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CE IVD

**For professional use only**

# Fetal Gender REAL-TIME PCR Detection Kit

# INSTRUCTION FOR USE


EC REP

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R1-H803-23/9EU
R1-H803-UA/9EU

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## 1. INTENDED USE

The **Fetal Gender REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **Fetal Gender REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) - detection-based product. The **Fetal Gender REAL-TIME PCR Detection Kit** is intended for the detection of multi-copy fragment of Y chromosome in samples of cell-free fetal DNA extracted from the blood of pregnant women by Real-Time PCR method. Peripheral blood is used as a sample.

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

The **Fetal Gender REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: personnel qualified in molecular diagnostics methods and in working with pathogenic microorganisms 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

Method: polymerase chain reaction (PCR) with real-time detection of results; 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 (package S) or Taq-polymerase blocked by antibodies (package U). The polymerase chain reaction starts only when paraffin is melted or after the Taq-polymerase/antibody complex temperature dissociating. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The **Fetal Gender REAL-TIME PCR Detection 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 Gender REAL-TIME PCR Detection Kit** includes PCR-mix specific for determining the presence of a multi-copy fragment of Y chromosome 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.

DNA probe used for the detection of the multi-copy fragment of Y chromosome product amplification includes fluorescent dye Fam. DNA probe used for the detection of SIC includes the fluorescent dye Hex. The application of two 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** |
|---|---|---|---|---|
| Y chromosome fragment | SIC | - | - | - |

The automatic analysis is available on "DNA-Technology" made instruments: DTlite or DTprime REAL- TIME Thermal Cyclers for **Fetal Gender REAL-TIME PCR Detection 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.

## 3. CONTENT

The **Fetal Gender REAL-TIME PCR Detection Kit** is presented in Tables 2, 3.

Table 2. The **Fetal Gender REAL-TIME PCR Detection Kit** content, package S (standard) for R1-H803-S3/9EU and R1-H803-23/9EU

| **Reagent** | **Description** | **Total volume** | **Amount** |
|---|---|---|---|
| Paraffin sealed PCR-mix | Colorless transparent liquid under waxy white fraction | 1920 µL (20 µL in each tube) | 96 tubes 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 | | |

Table 3. The **Fetal Gender REAL-TIME PCR Detection Kit** content, package U for R1-H803-UA/9EU

| **Reagent** | **Description** | **Total volume** | **Amount** |
|---|---|---|---|
| PCR-mix | Colorless or slightly pink transparent liquid | 600 µL | 1 tube |
| TechnoTaq MAX polymerase | Colorless transparent viscous liquid | 30 µL | 1 tube |
| PCR-buffer | Colorless transparent liquid | 600 µL | 1 tube |
| Positive control | Colorless transparent liquid | 130 µL | 1 tube |

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

The **Fetal Gender REAL-TIME PCR Detection Kit**, including 96 tubes, is intended for single use and designed for 48 tests (no more than 46 analyzed samples in doubles, negative controls (in 3 repeats) and positive control).

[1] - for detection kit packaged in strips REF R1-H803-S3/9EU

## 4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

### 4.1. Specimen collection

- For blood collection: 4.5 mL Vacuette blood collection tubes with anticoagulant 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(g) no less than 1150) for 4.5 mL tubes;
- High speed centrifuge (RCF(g) no less than 17000) for 1.5 mL tubes;
- Solid-state thermostat (temperature range 65-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Freezing container, e.g. IsoFreeze 24x1.5/2 mL (SSI), or CoolRack M15, 15x1.5/2 mL (Biocision), or other analogous equipment;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- RNase and DNase free pipette tips for aspirator with trap flask;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA-FET DNA 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 and 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);
- DTstream M1 dosage instrument (only for automated dosing using detection kit in the package U);
- Device for tray sealing DTpack ("DNA-Technology", LLC) (only for automated dosing using detection kit in the package U);
- Centrifuge for microtrays (only for automated dosing using detection kit in the package U);

- Polymer thermal seal for microtray sealing (only for automated dosing using detection kit in the package U);
- PCR mictotray (only for automated dosing using detection kit in the package U);
- 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 **Fetal Gender REAL-TIME PCR Detection Kit** except the TechnoTaq MAX polymerase must be stored at temperatures from 2 °C to 8 °C during the storage period. TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to minus 22 °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, except the TechnoTaq MAX polymerase, 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.

It is allowed to transport the TechnoTaq MAX polymerase in termobox 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 except TechnoTaq MAX polymerase 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 kits stored in under undue regime should not be used.

An expired **Fetal Gender REAL-TIME PCR Detection 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 Gender REAL-TIME PCR Detection 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 Gender REAL-TIME PCR Detection 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 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 Gender REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from peripheral blood.

**ATTENTION**! The patient's pregnancy period should be at least 8 embryological or 10 obstetric weeks.

**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 room temperature (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 Kit.**

1 Centrifuge the tube with blood at RCF(g) 1000-2000 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 fetDNA extraction 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 included in the **PREP-NA-FET DNA Extraction Kit** should go through all stages of DNA extraction in volumes as indicated.

**Assay procedure**

### 8.1 Preparing PCR for package S

**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, strips ( REF R1-H803-S3/9EU), strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

8.1.1 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 |

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

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

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

8.1.5 Vortex the tubes with DNA samples, positive control and negative control for 3-5 seconds, then spin down 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 strip to prevent contamination. Close the tubes/strips tightly. Use filter tips.

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

8.1.7 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.

8.1.8 Spin the tubes/strips briefly for 1-3 seconds in vortex mixer.

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

8.1.10 Launch the operating software for DT instrument[2]. Add corresponding test[3], specify the number and ID's of the samples, positive and negative controls. Specify the position of the tubes/strips in the thermal unit (see 8.1.9) and run PCR. See Table 4.

Table 4. The PCR program for DTlite and DTprime Thermal Cyclers (package S)

| 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[1] | | … | Holding | | Holding |
| √ - optical measurement<br>[1] – holding at 25°C is allowed | | | | | | |

### 8.2 Preparing PCR for package U, manual dosing

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

8.2.1. Mark the required number of 0.2 mL tubes: 2 for each test sample, 3 for negative control (C-) and 1 for positive control (C+).

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

8.2.2. Vortex the tube with PCR-mix for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

8.2.3. Add to each tube 6.0 µL of PCR-mix.

8.2.4. Vortex the PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin for 1-3 seconds.

**ATTENTION**! TechnoTaq MAX polymerase should be got out from the freezer immediately prior to use.

[2] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[3] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

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

- 6.0 x (N+1) µL of PCR-buffer,
- 0.3 x (N+1) µL of TechnoTaq MAX polymerase,

N is a quantity of the samples to be tested taking to account "C-", "C+".

**Example**: for simultaneous testing of 2 samples, "C-" and "C+" in one PCR run, mark 8 tubes (4 tubes for samples to be tested, 1 tube for "C+" and 3 tubes for "C-"). Prepare the mixture of PCR-buffer and Taq-polymerase for 9 (8+1) tubes. Mix 54 µL of PCR-buffer and 2.7 µL of TechnoTaq MAX polymerase.

8.2.6. Vortex the tube with the mixture of PCR-buffer and TechnoTaq MAX polymerase (3-5 seconds) and spin briefly in vortex mixer (1-3 seconds).

**ATTENTION**! Mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared immediately prior to use.

8.2.7. Add 6.0 µL of PCR-buffer and TechnoTaq MAX polymerase mixture into each tube with PCR-mix.

**ATTENTION**! Follow the steps listed in pp. 8.2.8 – 8.2.13 within two hours after addition of PCR-buffer and TechnoTaq MAX polymerase mixture to PCR-mix.

8.2.8. Vortex the tubes with samples, "C+" and "C-" for 3-5 seconds and spin down 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. Close the tubes tightly. Use filter tips.

8.2.9. Add 6.0 µL of DNA sample into corresponding tubes (2 tubes for each sample). Do not add DNA into the "C+", "C-" tubes.

8.2.10. Add 6.0 µL of negative control (C-) which passed whole DNA extraction procedure into corresponding tubes (3 tubes). Add 6.0 µL of positive control (C+) into corresponding tube.

8.2.11. Spin tubes for 3-5 seconds.

8.2.12. Set the tubes into the Real-time Thermal Cycler.

8.2.13. Launch the operating software for DT instrument[4]. Add corresponding test[5], specify the number and ID's of the samples, positive and negative controls. Specify the position of the tubes in the thermal unit (see 8.2.12) and run PCR. See Table 5.

[4] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[5] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Table 5. The PCR program for DTlite and DTprime Thermal Cyclers (package U)

| Step | Temperature, °C | Min. | Sec. | Number of cycles | Optical measurement | Type of the step |
|---|---|---|---|---|---|---|
| 1 | 80 | 0 | 5 | 15 | | Cycle |
| | 94 | 0 | 5 | | | |
| | | | | | | |
| 2 | 94 | 5 | 00 | 1 | | Cycle |
| | | | | | | |
| 3 | 94 | 0 | 30 | 5 | | Cycle |
| | 64 | 0 | 15 | | √ | |
| | | | | | | |
| 4 | 94 | 0 | 10 | 45 | | Cycle |
| | 64 | 0 | 15 | | √ | |
| | | | | | | |
| 5 | 94 | 0 | 5 | 1 | | Cycle |
| | | | | | | |
| 6 | 10[1] | | | Holding | | Holding |
| [1] – holding at 25°C is allowed | | | | | | |

### 8.3 Preparing PCR for package U, using DTStream

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

8.3.1. Vortex the tube with PCR-mix for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

8.3.2. Vortex the TechnoTaq MAX polymerase and PCR-buffer for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

**ATTENTION**! TechnoTaq MAX polymerase should be got out from the freezer immediately prior to use.

8.3.3. Prepare the mixture of PCR-buffer with TechnoTaq MAX polymerase according to the user manual for dosing device DTstream.

8.3.4. Vortex the tube with the mixture for 3-5 seconds, the spin in vortex for 1-3 seconds to collect the drops.

8.3.5. Vortex the tubes with DNA samples, “C-” and “C+” for 3-5 seconds and spin down the drops in vortex for 1-3 seconds.

8.3.6. Set the tubes with PCR-mix, the mixture of PCR-buffer and TechnoTaq MAX polymerase, DNA samples, positive and negative controls and PCR microtray on the DTstream working table and conduct dosage of the components according to DTstream user manual.

8.3.7. After the end of dosing program on DTstream put the PCR microtray without shaking on the working table of DTpack sealing device.

8.3.8. Run the process of sealing of PCR microtray according to the user manual of DTpack sealing device.

8.3.9. Centrifuge the microtray on RCF(g) 500 for 30 seconds.

8.3.10. Set the PCR microtray into the Real-time Thermal Cycler.

8.3.11. Launch the operating software for DT instrument[6]. Add corresponding test[7], specify the number and ID’s of the samples, positive and negative controls. Specify the position of the tubes in the thermal unit (see 8.3.10) and run PCR. See Table 5.

[6] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.

[7] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website

## 9. CONTROLS

The **Fetal Gender REAL-TIME PCR Detection Kit** contains positive control. 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 should go through all stages of DNA extraction. Use the negative control included in the **PREP-NA-FET DNA Extraction Kit** in volumes as indicated.

The test result is considered valid when fetal gender is defined.

The test result is considered invalid when fetal gender is not defined.

If Cp >35 for “C+”, repeat of amplification of the whole series is required.

If Cp is specified or ≤39 for “C-”, whole test of current batch is considered false. Decontamination is required.

## 10. DATA ANALYSIS

In case of using DNA-Technology made 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.

The 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) 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 Cp values for the Fam channel (a specific fragment of the Y chromosome) and for the Hex channel (SIC) 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 Cp 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 case of multiple pregnancies, the test result "the gender of the fetus is female" will mean that all the fetuses are female, and the test result "the gender of the fetus is male" will mean that at least one fetus is male.

In the samples with an insufficient amount of DNA for analysis (Cp>35.0 on the SIC detection channel), incorrect Cp 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 controls, the results must correspond to those from the Table 6.

Table 6 – The results of the test for positive and negative controls

| Entered material | Detection channel | |
|---|---|---|
| | **Fam** (Y chromosome fragment) | **Hex** (SIC) |
| “C+” | Cp ≤35 | Cp ≤35 |
| “C-” (3 repeats) | Cp is not specified or >39 | Cp is not specified or >39 |

If results for negative control differ from those in the Table 6, the results of the whole series are

https://www.dna-technology.com/assaylibrary.

considered invalid. In this case decontamination is required.

If results for positive control differ from those in Table 6, repeat of amplification of the whole series is required.

## 11. SPECIFICATIONS

**a.** The analytical **specificity** of the **Fetal Gender REAL-TIME PCR Detection 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 amplification of a fetal DNA sample containing Y chromosome fragment should result in positive results for Fam and Hex channels. In this case, the sex of the fetus is male.

The amplification of a fetal DNA sample isolated from the blood of a pregnant woman, and not containing the Y chromosome fragment should result in a negative result for the Fam channel but a positive result for the Hex channel. In this case, the sex of the fetus is female.

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

**b.** Analytical **sensitivity** of the **Fetal Gender REAL-TIME PCR Detection 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 detection channel (Hex). When using a smaller amount of DNA (Cp>35.0 on the SIC detection channel), unreliable results will be obtained.

**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** ( REF P-027/2EU).

## 12. TROUBLESHOOTING

Table 7. Troubleshooting

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

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 **Fetal Gender 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 |
|  | 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.

Results interpretation principles

| Cp on Fam channel | Result on Fam channel | Cp on Hex channel | Result on Hex channel | Interpretation |
|---|---|---|---|---|
| Cp ≤35 | + | Cp ≤35 | + | Fetal gender is male |
| Cp is not specified or >37 | - | Cp ≤35 | + | Fetal gender is female |

**ATTENTION**! The results in duplicates for each sample must match qualitatively (the presence or absence of a fluorescence signal in the Cp intervals indicated in the table).