vacuum plastic tube. It may be 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant. After taking the material, it is necessary to mix the blood with anticoagulant turning the tube 2 – 3 times.

**ATTENTION!** It is not allowed to use heparin as an anticoagulant.

### **Dried blood spots**

The blood is applied to the sampling paper in an amount sufficient to obtain DBS with a diameter of at least 1 cm, and the blood must penetrate the paper. After applying the sample, the filter card is dried horizontally on a clean, degreased surface for at least two hours at room temperature (from 18 °C to 25 °C) without any external source of heat and direct sunlight.

**ATTENTION!** To avoid incorrect results, no one other than the patient being tested should be allowed to touch the sampling paper without gloves.

Punch out 3 3.0 mm discs from the central part of the DBS with a hand puncher and place them in a 1.5 mL tube.

**ATTENTION!** It is recommended to punch out the discs immediately before the DNA extraction procedure.

### **Buccal epithelium**

**ATTENTION! Sampling procedure is carried out using a dry swab.** It is necessary to keep the solutions away from skin, eyes, and mucous membranes. If the contact occurs, it is necessary to wash the affected area immediately and obtain medical attention.

Sampling procedure is carried out using special sterile disposable instruments – swabs, cytobrushes or tampons in accordance with established procedure.

It is recommended to abstain from eating, smoking, and taking medication perorally two hours prior the biological material sampling. Before sampling rinse mouth with water twice.

Sampling is carried out in 1.5 mL tube with lysis solution or transport medium.

Order of taking:

- 1 In order to take the swab, rotate the sterile swab slightly pressing the buccal region for 30 seconds.
- 2 Open the tube.
- 3 Put the swab into the tube with lysis solution, rotate the swab for 10-15 seconds and rinse it thoroughly. Avoid spraying of solution.
- 4 Remove swab from solution, press it to the wall of tube and squeeze the rest of the liquid. Throw out the swab.
- 5 Close the tube tightly and mark it.

### **Transportation and storage of the samples**

Whole peripheral blood can be stored at 2 °C to 8 °C for no more than 24 hours. If the material cannot be delivered to the laboratory within 24 hours, it can be frozen once. Frozen material can be stored at minus 20 °C for up to one month.

Dried blood spots can be stored for up to one year after drying in individual packages in low humidity

conditions (max. 30%) at 2 °C to 8 °C. For longer storage periods, samples of dried blood spots should be stored as recommended by the filter card manufacturer.

Buccal epithelium can be stored at 2 °C to 8 °C for a no more than 24 hours. If the material cannot be delivered to the laboratory within 24 hours, it can be frozen once. Frozen material can be stored at minus 20 °C for up to one month.

**ATTENTION!** The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-RAPID Genetics**, **PREP-GS Genetics**, **PREP-CITO DBS**, **PREP-OPTIMA**, **PREP-OPTIMA MAX** and **PREP-MB MAX** DNA extraction kits user manuals.

## 8. PROCEDURE

### DNA extracting from biological material

DNA extraction is carried out according to the extraction kit instructions. **PREP-RAPID Genetics**, **PREP-GS Genetics**, **PREP-CITO DBS**, **PREP-OPTIMA**, **PREP-OPTIMA MAX** and **PREP-MB MAX** extraction kits are recommended. It is allowed to use any kits of reagents registered as a medical device and recommended by manufacturers for the extraction of DNA from the corresponding types of biological material.

**ATTENTION!** Independently of DNA extraction kit used, a negative control sample should go through all stages of DNA extraction. Physiological saline solution can be used as a negative control in volumes as indicated.

### Assay procedure

**ATTENTION!** The reagents and tubes should be kept away from direct sun light.

- 8.1** Mark the required number of 0.2 mL PCR-tubes for each polymorphism to be tested (one tube for each sample to be tested and one extra for negative control (C-)).

**Example:** there are 5 samples to be tested. If Set № 1 of the kit is being used, mark 10 tubes for samples and 2 tubes for "C-". The resulting number of tubes is 12. If Set № 2 of the kit is being used, mark 10 tubes for samples, 2 tubes for "C-" and 4 tubes for "CS". The resulting number of tubes is 16.

- 8.2** Vortex the tubes with PCR-mixes for 3-5 seconds, then spin for 1-3 seconds to collect the drops.
- 8.3** Add 20 µL of corresponding PCR-mix into the marked tubes (use a new pipette tip for each type of PCR-mix).
- 8.4** Vortex the tubes with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

**ATTENTION!** TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to minus 22 °C. Room temperature exposure is permitted only for a short time. Remove from freezer just prior to use and place on ice.

- 8.5** Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase. Add into one tube:

- 10×(N+1) µL of PCR-buffer,
- 0.5×(N+1) µL of TechnoTaq MAX polymerase,

N — number of the marked tubes including "C-", "CS" (frequent homozygous), "CS" (rare homozygous).

**Example:** 5 samples for 2 polymorphisms to be tested using the Set № 2 of the kit. There are 16 marked tubes. A mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared for 17 (16+1) tubes, i.e. 170 µL PCR-buffer must be mixed with 8.5 µL TechnoTaq MAX polymerase.

- 8.6** Vortex the tube with mixture of PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

**ATTENTION!** The mixture of PCR-buffer and Taq-AT-polymerase must be prepared just prior to use.

- 8.7** Add 10 µL of PCR-buffer and Taq-AT-polymerase mixture into each PCR-tube.

**ATTENTION!** Follow the steps listed in pp. 8. 8 – 8.14 within two hours after addition of PCR-buffer and Taq-AT-polymerase mix to amplification mix.

- 8.8** Add one drop (~20 µL) of mineral oil into each tube. Close the tubes.

- 8.9** Vortex the tubes with samples, “C-” and “CS” for 3-5 seconds and spin down drops for 1-3 seconds.

**ATTENTION!** In case of using **PREP-GS Genetics DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

If the reagent kit **PREP-MB MAX DNA Extraction Kit** is used for DNA extraction, the tubes containing the DNA preparation must be placed on a magnetic rack after vortexing. If the supernatant containing the isolated DNA has been transferred to new tubes after extraction, centrifuge the DNA after vortexing for 3-5 seconds.

**ATTENTION!** Open the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample to prevent contamination. Close the tubes tightly. Use filter tips.

- 8.10** Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the “C-” and the “CS” tubes.

- 8.11** Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into “C-” tube into corresponding tube.

- 8.12** If Set № 2 of the kit is being used, add 5.0 µL of the control samples into each corresponding “CS” tube.

- 8.13** Spin tubes for 1-3 seconds.

- 8.14** Set the tubes into the Real-time Thermal Cycler.

- 8.15** Launch the operating software for DT instrument<sup>1</sup>. Add corresponding test<sup>2</sup>, specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (see 8.14) and run PCR. See Table 6.

**ATTENTION!** The type of the negative control and control sample (CS) tubes must be specified as “Sample”.

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<sup>1</sup> Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.

<sup>2</sup> Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website <https://www.dna-technology.com/assaylibrary>.

Table 6. The PCR program for DTlite and DTprime Thermal Cyclers

Step	Temperature, °C	min	sec	Number of cycles	Optical measurement	$\Delta t$ , °C	Type of the step
1	80	2	00	1			Cycle
	94	5	00				
2	94	0	30	5			Cycle
	67	0	15		√		
3	94	0	5	45			Cycle
	67	0	15		√		
4	25	0	30	1			Cycle
5*	25	0	15	50	√	1.0	«Melting curve», $\Delta t=1$ °C; $T_{con}=75$ °C
6	10	...	...	Storage			Storage
* When creating block 5, tick the "Melting curve" type							

**ATTENTION!** The arrangement of the tubes on the matrix of the thermoblock must strictly follow the order of the tubes on the block. For multi-test mode, the order of the tubes must match the sequence of the tests in the group.

## 9. CONTROLS

The **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** contains amplification system for human genomic DNA intended to sample intake control (IC). IC allows to determine sufficiency of the extracted DNA for analysis. To reveal possible contamination a negative control is required.

**ATTENTION!** A negative control sample should go through all stages of DNA extraction. Physiological saline solution can be used as a negative control sample in volumes indicated in supplied instructions.

The corresponding genotype (see Table 3), which is shown in the table in the column "Polymorphism", must be determined in the control samples (CS).

The test result is considered valid when genotype is defined.

The test result is considered invalid when the Cp of IC (Cy5) is less than 32 or absent.

For negative control samples the software registers invalid result.

In case of obtaining positive result (genotype determination) for negative control sample all results of the current PCR run are considered false. In this case conduction of special procedures against possible contamination is required.

## 10. DATA ANALYSIS

Registration and interpretation of the PCR results held in automatic mode. The graph will show the fluorescence dependence of the melting temperature for each tube in the thermoblock. The table will show the sample ID, the name of the polymorphism being detected, and the genotyping result of each sample. It is possible to create and print a report based on the analysis results. Please refer to DTlite or DTprime thermal cycler's user manual for details on working with software.

The software registers the result of human genomic DNA amplification (IC) for all samples. For samples containing a sufficient quantity of DNA for correct analysis, the software defines the genotype, which is displayed in the table in the "Polymorphism" column.

The corresponding genotype (see Table 3), which is shown in the table in the column "Polymorphism", must be determined in the control samples (CS).

The samples containing an insufficient quantity of DNA (less than 1.0 ng per reaction or  $C_p > 32$ ) will be analyzed as "invalid" (uncertain result).

In the case of uncertain result, PCR method with the same DNA sample, or DNA extraction and PCR, or blood taking (carry out sequentially) is required to repeat.

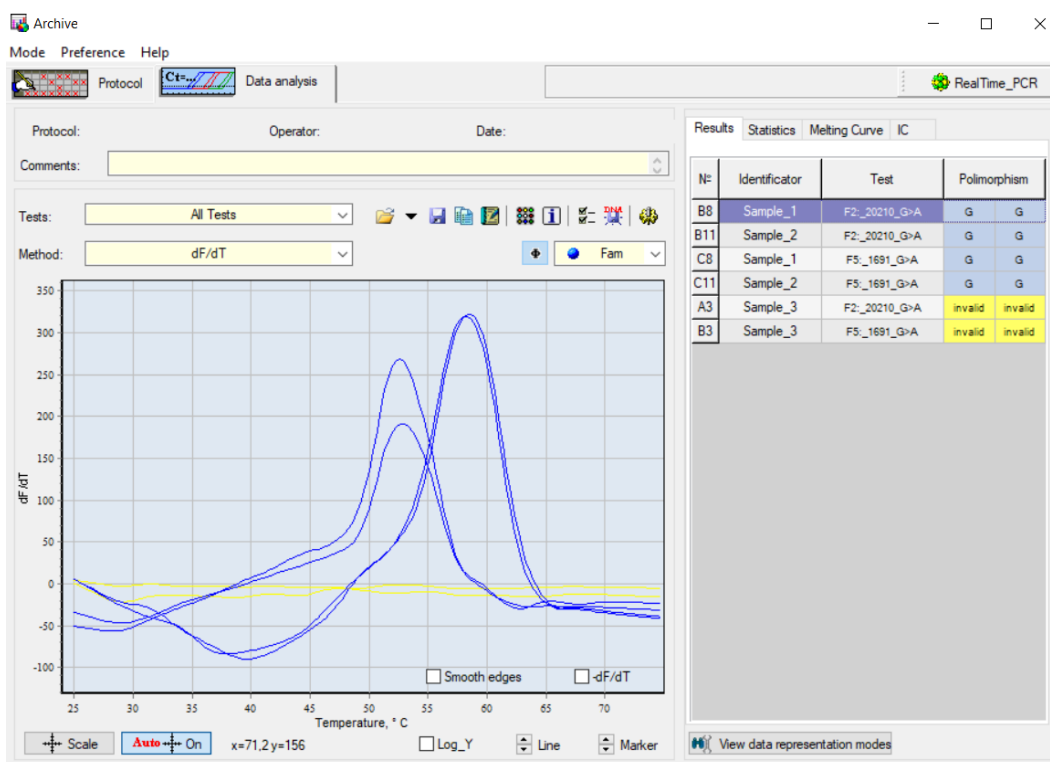
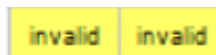
For samples with sufficient amount of DNA ( $C_p \leq 32$ ) the software can register uncertain result




according to the following criteria of polymorphism analysis:

- “Analysis on temperature peak” – the melting temperature of PCR products differs from predetermined more than on 3.0 °C;
- “Differential analysis” - the difference between predetermined and obtained temperatures on Fam and Hex channels for one genotype is more than 2.0 °C;
- “Lower line of temperature peak” - the temperature peak ( $dF/dT$ ) < 5.0. For such samples retesting is required.

For negative control, the software records an invalid result.



Operator can create, save and print a Specific report. To create a Specific report press the “Form the answer button” .

#### Genotyping report

Date  
Number of tube ...  
Patient name ...  
Sex ...  
Age ...  
Organization ...  
Clinician name ...  
CommentsUnique ...

Sample ID: Sample\_1

No	Name of research	Results
		Genotype
1	F2_20210_G>A	G G
2	F5_1691_G>A	G G

Study was carried out

Date  
Signature

Logotype

Information about laboratory

If a positive value is obtained (genotype determination) in a negative control, the results of the whole production series are considered invalid. In this case special measures are required to eliminate contamination.

**ATTENTION!** DNA-Technology Genotyping assays provide genetic information for some, but not all polymorphic loci known to be associated with certain medical conditions. This information estimates a probability of disease development but does not provide a definitive diagnosis, since other genes may contribute to the odds of disease onset. Moreover, the professional medical consultation regarding complex diseases cannot solely rely on genetic testing. The medical recommendations should also consider behavioral, physical, nutritional and familial information of a patient. On the basis of DNA-Technology Genotyping assays, a specialist can conclude whether a person of a certain genotype has lower or higher chance of disease development in relation to average risk. The definitive diagnosis is a derivative of a physicians' experience and the depth of clinical information.

At the assay development stage, we review the most up-to-date scientific literature on genetic associations repeatedly confirmed by independent research. We restrict our genotyping assays to a relatively small set of genetic markers because we believe they provide the most helpful and unbiased information about possible genetic susceptibility to common diseases.

## 11. SPECIFICATIONS

a. The analytical **specificity** of the **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

b. In a determination of analytical **sensitivity**, the **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit** demonstrated the ability to reproducibly detect 1 or more genome equivalents per PCR reaction. The lower limit of detection is not less than 1.0 ng of human DNA per amplification tube, which corresponds to Cp≤32 on the IC detection channel. When the amount of DNA is smaller (CP>32 on the IC detection channel), the manufacturer does not guarantee the correct result of the kit.

After the amplification reaction for samples with insufficient quantity of DNA (less than 1.0 ng per amplification tube), the result is defined as unreliable.

c. Diagnostic characteristics

The analytical sensitivity and analytical specificity values of the tested medical product are calculated as the proportion of correctly identified rare alleles detected among the entire pool of alleles detected by the **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit**.

Polymorphism F2: 20210 G>A

Biological material	F2: 20210 G>A		Diagnostic sensitivity (%)	Diagnostic specificity (%)
	Allele			
	A	G		
	rare	frequent		
Whole peripheral blood	3	107	100% (29.24-100)	100% (96.61-100)
Buccal epithelium	1	49	100% (2.50-100)	100% (92.75-100)
Dried blood spots	1	49	100% (2.50-100)	100% (92.75-100)
Total	5	205	1000% (47.82-100)	100% (98.22-100)

Polymorphism F5: 1691 G>A (Arg506Gln)

Biological material	F5: 1691 G>A (Arg506Gln)		Diagnostic sensitivity (%)	Diagnostic specificity (%)
	Allele			
	A	G		
	rare	frequent		
Whole peripheral blood	3	107	100% (29.24-100)	100% (96.61-100)
Buccal epithelium	1	49	100% (2.50-100)	100% (92.75-100)
Dried blood spots	1	49	100% (2.50-100)	100% (92.75-100)
Total	5	205	1000% (47.82-100)	100% (98.22-100)

**d.** Within-batch and between-batch precision

Within-batch precision amounts at 100% (95% confidence interval 83.16–100%).

Between-batch precision amounts at 100% (95% confidence interval 83.16–100%).

## 12. TROUBLESHOOTING

Table 7. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	Genotype is defined	Contamination	Dispose current batch Perform decontamination procedures
IC	Invalid	PCR inhibition Insufficient amount of DNA	Repeat whole test Resample

If you face to any undescribed issues contact our customer service department regarding quality issues with the kit:

Phone: +7(495) 640.16.93

E-mail: [hotline@dna-technology.ru](mailto:hotline@dna-technology.ru)

<https://www.dna-technology.com/support>

### 13. QUALITY CONTROL

“DNA-Technology Research&Production”, LLC declares that the abovementioned products meet the provision of the Council Directive 98/79/EC for *in vitro* Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of IVDD products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our official representative in EU by quality issues of **Hemostasis F2, F5 mutations REAL-TIME PCR Genotyping Kit**.

Technical support:

E-mail: [hotline@dna-technology.ru](mailto:hotline@dna-technology.ru)

<https://www.dna-technology.com>

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<https://www.dna-technology.com>

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int. ter. Municipal District Chertanovo Severnoye,

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#### 14. KEY TO SYMBOLS

	<i>In vitro</i> diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests		Catalogue number
	Use-by date		Manufacturer
	Batch code		Keep away from sunlight
	Version		Positive control
	Authorized representative in the European Community		Caution



R1-H958-N3/4EU  
R1-H959-N3/4EU



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