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INSTRUCTIONS FOR USE

Reagent kit for the isolation of circulating free DNA from blood plasma "DNA-Plasma-M-RT"

TS 21.20.23-010-97638376-2017

Version 3 dated 16.09.2019

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Introduction

Target analyte. DNA-Plasma-M-RT reagent kit is used at the sample preparation stage for subsequent testing. The kit is not designed for the obtained human DNA detection as a target analyte.

Scientific validity.

DNA isolation is an important step in sample preparation. Many techniques, such as amplification, reverse transcription, amplification product accumulation detection by real-time PCR, etc., cannot be performed directly on biological samples without prior nucleic acids purification.[1]

DNA isolation is necessary for genetic testing, which is used for scientific and medical purposes. In medical practice, it is used to diagnose hereditary diseases and determine the risk of developing various hereditary diseases.

Circulating cell-free DNA are double–stranded low-molecular-weight genomic DNA molecules, fragmented into short (70-200 base pairs) and long (up to 21 000 base pairs) segments, RNases and proteinases resistant, but cleaved with DNase. There are two possible sources of cfDNA: passive DNA release with apoptotic cells and necrotic cells and active DNA release by cellular secretion[2].

Circulating cell-free nucleic acids (ccfNAs) in plasma or blood serum obtained after the isolation stage are an extremely valuable object of subsequent molecular genetic testing.

The scope of the reagent kit is clinical laboratory diagnostics.

Indications and contraindications for use.

Indications for use: DNA-Plasma-M-RT reagent kit is recommended for circulating cell-free DNA (cfDNA) isolation from human peripheral venous blood plasma for subsequent testing in clinical laboratory diagnostics by allele-specific real-time PCR.

Contraindications for use: none.

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[1] Antonova, O.S., Korneva, N.A., Belov, Y.V., Kurochkin, V.E. Effective methods of nucleic acid isolation for testing in molecular biology. Review. // Scientific instrument engineering. - 2010. - Volume 20, No. 1. - pp. 3-9.

[2] Tamkovich S.N., Vlasov V.V., Laktionov P.P. Circulating blood DNA and their use in medical diagnostics / Tamkovich S.N., Vlasov V.V., Laktionov P.P. // Molecular Biology. - 2008. - Vol. 42, No. 1. - pp. 12-23.

1. Intended use

Intended use: DNA-Plasma-M-RT reagent kit is designed to isolate circulating cell-free DNA (cfDNA) from human peripheral venous blood plasma by a method based on reversible binding of nucleic acids on the magnetic beads surface for subsequent testing in clinical laboratory diagnostics by allele-specific real-time PCR.

Functional purpose: DNA-Plasma-M-RT reagent kit is designed to provide a preanalytical testing stage. Circulating free DNA (cfDNA) isolated from human blood plasma is not the basis for diagnosis, but it can be used for subsequent testing in clinical laboratory diagnostics, particularly in prenatal diagnostics and oncology, when tested by allele-specific real-time PCR.

For example, during subsequent allele-specific PCR, the following medical devices can be used together:

- A reagent kit for determination of EGFR gene mutation status by real-time polymerase chain reaction with hybridization-fluorescence detection (PCR-RT) in cell-free DNA from human blood plasma (Test-EGFR-plasma) according to TS 9398-004-97638376-2015 in configuration forms: 1) Test-EGFR-12 for 12 detections, 2) Test-EGFR-24 for 24 detections, manufactured by TestGene LLC (Registration certificate No. RZN 2017/6267 dated September 19, 2017),
- Reagent kits for qualitative determination of fetal RHD gene in blood plasma sample of a pregnant woman with negative Rh factor by real-time polymerase chain reaction with hybridization-fluorescence detection (PCR-RT) “RHD-test” according to TS 9398-001-97638376-2012, manufactured by TestGene LLC (Registration certificate No. RZN 2015/2703 dated February 03, 2016)", and similar.

Potential consumers of a medical device:

The kit is intended for professional use in medical centers and clinical diagnostic laboratories. Professional level of potential users – clinical laboratory diagnostics doctor, medical technologist, medical laboratory technician.

2. Method principle

Test sample type

Material for circulating cell-free DNA isolation is human peripheral venous blood plasma.

Method principle

The method principle used in the kit is based on reversible DNA binding on the magnetic beads surface. After the sample is lysed, the nucleic acids it contains bind to magnetic beads. Then wash them with Wash Solutions No. 1 and No. 2, included in the kit. After several washing cycles, the magnetic beads sediment must be dried, after which the nucleic acids can be eluted.

Total time of DNA isolation from 1 sample is 70 minutes.

3. Reagent kit components

Configuration forms

DNA-Plasma-M-RT reagent kit is designed in three configuration forms:

1) DNA-Plasma-M-RT-25 for 25 isolations,

2) DNA-Plasma-M-RT-50 for 50 isolations,

3) DNA-Plasma-M-RT-50 with a magnetic separation rack for 50 isolations.

DNA-Plasma-M-RT reagent kit is designed for isolation from 2 ml of plasma.

Reagent kit components

Table 1 – DNA-Plasma-M-RT reagent kit components

No.	Reagent name	Description	Configuration form		
			DNA-Plasma-M-RT-25 for 25 isolations	**DNA-Plasma-M-RT-50 for 50 isolations**	**DNA-Plasma-M-RT-50 with a magnetic separation rack for 50 isolations**
1	DNA Binding Buffer	Transparent colorless liquid	1 bottle (90 ml)	2 bottles (90 ml each)	2 bottles (90 ml each)

2	Lysing Solution	Transparent colorless liquid	1 bottle (20 ml)	1 bottle (40 ml)	1 bottle (40 ml)
3	Magnetic beads, MB	Brown liquid	1 tube (600 µl)	1 tube (1200 µl)	1 tube (1200 µl)
4	Wash Solution No.1	Transparent colorless liquid	1 bottle (16 ml)	1 bottle (30 ml)	1 bottle (30 ml)
5	Wash Solution No.2	Transparent colorless liquid	1 bottle (8 ml)	1 bottle (15 ml)	1 bottle (15 ml)
6	Eluent	Transparent colorless liquid	1 bottle (3 ml)	1 bottle (6 ml)	1 bottle (6 ml)
7	Magnetic separation rack	1.5 ml and 15 ml tube holder with magnet 100*15*15 mm	-	-	1 piece

Calibrators and control materials are not used in the isolation kit.

The kit contains no products for medical use, materials of human or animal origin.

Note: The kit does not contain any other components that may affect the procedure.

4. Reagent kit characteristics

4.1 Technical and functional characteristics

Table 2 – DNA-Plasma-M-RT reagent kit technical and functional characteristics

Indicator name	Characteristics and standards
1. Technical characteristics	
1.1. Appearance	
1.1.1 DNA-Plasma-M-RT-25 reagent kit for 25 isolations	
DNA Binding Buffer	Transparent colorless liquid
Lysing Solution	Transparent colorless liquid

Magnetic Beads, MB	Brown liquid
Wash Solution No. 1	Transparent colorless liquid
Wash Solution No. 2	Transparent colorless liquid
Eluent	Transparent colorless liquid
1.1.2 DNA-Plasma-M-RT-25 reagent kit for 50 isolations	
DNA Binding Buffer	Transparent colorless liquid
Lysing Solution	Transparent colorless liquid
Magnetic Beads, MB	Brown liquid
Wash Solution No. 1	Transparent colorless liquid
Wash Solution No. 2	Transparent colorless liquid
Eluent	Transparent colorless liquid
1.1.3 DNA-Plasma-M-RT-50 reagent kit with a magnetic separation rack for 50 isolations	
DNA Binding Buffer	Transparent colorless liquid
Lysing Solution	Transparent colorless liquid
Magnetic Beads, MB	Brown liquid
Wash Solution No. 1	Transparent colorless liquid
Wash Solution No. 2	Transparent colorless liquid
Eluent	Transparent colorless liquid

Magnetic separation rack	1.5 ml and 15 ml tube holder with magnet 100*15*15 mm
1.2 Physicochemical parameters	
Hydrogen ion concentration values, pH	
DNA Binding Buffer	min 6.0 pH, max 8.0 pH
Wash Solution No. 1	min 6.0 pH, max 8.0 pH
Wash Solution No. 2	min 6.0 pH, max 8.0 pH
1.3. Completeness	According to clause 1.4 TS 21.20.23-010-97638376-2017
1.4. Labelling	According to clause 1.5 TS 21.20.23-010-97638376-2017
1.5. Packaging	According to clause 1.6 TS 21.20.23-010-97638376-2017
2. Functional characteristics	
2.1. DNA isolation efficiency, %, at least	20
2.2. DNA isolation purity, A260/280, at least	1.6

4.3 Clinical efficiency characteristics:

Since the clinical trials purpose was to verify the medical device functional properties and/or efficiency when used in accordance with its intended purpose, specified in the manufacturer's documentation, so based on the clinical trials results, in a series of 150 experiments with isolated circulating cell-free DNA samples, the tested medical device functional properties (DNA isolation efficiency - at least 20%, DNA isolation purity, A260/280 - at least 1.6) and effectiveness when used in accordance with its intended purpose (DNA isolated from blood plasma samples is suitable for subsequent testing in clinical laboratory diagnostics by allele-specific real-time PCR) were confirmed at 98.1 % level of diagnostic sensitivity and 90% of confidence probability.

5. Risks associated with DNA-Plasma-M-RT reagent kit use

The border risk zone includes the following hazards:

- loss of functional properties of the reagents included in the kit due to transportation, storage or use under inappropriate conditions,
- contaminant presence in DNA;
- carrying out the DNA isolation procedure from an insufficient blood plasma amount;
- failure to comply with the requirements for sample preparation, testing and disposal due to the unqualified personnel work;
- use of an unsuitable kit (use after the expiration date or in case of damaged packaging);

The cumulative residual risk of using a "Reagent kit for the isolation of circulating free DNA from blood plasma "DNA-Plasma-M-RT" manufactured by TestGene LLC is acceptable, the benefits of its use exceed the risk.

6. Safety precautions

Potential risk class - is 2a - in accordance with the Nomenclature Classification of Medical Devices approved by the Order of the Ministry of Health of the Russian Federation No. 4n dated 06.06.2012.

The work should be carried out in a laboratory performing molecular biological (PCR) assays of clinical material, in compliance with the sanitary and epidemiological rules of SP 1.3.2322-08 "Safety of working with microorganisms of pathogenicity (danger) groups III-IV and pathogens of parasitic diseases", SanPiN 2.1.7.2790-10 "Sanitary and epidemiological requirements for the treatment of medical waste" and methodological guidelines MU 1.3.2569-09 "Organization of work of laboratories using methods of nucleic acid amplification when working with material containing microorganisms of pathogenicity groups I– IV".

The following requirements should always be met when working:

▪ the test samples should be considered as infectious and dangerous, and work and storage should be organized in accordance with SP 1.3.2322-08 "Safety of work with microorganisms of pathogenicity (danger) groups III–IV and parasitic diseases pathogens ";

▪ clean and disinfect spilled samples or reagents using

disinfectants in accordance with SP 1.3.2322-08 "Safety of working with microorganisms of pathogenicity (danger) groups III–IV and parasitic diseases pathogens";

▪ the laboratory process should be unidirectional. The testing is carried out in separate rooms (areas). Work should begin in the Isolation Area and continue in the Amplification and Detection Area. Do not return samples, equipment and reagents to the area where the previous process stage was carried out;

▪ unused reagents, expired reagents as well as used reagents should be disposed of in accordance with the requirements of SanPiN 2.1.7.2790-10 "Sanitary and epidemiological requirements for medical waste management";

▪ use and change disposable filter tips for automatic dispensers during each operation. Disposable plastic items must be disposed of in a special container with a disinfectant that can be used to disinfect medical waste;

▪ table surfaces, as well as rooms where PCR is performed, should be exposed to ultraviolet radiation for 30 minutes before and after work completion;

▪ use the kit strictly for its intended purpose, according to these instructions;

▪ only specially trained personnel is allowed to work with the kit;

▪ do not use the kit after the expiry date;

▪ do not use the reagent kit if the inner packaging is damaged or the reagent appearance does not match the description;

▪ use disposable gloves, lab coats and eye protection while working with samples and reagents. Wash hands thoroughly when work finishing work;

▪ all kit components are non-toxic to humans in the stated concentrations. In case of kit components contact with the skin or mucous membranes, rinse the affected area with plenty of water.

The necessary precautions regarding the effects of magnetic fields, external electrical influences, electrostatic discharges, pressure or pressure changes, overload of sources of thermal inflammation are not provided.

The kit contains no substances of human or animal origin with a

potential infectious nature, therefore, precautions against any special, unusual risks during the product use or sale are not provided.

7. Required equipment and materials

Equipment:

1. Sterile laminar flow cabinet (e.g., BAVp-01-Laminar-C-1.2, Lamsystems, Russia),
2. Thermostat for Eppendorf type tubes, maintaining temperatures from +25 to +100°C (e.g., Termo 24-15, Biocom, Russia),
3. Medical vacuum aspirator with a trap flask (e.g., OM-1, Ulyanovsk, Russia),
4. Vortex (e.g., TETA-2, Biocom, Russia),
5. Separate set of automatic variable volume dispensers (e.g., Eppendorf, Germany),
6. Refrigerator from +2 to +8°C with freezer below -16°C.

Materials and reagents not included in the kit:

1. Ethyl alcohol (95%),
   2. Disposable polypropylene screw-on or tightly closed 1.5 ml microtubes, extraneous DNA and DNase free (e.g., Axygen, USA),
3. Disposable polypropylene screw-on or tightly closed 15 ml microtubes, extraneous DNA and DNases free (e.g., Axygen, USA),
   4. Racks for 15 ml and 1.5 ml tubes (e.g., InterLabService, Russia) and pipette tips (e.g., Axygen, USA),
5. Magnetic separation rack for 1.5 and 15 ml tubes (e.g., InterLabService, Russia),
6. Disposable pipette tips for 100 µl, 1000 µl and 5 µl variable volume dispensers with an aerosol barrier, extraneous DNA and DNases free (e.g., Axygen, USA),
7. Disposable pipette tips for 100 µl and 1000 µl variable volume dispensers, extraneous DNA and DNases free (e.g., Axygen, USA),
8. Disposable or separate lab coats and disposable gloves,
9. Containers with disinfectant solution.

Measuring equipment is not required during the kit operating.

8. Test samples

Before starting work, review the guidelines "Sampling, transporting, and storing clinical material for PCR diagnostics" developed by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Moscow, 2012.

The material for the circulating cell-free DNA isolation procedure is human peripheral venous blood plasma.

8.1 Biological material sampling.

Sampling.

To obtain plasma, peripheral venous blood is placed into a test tube with CPDA or EDTA-K2 added as an anticoagulant. Turn the tube upside down 2-3 times to mix the blood with the anticoagulant after material sampling.

Initial clinical material transportation and storage conditions:

When using tubes with CDPA as an anticoagulant, whole blood can be transported to the laboratory within 2 days at 4-8°C. No more than 48 hours should pass from the moment of blood collection and plasma separation.

When using tubes with EDTA-K2 as an anticoagulant, blood must be delivered to the laboratory within 1 hour. From the moment of blood collection and plasma separation should pass no more than 2-3 hours.

ATTENTION! Avoid the blood tube freezing and heating above 25°C.

Sample preparation.

Centrifugate the tube with blood for 10-15 minutes at 2000-3000 g, then carefully remove the top plasma layer and transfer it into a separate disposable tube, avoiding leukocyte clots and erythrocyte layers. Centrifuge the plasma for 15 minutes at 13000g or 10 minutes at 16000g, transfer the top layer into a separate tube again, without the sediment at the tube bottom. The obtained plasma can be used for isolation.

8.2 Interfering substances and limitations on the test material use

The effect of potentially interfering substances on DNA-Plasma-M-RT reagent kit performance was tested for potentially interfering substances that will occur during human whole peripheral blood

collection and during DNA-Plasma-M-RT reagent kit normal use, and presumably influence the result of circulating cell-free DNA isolation from human blood plasma of the appropriate quality and quantity required for testing in clinical laboratory diagnostics by allele-specific real-time PCR.

Potentially interfering substances that may occur during the human whole peripheral blood collection, can get into the DNA test samples and affect DNA Plasma-M-RT reagent kit ability to isolate circulating cell-free DNA from human blood plasma, and the range of tested concentrations is shown in Table 4.

Table 4 – Potentially interfering substances at whole peripheral blood collection stage, excess of which causes PCR inhibition.

Interfering substances	Maximum concentration	Minimal concentration
Heparin (anticoagulant)	0.15 U/ml	0.075 U/ml
Sodium citrate (anticoagulant)	1 mmol/l	0.5 mmol/l
Hemoglobin - high molecular weight protein fraction (hemolysis)	1 mg/ml	0.5 mg/ml
Triglycerides (chylous plasma)	0.5 mmol/l	0.25 mmol/l

For the potentially interfering substances study under DNA-Plasma-M-RT reagent kit normal use, ethyl alcohol (95%), which is added to Wash Solution No. 1 and Wash Solution No. 2 at the kit components preparation for the testing stage, was chosen as an interfering substance due to its potential inhibitory effect on PCR. The potentially interfering substance concentrations are shown in Table 5.

Table 5 – Range of interfering substances concentrations tested during the assay

Interfering substances	Maximum concentration (µl / 200 µl of DNA solution)	Minimum concentration (µl / 200 µl of DNA solution)

Ethyl alcohol (95%)	$1.35*10^{-3}$	$3.38*10^{-4}$

To evaluate the potentially interfering substances effect, the study was performed by analyzing their effects at two concentrations (maximum and minimum), the range expected to occur during human whole peripheral blood collection and during DNA-Plasma-M-RT reagent kit normal use, on the DNA isolation purity values (expressed as a ratio of the optical densities of the isolated DNA solution, A260/280) and the DNA isolation efficiency (expressed in %), followed by allele-specific real-time PCR assay.

Based on the results obtained, substances in DNA samples can interfere at concentrations exceeding permissible levels:

Interfering substances	**Inhibiting PCR concentrations**
Heparin (anticoagulant)	$\geq$ 0.15 U/ml
Sodium citrate (anticoagulant)	>1 mmol/L
Hemoglobin - high molecular weight protein fraction (hemolysis)	$\geq$ 1 mg/ml
Triglycerides (chylous plasma)	>0.5 mmol/L

The study results indicate that, ethyl alcohol, evaluated at concentrations that may occur during the reagent kit normal use, does not affect DNA-Plasma-M-RT reagent kit ability to isolate circulating cell-free DNA (cfDNA) from blood plasma of the appropriate quality and quantity required for testing in clinical laboratory diagnostics using allele-specific real-time PCR.

8.3 Limitations on the test material use:

- To obtain plasma, blood is taken into a tube with CPDA or EDTA-K2 added as an anticoagulant. When using tubes with CPDA as an anticoagulant, it is allowed to transport whole blood to the laboratory within 2 days at 4-8°C. When using EDTA-K2, plasma should be separated within 2-3 hours from the moment of blood sampling.
- It is not allowed to freeze blood before the obtaining plasma procedure.

- Do not use hemolyzed and chylous blood; when testing such samples, the results may be unreliable.

- When separating plasma, it is not allowed to contaminate it with blood cells.

- Only one-time plasma freezing or thawing is allowed.

- Start PCR reaction right after DNA isolation procedure completion.

- Operator's errors during blood collection, plasma separation and DNA isolation procedure, the recommended instruction violation can lead to unreliable results.

8.4 Test samples storage conditions

Initial clinical material transportation and storage conditions:

When using tubes with CPDA as an anticoagulant, whole blood can be transported to the laboratory within 2 days at 4-8°C. No more than 48 hours should pass from the moment of blood collection and plasma separation. When using EDTA-K2 tubes as an anticoagulant, the blood must be delivered to the laboratory within 1 hour. From the moment of blood collection and plasma separation should pass no more than 2-3 hours.

ATTENTION! Avoid the blood tube freezing and heating above 25°C.

Plasma storage conditions:

- at a temperature below 4-8°C — up to 5 days;
- at a temperature of below -20°C — up to a month;
- at -70°C — for a long time.

ATTENTION! Only one-time material freezing or thawing is allowed.

ATTENTION! Do not use hemolyzed and chylous blood. When testing such samples, the results may be unreliable!

Storage conditions of a circulating cell-free DNA (cfDNA) sample isolated from blood plasma:

The obtained DNA should be stored at temperatures from +2 to +8°C up to 12 hours before the testing, at -20°C – up to 3 months or at -70°C – up to 1 year.

9. Components preparation for testing

It is not required to install, assemble, adjust, calibrate the medical device for commissioning.

If the plasma sample was stored in a refrigerator, warm the plasma to room temperature before the isolation procedure.

!!!The magnetic beads suspension is two-phase, it forms clearly separable phases easily and quickly. Before starting work and before each operation with magnetic bead solution, completely resuspend the magnetic bead solution on the vortex or by pipetting.

Layering or crystal sediment formation does not affect the solution quality. In case of crystal sediment formation or component layering, it is required to heat the bottles at 50°C and mix thoroughly until the sediment dissolves completely and the solutions homogenize.

Mix thoroughly all kit components before starting work.

Prepare Wash Solution No. 1 and Wash Solution No. 2 before starting work.

For DNA-Plasma-M-RT-25:

1) add 8 ml of ethyl alcohol (95%) to Wash Solution No. 1.
2) add 32 ml of ethyl alcohol (95%) to Wash Solution No. 2.

Make a note on the bottle label about the operation.

For DNA-Plasma-M-RT-50 and DNA-Plasma-M-RT-50 with a magnetic separation rack:

1) add 15 ml of ethyl alcohol (95%) to Wash Solution No. 1.
2) add 60 ml of ethyl alcohol (95%) to each bottle of Wash Solution No. 2.

Make a note on the bottle label about the operation.

10. Testing procedure

Only specially trained personnel with PCR analysis skills are allowed to work with the kit.

The isolation protocol can be modified to scale when more final material is required:

Plasma volume	Lysing Solution volume, µl	DNA Binding Buffer volume, µl	MB volume, µl	Wash Solution No. 1 volume, µl	Wash Solution No. 2 volume, µl
2 ml	600	3 000	20	700	700
3 ml	900	4 500			
4 ml	1 200	6 000			
5 ml	1 500	7 500			

DNA is isolated from 2 ml of plasma sample.

For each sample prepare 1 tube (15 ml) and 2 tubes (1.5 or 2 ml). Label the tubes according to the samples.

1. Add 2 ml of plasma into a 15 ml tube, add 600 µl of Lysing Solution and mix turning over 3-5 times.

2. Incubate at room temperature for 5 minutes, mixing the solution periodically, turning the tube over 3-5 times.

3. After incubation add 3 ml of Binding Buffer and 20 µl of well-resuspended magnetic bead solution into the tube. Mix by turning the tube 3-5 times over.

4. Incubate at room temperature for 15 minutes, mixing the solution periodically, turning the tube over 3-5 times to prevent magnetic bead sedimentation.

5. Place the tube in a magnetic separation rack, wait until the beads are completely collected on the tube wall (usually it takes 3-5 minutes), and remove the supernatant.

6. Add 700 µl of Wash Solution No. 1 into the tube. Resuspend completely the magnetic beads in the solution by pipetting and transfer the resulting suspension of magnetic beads in the Wash Solution No. 1 into a 1.5 ml tube.

7. Place the tube in a magnetic separation rack, wait until the beads are completely collected on the tube wall, and remove the supernatant (usually it takes 1 minute).

8. Add 700 µl of Wash Solution No. 2 into the tube and mix thoroughly on a vortex. Discharge the drops by short centrifugation.

9. Place the tube in a magnetic separation rack, wait until the beads are completely collected on the tube wall, and completely remove the supernatant (usually it takes 1 minute).

10. Repeat steps 8 and 9.

11. Place the tube with the lid open in a thermostat and incubate at 60°C for 10 minutes to dry magnetic beads and remove residual ethanol.

12. Add 60-100 µl of Eluent into the tube. Carefully resuspend the beads by pipetting.

13. Incubate the tube in a thermostat at 60°C for 10 minutes, shaking the solution periodically.

14. Place the tube in a magnetic separation rack, wait until the beads are completely collected on the tube wall, and transfer the supernatant containing the isolated DNA into a new tube.

When performing PCR, it is required to keep the tube with the isolated DNA on a magnetic separation rack.

If required the eluent volume can be increased, but the DNA concentration will decrease. The DNA concentration can be increased by reducing the eluent volume.

ATTENTION! If the isolation purpose is to test fetal DNA present in the maternal blood, the PCR reaction should begin right after the isolation procedure completion. Fetal DNA is present in the maternal blood at very low concentrations and in a degraded state. During storage, fetal DNA can be destroyed, which can lead to false negative results.

11. Possible problems and their solutions

1. Low DNA yield, reason and possible solution:

▪ sample condition (the sample contains insufficient DNA; the sample was stored for a long time or improperly stored or was frozen-thawed several times) – possible solutions: take more initial material or elute into less buffer; recollect the material;

▪ magnetic beads are poorly collected on the magnet, which makes it impossible to remove the supernatant after incubation (step 6), the supernatant is cloudy – heat the tube at 60˚C until the solution clears completely. If plasma was stored in the refrigerator, warm it up to room temperature before carrying out the isolation procedure.

▪ incomplete beads drying before adding the eluent – completely remove Wash Solution No. 2, increase the drying time after removing Wash Solution No. 2;

▪ magnetic beads overdrying – dry magnetic beads for 10 minutes;

▪ incomplete lysis – after adding Lysing Solution, suspend the sample as thoroughly as possible;

▪ large eluent volume – take an optimal eluent volume to obtain the desired DNA concentration.

2. Protein contaminants – it is required to achieve the most thorough magnetic bead suspension.

3. Possible DNA degradation, reason and possible solution – an old sample, or the sample was frozen-thawed - it is required to recollect the material. Do not freeze a sample during transportation and storage.

If you have any questions or need advice, please contact the TestGene Technical Support Service - see Section 14.

12. Storage, transportation and operation conditions

Storage.

Store DNA-Plasma-M-RT reagent kit in the manufacturer's packaging at a temperature below +30°C and relative humidity up to 90%. Atmospheric pressure is not subject to control as it does not affect the product quality.

A reagent kit stored in violation of the regulated regime cannot be used.

Transportation.

Transport DNA-Plasma-M-RT reagent kit in all types of covered vehicles in accordance with the transportation rules applicable to this transport type.

Transport a reagent kit at a temperature below +30°C and relative humidity up to 90%. Atmospheric pressure is not subject to control as it does not affect the product quality.

Reagent kits transported with violation of the temperature regime, cannot be used.

Shelf life.

DNA-Plasma-M-RT kit shelf life is 12 months from the acceptance date of the manufacturer's QCD, if all transportation, storage and operation conditions are met. A reagent kit with expired shelf life cannot be used.

Shelf life of the opened kit components is 12 months from the acceptance date of the manufacturer's QCD, if stored at a temperature below +30°C.

After opening the bottles and adding ethyl alcohol (95%) to Wash Solution No. 1 and Wash Solution No. 2 shelf life is 6 months.

A reagent kit stored in violation of the regulated regime cannot be used.

13. Disposal

Reagent kits that have become unusable, including due to expiration dates, must be disposed of in accordance with the requirements of SanPiN 2.1.7.2790-10 "Sanitary and epidemiological requirements for the treatment of medical waste".

According to the classification of medical waste, the kits belong to class A (epidemiologically safe waste, similar in composition to solid household waste). Unused reagents in accordance with clause 4.28 SanPiN 2.1.7.2790-10 "Sanitary and epidemiological requirements for the management of medical waste" are collected in disposable labeled packaging of any color (except yellow and red).

The remaining tubes and materials after the work are disposed of in accordance with MU 287-113 (Guidelines for disinfection, pre-sterilization cleaning and sterilization of medical devices).

Liquid components (reagents) are destroyed by draining into the sewer with preliminary reagent dilution with 1:100 tap water and removal of the remaining packaging as industrial or household waste.

DNA-Plasma-M-RT reagent kit consumer packaging is subject to mechanical destruction with the removal of residues as industrial or household waste.

Personnel destroying a reagent kit must comply with the safety rules of a particular destruction method.

14. Warranty, contacts

The manufacturer guarantees DNA-Plasma-M-RT reagent kit quality and safety during the shelf life if all transportation and storage requirements as well as operation rules are met.

In case of complaints about the reagent kit quality, undesirable events or incidents, submit information to:

Limited Liability Company TestGene (TestGene LLC),
9, 44th Inzhenerny Proezd, office 13, Ulyanovsk, 432072
Phone number: +7 (499) 705-03-75
www.testgene.com
Technical Support Service:
Phone number: +7 927 981 58 81
E-mail: help@testgen.ru

Annex A

List of applicable national standards

GOST ISO 14971-2011	Medical products. Application of risk management to medical devices.
GOST R 15.309-98	System of product development and launching into manufacture. Test and acceptance of produced goods. Principal positions.
GOST R 51088-2013	In vitro diagnostic medical devices. Reagents, kits, the test-systems, control materials, culture media. Requirements to devices and to supporting documentation.
GOST R 51352-2013	In vitro diagnostics medical devices. Test methods.
GOST R EN 13612-2010	Performance evaluation of in vitro diagnostic medical devices.
GOST R 56894-2016	Summary technical documentation for demonstrating conformity to the essential principles of safety and performance of in vitro diagnostic medical devices.
GOST R ISO 18113-1-2015	In vitro diagnostic medical devices. Information supplied by the manufacturer (labelling). Part 1. Terms, definitions and general requirements.
GOST R ISO 18113-2-2015	In vitro diagnostic medical devices. Information supplied by the manufacturer (labelling). Part 2. In vitro diagnostic reagents for professional use.
GOST R ISO 23640-2015	In vitro medical devices. Evaluation of stability of in vitro diagnostic reagents.
GOST R ISO 15223-1-2014	Medical devices. Symbols to be used with medical device labels, labelling, and information to be supplied. Part 1. General requirements.
GOST ISO 13485-2017	Medical devices. Quality management systems. Requirements for regulatory purposes.

Note – The above standards were in force at the time of the instructions for use approval. In the future, when using the document, it is advisable to check the validity of the reference normative documents at the current moment. If the reference document is replaced or modified, then the replaced (modified) document should be used when applying this document.