DNA-TECHNOLOGY

727 2022-03-16

CE IVD

For professional use only

Fetal RHD Genotyping REAL-TIME PCR Kit

INSTRUCTION FOR USE

EC REP

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REF R1-H802-S3/9EU
R1-H802-23/9EU

VER 727.2022.03.16

Digitally signed by Ceaicovschi Tudor
Date: 2023.10.18 11:27:19 EEST
Reason: MoldSign Signature
Location: Moldova

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1. INTENDED USE

The **Fetal RHD Genotyping REAL-TIME PCR Kit** is intended for research and diagnostic applications. The **Fetal RHD Genotyping REAL-TIME PCR Kit** is an *in vitro* Nucleic Acid Test (NAT) – human genotyping-based product. The **Fetal RHD Genotyping REAL-TIME PCR Kit** is designed to detect cell-free fetal DNA of RHD gene in the blood of Rhd-negative pregnant women with an aid of Polymerase Chain Reaction (PCR) method in order to predict the risk of Rh-disease and hemolytic disease of the fetus and newborn. Peripheral blood is used as a sample.

The obtained results can be used in the framework of prenatal screening of pregnancy complications in Rh-negative women. Starting from 8-10 weeks of pregnancy.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **Fetal RHD Genotyping REAL-TIME PCR Kit**.

The **Fetal RHD Genotyping REAL-TIME PCR 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 user manual.

2. METHOD

Method: polymerase chain reaction (PCR) with real-time results detection; multiplex qualitative analysis.

The implemented PCR method is based on amplification of a target DNA sequence. The process of amplification includes repeating cycles of thermal DNA denaturation, annealing of primers with complementary sequences and their extension by Taq-polymerase.

To increase the sensitivity and specificity of amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The polymerase chain reaction starts only when paraffin is melted. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The **Fetal RHD Genotyping REAL-TIME PCR Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The **Fetal RHD Genotyping REAL-TIME PCR Kit** includes PCR-mix specific for two exons of RHD gene (7 and 10) and human genomic DNA (sample intake control (SIC)). The SIC allows to exclude preanalytical error. If the amount of collected material is insufficient for the analysis, it is necessary to repeat sampling procedure. The calculation of the results is based on the evaluation of the Cp values of the indicator cycles of the studied targets (ΔCp).

DNA probe used for the detection of the 7 and 10 RHD gene exons fragments product amplification includes fluorescent dye Fam and Rox respectively. DNA probe used for the detection of SIC product amplification includes the fluorescent dye Hex. The application of several fluorescent dyes makes it possible to register the results of different amplification reactions taking place simultaneously in one tube. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam	Hex	Rox	Cy5	Cy5.5
RHD gene, 7 exon	SIC	RHD gene, 10 exon	-	-

The automatic analysis is available on "DNA-Technology" made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **Fetal RHD Genotyping REAL-TIME PCR Kit** (see the catalogue at www.dna-technology.com to see available supply options).

The current version of the software is available for download at http://dna-technology.com/software.

3. CONTENT

The **Fetal RHD Genotyping REAL-TIME PCR Kit** contains paraffin sealed PCR-mix, Taq-polymerase solution, mineral oil and positive control. The detailed description of content is presented in Table 2.

Table 2. The **Fetal RHD Genotyping REAL-TIME PCR Kit** content, package S (standard) for R1-H802-S3/9EU and R1-H802-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	96 tube or 12 8-tube strips
Taq-polymerase solution	Colorless transparent liquid	1000 µL (500 µL in each tube)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each tube)	2 tubes
Positive control	Colorless transparent liquid	75 µL	1 tube
Strip's caps [1]	12 8-caps		

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

The **Fetal RHD Genotyping REAL-TIME PCR Kit** is intended for single use and designed for 96 tests (no more than 46 analysed samples in doubles, negative control samples (in 3 repeats) and positive control sample).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- For blood collection: 4.5 mL Vacuette blood collection tubes with anticoagulant, for example, salt of EDTA at a final concentration of 2.0 mg/mL.

Please use only salt of EDTA 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 1150 x g) for 4.5 mL tubes;
- High speed centrifuge (RCF 17000 x g) for 1.5 mL tubes;
- Solid-state thermostat (temperature range 65-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;

[1] - for detection kit packaged in strips REF R1-H802-S3/9EU

- Freezing container, e.g. IsoFreeze 24x1.5/2 mL (SSI), or CoolRack M15, 15x1.5/2 mL (Biocision), or other analogous equipment;
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- 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-NA-FET Extraction Kit** (REF P-027/2EU), are recommended);
- 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;
- Tube rack for 0.2 mL strips;
- Tube rack for 0.2 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 http://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 **Fetal RHD Genotyping REAL-TIME PCR Kit** must be stored at temperatures from 2 °C to 8 °C during the storage period. The PCR-mix for amplification must be stored 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 is allowed in termobox 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.

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;

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

An expired **Fetal RHD Genotyping REAL-TIME PCR 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 **Fetal RHD Genotyping REAL-TIME PCR 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 **Fetal RHD Genotyping REAL-TIME PCR 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 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 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. Work surfaces, as well as rooms where PCR is performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work. 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 **Fetal RHD Genotyping REAL-TIME PCR Kit** is designed to detect DNA extracted from peripheral blood.

Sample collection

The required blood volume is 4.0-4.5 mL. Peripheral blood sampling is carried out in vacuum plastic tubes, for example 4.5 mL Vacuette tubes containing ethylenediaminetetraacetic acid disodium salt (EDTA) at final concentration of 2.0 mg/mL as an anticoagulant. After taking the material, it is necessary to mix the blood with anticoagulant inverting the tube 2 – 3 times.

ATTENTION! It is not allowed to use heparin and sodium citrate as an anticoagulant.

Transportation and storage of the samples

It is recommended to start blood processing in the first two hours after sample intake.

When it is impossible to start blood processing in the first two hours, it is allowed to store blood at temperatures from 18 °C to 25 °C for no more than 4-8 hours.

Sample preparation

It is necessary to perform pretreatment before DNA extraction by the **PREP-NA-FET DNA Extraction Kits.**

1 Centrifuge the tube with blood at 1000-2000 x g for 20 minutes at room temperatures from 18 °C to 25 °C.

2 Mark the required number of 1.5 mL tubes (two for each tested sample).

3 Without touching the lower (cellular) fraction, take 900 mL of the upper fraction (plasma) with an automatic dispenser and transfer it to two marked tubes.

ATTENTION! Only one test tube is used for DNA extraction! The second tube can be frozen at minus 20 °C or lower and, if necessary, used for re-extraction of DNA.

Prior to the start of DNA extraction, the tubes with plasma can be stored at temperatures from 4 °C to 8 °C for 8 hours.

When planning to extraction fetDNA the next day or later, the tubes with plasma should be frozen at minus 20 °C or lower. Frozen plasma can be stored for no more than 3 months. Before starting extraction, one tube of each sample must be thawed at room temperature.

ATTENTION! The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-NA-FET DNA Extraction Kit**'s user manual.

8. PROCEDURE

DNA extraction from biological material

DNA extraction is carried out according to the **PREP-NA-FET DNA Extraction Kit**'s instruction.

ATTENTION! When using kits for the extraction of fetDNA from other manufacturers, incorrect results may be obtained.

ATTENTION! Simultaneously with the DNA extraction from plasma, a negative control sample included in the **PREP-NA-FET DNA Extraction Kit** should go through all stages of DNA extraction in volumes as indicated.

Assay procedure

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

ATTENTION! Due to the small amount of fetal DNA in the blood of pregnant women, analysis of each DNA sample must be done in duplicate, otherwise can be obtained incorrect results.

ATTENTION! When using package S (R1-H802-S3/9EU), strips, strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

8.1 Mark 2 strip tubes (or 2 separate tubes) with PCR-mix for each test sample, 3 for negative control (C-) and 1 for positive control (C+).

Example: to test 2 samples, mark 4 tubes for the samples, 3 tubes for "C-" and 1 tube for "C+". The resulting number of tubes is 8.

Tube marking

Sample 1	Tubes 1-2
Sample 2	Tubes 3-4
C+	Tube 5
C-	Tubes 6-8

8.2 Vortex the tube with Taq-polymerase solution for 3-5 seconds, then spin briefly for 1-3 seconds to collect the drops.

8.3 Add 10 µL of Taq-polymerase solution into each tube. Avoid paraffin layer break.

8.4 Add one drop (~20 µL) of mineral oil into each tube. Close the strips.

8.5 Vortex the tubes with DNA samples, positive control and negative control for 3-5 seconds, then spin down the drops for 1-3 seconds.

ATTENTION! Open the cap of the tube, add DNA sample (or control sample), then close the tube before proceeding to the next tube to prevent contamination. In case of using tubes in strips, close the strip before proceeding to the next DNA sample to prevent contamination. Close the tubes/strips tightly. Use filter tips.

8.6 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the "C-", "C+" tubes. Avoid paraffin layer break.

8.7 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure and positive control (C+) into corresponding tube. Avoid paraffin layer break.

8.8 Spin the tubes/strips briefly for 1-3 seconds on vortex mixer.

8.9 Set the tubes/strips into the Real-time Thermal Cycler.

8.10 Launch the RealTime_PCR application in "Device operation" mode. Upload the «RHD_en.ini» file supplied with the kit before first run. Please refer to DTlite or DTprime thermal cycler's user manual for details on working with .ini files. In subsequent runs add corresponding test to the protocol, specify the number and IDs of the samples, specify the position of the tubes/strips in the thermal unit (p. 8.9), and run PCR. See Table 3.

Table 3. The PCR program for DTlite and DTprime Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		√	
3	94	0	10	45		Cycle
	64	0	15		√	
4	94	0	5	1		Cycle
5	10		…	Holding		Holding
√ - optical measurement						

9. CONTROLS

The **Fetal RHD Genotyping REAL-TIME PCR Kit** contains positive control sample. Positive control is a cloned part of the genome detected by the kit. It is produced with genetic engineering techniques and is characterized by automatic DNA sequencing. To reveal possible contamination, a negative control is required.

ATTENTION! A negative control sample should go through all stages of DNA extraction. Use the negative control sample included in the **PREP-NA-FET DNA Extraction Kit** in volumes as indicated.

The test result is considered valid when fetal Rh factor is defined.

The test result is considered invalid when fetal Rh factor is not defined.

If Cp <28 or >32 for “C+“, repeat of amplification of the whole series is required.

If Cp is specified, or ≤39 on Hex channel or ≤41 on Fam and Rox channels for “C-“, whole test of current batch considered false. Decontamination is required.

10. DATA ANALYSIS

In case of using DNA-Technology Real-Time PCR Thermal Cyclers, the analysis is performed automatically. In other cases, the analysis is based on the presence or absence of specific signal.

Real-time PCR Thermal Cyclers detects and interprets results automatically. Analysis will be performed by Real-Time PCR application. The resulting graph will display the dependence of fluorescence intensity on the cycle number for each tube for all detection channels used. Sample ID, threshold cycles for two channels (Cp), ΔCp (dCp) and test result for duplicates will be displayed in the right module of the window. Operator can create, save, and print a report.

The test result for each sample is determined automatically by the software, taking into account the SIC Cp values for the Fam channel and ΔCp for Fam and Rox channels in total by duplicates for this sample (see Annex A).

In the samples passed PCR and containing a sufficient amount of DNA, for which the correct ΔCp values are obtained, the program determines the genotype of the test sample, which is displayed in the table in the "Result" column. In this case, a conclusion based on the results is issued.

In the samples with an insufficient amount of DNA for analysis (Cp>35.0 on the Hex detection channel),

incorrect ΔCp values, or if the results for duplicates do not match, the program determines doubtful or invalid results. "?" or “invalid" will be indicated in the "Result" column respectively. In this case, repeated PCR with the existing DNA preparation is required, or repeated DNA extraction and PCR restaging, or repeated taking of the clinical material (performed sequentially).

For positive and negative control samples the results must correspond to those from the Table 4.

Table 4 – The results of the test for positive and negative control samples

Entered material	Detection channel		
	Fam (RHD gene, 7 exon)	Hex (SIC)	Rox (RHD gene, 10 exon)
“C+”	28≤ Cp ≤32	28≤ Cp ≤32	28≤ Cp ≤32
“C-“ (3 repeats)	Cp is not specified[1] or >41	Cp is not specified or >39	Cp is not specified or >41

[1] A dash in the result table.

If results for negative control sample differ from those in the Table 4, the results of the whole series are considered invalid. In this case decontamination is required.

If results for positive control sample differ from those in Table 4, repeat of amplification of the whole series is required.

11. SPECIFICATIONS

a. The analytical **specificity** of the **Fetal RHD Genotyping REAL-TIME PCR 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.

The DNA samples extracted from blood of Rhd-negative pregnant women and containing RHD gene with 7 exon are to be registered positive on Fam and Hex channels, with 10 exon – on Rox and Hex channels. The presence of two or one of any exons of the RHD gene in fetal DNA sample means the positive Rh factor of the fetus.

The DNA samples extracted from blood of Rhd-negative pregnant women and not containing RHD gene are to be registered negative on Fam and Rox channels, but positive through Hex channel.

The interpretation of the test results is carried out automatically using the software for the device (see Annex A).

b. The analytical **sensitivity** of the **Fetal RHD Genotyping REAL-TIME PCR Kit** using the **PREP-NA-FET DNA Extraction Kit** is 150 copies of genomic DNA (the total DNA of the mother and fetus) in 1.0 mL of the blood plasma sample.

The amount of analyzed total DNA of the mother and fetus should be at least 0.1 ng per amplification tube, which approximately corresponds to Cp≤35.0 on the SIC (Hex) detection channel. When using a smaller amount of DNA (Cp>35.0 on the SIC detection channel), unreliable results will be obtained associated with an insufficient amount of fetal DNA for analysis.

c. Diagnostic characteristics

Diagnostic sensitivity (100% CI) - 100% (92-100%);

Diagnostic specificity (100% CI) – 100% (90-100%).

ATTENTION!The claimed specifications are guaranteed when DNA extraction is performed with **PREP-NA-FET DNA Extraction Kit**.

12. TROUBLESHOOTING

Table 5. Troubleshooting

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

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

Phone: +7(495) 640.16.93

E-mail: hotline@dna-technology.ru

http://dna-technology.com/support

13. QUALITY CONTROL

"DNA-Technology Research&Production", LLC declares that the above mentioned 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 **Fetal RHD REAL-TIME PCR Detection Kit**:

Technical support:

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Manufacturer
	Temperature limit		Date of manufacture
Σ	Contains sufficient for <n> tests		Consult instructions for use
	Use by date	REF	Catalogue number
LOT	Batch code		Keep away from sunlight
VER	Version	CONTROL +	Positive control
EC REP	Authorized representative in the European Community		Caution

Annex A

Results calculation and interpretation principles

Detection and data analysis are made by software automatically. The principles of calculating the results set out in this annex are informative and not intended for users to perform independent calculations.

The calculation of the results is based on the evaluation of the Cp values of the indicator cycles of the studied targets (ΔCp).

Results interpretation principles

Result on Fam channel (Fam Cp)	Result on Hex channel (Hex Cp)	Result on Rox channel (Rox Cp)	ΔCp (Fam Cp – Hex Cp)	ΔCp (Rox Cp - Hex Cp)	Interpretation
Cp is not specified[1] or >41,0	Cp ≤35	Cp is not specified or >41.0	-	-	Fetal Rh factor: genotypically negative
Cp ≤41.0		Cp ≤41.0	Not less than 2.0	Not less than 2.0	Fetal Rh factor: genotypically positive
Cp is not specified or >41.0		Cp ≤41.0	-	Not less than 2.0	
Cp ≤41.0		Cp is not specified or >41.0	Not less than 2.0	-	
Cp ≤41.0		Cp is not specified or >41.0	Less than 1.0	-	The Rh factor of a pregnant woman: genotypically positive, the Rh factor of the fetus cannot be determined by this method
Cp is not specified or >41.0		Cp ≤41.0	-	Less than 1.0	
Cp ≤41.0		Cp ≤41.0	Less than 1.0	Less than 1.0	

[1] - A dash in the result table.

ATTENTION! ΔCp can take negative values if a pregnant woman is genotypically Rh-positive.

REF R1-H802-S3/9EU
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VER 727.2022.03.16